Founder: Federal Scientific Center
for Medical and Preventive
Health Risk Management
Technologies Federal Service
for Surveillance on Consumer
Rights Protection and
Human Wellbeing

Contact Information:
82 Monastyrskaya Str.,
Perm, 614045, Russia
Tel/Fax: +7 (342) 237-25-34
E-mail: journal@fcrisk.ru
Site: journal.fcrisk.ru/eng

Editor and corrector – M.N. Afanaseva
Technical Editor – M.M. Tsinker,
A.V. Alekseeva
Translator – N.V. Dubrovskaya

The publication 30.06.2025.
Format 90×60/8.
Order no. 161/2025.
Edition is 125 copies.
The price is free.

The Journal is registered by The Federal Service For Supervision Of Communications, Information Technology, And Mass Media (Roscomnadzor). Register certificate СМИ – ПИ no. ФС 77-52552 issued on January 21, 2013

Address of the publisher and printing house:
29 Komsomolsky ave., Perm, 614990,
Russia, tel.: +7 (342) 219-80-33

Printed by the Publishing House of Perm National Research Polytechnic University (29 Komsomolsky ave., Perm, 614990, Russia, tel.: +7 (342) 219-80-33)

Subscription number:
catalog "Russian Post"
annual subscription – 04153
semi-annual subscription – 83927

ISSN (Print) 2308-1155
ISSN (Online) 2308-1163
ISSN (Eng-online) 2542-2308

This edition is provided financial support by the Perm Region Ministry for Education and Science

HEALTH RISK ANALYSIS

Theoretical and practical journal. Start of publication: 2013.
4 issues per year

2

April 2025 June

CONTENTS

UDC 613.6.027, 613.644
DOI: 10.21668/health.risk/2025.2.01.eng

Read online

Research article

METHODOLOGY FOR ASSESSING AND PREDICTING PERSONALIZED OCCUPATIONAL HEALTH RISKS BASED ON ADAPTIVE NEURAL FUZZY NETWORK FOR IMAGE RECOGNITION

N.V. Zaitseva[1,2], A.A. Savochkina[3], M.A. Zemlyanova[1,3], D.V. Goryaev[4], A.G. Fadeev[4]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation
[2]Russian Academy of Sciences, Department for Medical Sciences, 14 Solyanka St., Moscow, 109240, Russian Federation
[3]Perm National Research Polytechnic University, 29 Komsomolskii Av., Perm, 614990, Russian Federation
[4]Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, Krasnoyarsk Regional Office, 21 Karatanova St., Krasnoyarsk, 660097, Russian Federation

Health protection provided for industrial production workers is a national priority, which determines possibilities for preservation of occupational longevity. Given that, it is becoming especially vital to create and develop scientific grounds for analyzing occupational health risks associated with complex exposures to occupational and work-related risk factors with special emphasis placed on personalized estimates. In this study, we aimed to develop and test a methodology and software for assessing and predicting personalized occupational health risks based on an adaptive neural fuzzy network for image recognition.

The study design was based on an artificial intellect model as a mathematical structure trained to recognize regularities and establish whether an analyzed object belonged to a specific occupational health risk category per a system of indicators. Network training and validation were performed on an example sample made of workers employed at underground copper-nickel ore mining using data on their working conditions, exposure factors and individual biomedical indicators (175,000 parameters overall). The training sample equaled 80 % and the validating one 20 %. The network was tested on an independent sample of data about workers exemplified by blast-hole drillers as a basic occupation at the mine.

A methodology was developed and provided with relevant software; its theoretical ground was represented by an adaptive neural fuzzy network for image recognition. The network had a specific hybrid multilayer architecture, which ensured accuracy of predictive estimates and error minimization. Personalized occupational health risks for each worker in the validating sample were caused by vibrational disease associated with simultaneous exposure to occupational noise (10–40 dBA higher than MPL) and total vibration equal to 106–113 dB; these risks were ranked as 'high' and 'very high'. Health risks caused by sensorineural hearing loss (SHL) associated with combined exposure to noise (5–30 dBA higher than MPL) and adverse chemicals (2.0–2.5 times higher than single maximum MPC) were estimated as varying from medium to very high. Health prediction for workers of this occupation in the independent sample showed that vibration diseases accounted for 75 % of expected occupational and work-related diseases with risks varying from low to high; polyneuropathy, 48 %; SHL, 6 %; dorsopathy, 75 %; essential hypertension, 30 %. Profound medical examination of blast-hole drillers confirmed that these health risks were actually manifested as diseases in 87–89 % workers.

The developed and tested methodology is quite effective. The prediction accuracy is estimated to reach 89 ± 2 % and the prediction error trend comes to minimum. The methodology provides a considerably wider opportunity to obtain prompt

---

Nina V. Zaitseva – Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor, Scientific Director (e-mail: znv@fcrisk.ru; tel.: +7 (342) 237-25-34; ORCID: http://orcid.org/0000-0003-2356-1145).

Anna A. Savochkina – Senior Lecturer at the Higher Mathematics Department (e-mail: aidas_76@mail.ru; tel.: +7 (342) 239-16-97; ORCID: https://orcid.org/0000-0003-2591-6632).

Marina A. Zemlyanova – Doctor of Medical Sciences, Chief Researcher with the duties of the Head of the Department of Biochemical and Cytogenetic Methods of Diagnostics (e-mail: zem@fcrisk.ru; tel.: +7 (342) 236-39-30; ORCID: http://orcid.org/0000-0002-8013-9613).

Dmitrii V. Goryaev – Candidate of Medical Sciences, Head of the Administration, Chief State Sanitary Inspector for the Krasnoyarsk region (e-mail: office@24.rospotrebnadzor.ru; tel.: +7 (391) 226-89-50; ORCID: http://orcid.org/0000-0001-6450-4599).

Aleksei G. Fadeev – Head of the Department for Supervision of Working Conditions (e-mail: onut@24.rospotrebnadzor.ru; tel.: +7 (391) 227-66-43; ORCID: https://orcid.org/0000-0003-1712-9196).

and accurate personalized prediction of health risks for workers. The system is eligible for workers employed at variable productions and implements a transition from contact-based examinations to quantitative prediction without any information losses, which determines its scalability and possibility to replicate it.

Keywords: *occupational environment, work process, adverse and hazardous factors, exposure, occupational health risks, adaptive neural fuzzy network, image recognition.*

Protecting and promoting health of industrial workers is a national priority, which determines possibilities for preservation of occupational longevity in accordance with the pace of the socioeconomic development of the country and strengthening the national security [1–4]. Special attention is paid to achieving this goal among other priority trends of the state policy since it is a crucial factor for realization of strategic interests of the Russian Federation[1], which, among other things, include "…advanced growth of healthy life expectancy and a decrease in the total duration of temporary disability among employable population by 2030…"

Given prolonged occupational longevity [1, 4], issues of workers' health improvement, declining incidence of non-communicable diseases and losses of work activity associated with persistent or temporary disability are becoming especially vital [5–8]. The Federal State Statistics Service estimated the employed population to account for 62.0 % of the total RF population as of the beginning of 2025 (75.3 million people)[2]. Working conditions hold the most important place in the structure of factors determining occupational health risks as well as associated morbidity[3] [9–11]. Wide modernization and automation of major productions in leading brunches of the economy, implementation of energy-saving technologies etc. promote gradual changes in conventional work performed by workers with basic industrial occupations and make adverse occupational and work-related factors less pronounced [12]. At the same time, a considerably large proportion of workplaces cannot be considered conforming to the valid sanitary-hygienic requirements to working conditions[4]. This proportion reaches 55 % in ore mining and even 65 % in metal ore mining; it reaches 42 % in processing industry, 39 % in transport, 37 % in electric energy production and supply, and 30 % in oil production [13, 14]. Working conditions in these brunches are mostly assessed as hazardous (from medium to very high hazards, hazard classes 3.2–3.4) in conformity with the Federal State System of keeping data obtained by Special Assessment of Working Conditions (SAWC).

Hygienic studies have established that harmfulness and hazards of working conditions in leading industrial brunches are determined by a set of adverse factors, which affect approximately 47 % of the total employed population (as of the end of 2024)[5]. Of them, 30.4 % of workers are exposed to occupational noise and vibration; 11.8 %, chemicals in

---

[1] O natsional'nykh tselyakh razvitiya Rossiiskoi Federatsii na period do 2030 goda i na perspektivu do 2036 goda: Ukaz Prezidenta Rossiiskoi Federatsii ot 07.05.2024 g. № 309 [On national goals and strategic tasks of the Russian Federation development for the period up to 2030 and the future prospect up to 2036: the RF President Order dated May 07, 2024. No. 309]. *Prezident Rossii: the RF President Official Web-site*. Available at: http://www.kremlin.ru/acts/bank/50542 (June 08, 2025) (in Russian).

[2] Skol'ko lyudei v Rossii rabotaet: statistika trudosposobnogo naseleniya [How many people work in Russia: data on the employable population]. *Skypro*. Available at: https://sky.pro/wiki/profession/skolko-lyudej-v-rossii-rabotaet-statistika-trudosposobnogo-naseleniya/ (June 10, 2025) (in Russian).

[3] Professional'naya patologiya: natsional'noe rukovodstvo [Occupational pathology: national guide]. In: RAS Academician I.V. Bukhtiyarov ed. Moscow, GEOTAR-Media Publ., 2024, 904 p. (in Russian).

[4] Ob utverzhdenii sanitarnykh pravil SP 2.2.3670-20 «Sanitarno-epidemiologicheskie trebovaniya k usloviyam truda»: Postanovlenie Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 02.12.2020 g. № 40 [On Approval of sanitary Rules SP 2.2.3670-20 Sanitary-Epidemiological Requirements to Working Conditions: the Order by the RF Chief Sanitary Inspector dated December 02, 2020, No. 40]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573230583 (June 10, 2025) (in Russian).

[5] Udel'nyi ves rabotnikov organizatsii, zanyatykh na rabotakh vrednymi i (ili) opasnymiusloviyam truda po vidam ekonomicheskoi deyatel'nosti na konets 2024 goda [Specific weight of workers employed at workplaces with harmful and (or) hazardous working conditions per kinds of economic activity as of the end of 2024]. Available at: https://docs.yandex.ru/docs/view?tm=1750065411&tld=ru&lang=ru&name=73631(5).pdf (June 10, 2025) (in Russian).

workplace air and aerosols with predominantly fibrogenic effects; 35.2 %, work hardness and intensity [8, 15, 16]. Harmful working conditions create considerable occupational health risks and risks of work ability losses and cause occupational and work-related diseases [12]; this has a substantial effect on human potential preservation and the demographic situation in the country as a whole. According to Rosstat, deaths of employable population accounted for 58 % of the total deaths due to all causes as of the beginning of 2024; of them, deaths due to diseases of the circulatory system accounted for 31 %[6]. The number of employed people is declining in Russia and the process is further accelerated by a downward demographic trend and accompanied with their ageing. This is confirmed by trends predicted by the Institute for Demography of the Higher School of Economics, which show that the number of people aged between 20 and 59 will go down by 2.6 million people in the Russian Federation between 2022 and 2030; the proportion of employed people aged younger than 40 years will go down by 6.6 million whereas the proportion of employed people aged between 40 and 59 will grow by 4.0 million. This can have a considerable effect on the country economy, the labor market included [17].

As human potential is declining in the country and simultaneously undergoing quantitative transformation, protection of employable population's health is becoming an especially urgent challenge [18]. Occupational risk assessment as an advanced analytical instrument for decision-making is conducted in occupational groups exposed to the same occupational factors; any personalized risk realization mostly covers sex, age, and work records [2, 12]. In addition, working conditions for workers with most common occupations in most leading industries are characterized, as a rule, by a complex interrelated system of risk factors that influence each other. Conventional occupational risk assessment methods employed in conformity with the valid Guide R 2.2.3969-23[7] do not consider this complexity to the full; this indicates the necessity to develop and update existing methodical approaches to occupational risk quantification and personalized prediction [19].

Analysis of management decisions as regards occupational and work-related diseases clearly reveals their specific restrictive essence. The existing practices on the matter do not contain all necessary elements of occupational risk management; do not meet actual demands in early detection of pre-nosologic states in workers with high likelihood of occupational and / or work-related diseases; do not involve subsequent development and implementation of personalized medical and preventive measures [20–22]. Most developed and implemented measures including those designed as corporate programs for workers' health protection are represented by general recommendations and are aimed at all workers employed by an enterprise as a whole [2, 4, 6, 12, 18]. Periodical medical examinations are conducted in strict conformity with the valid regulatory and legislative acts and are aimed at detecting already developed clinical forms of diseases, which are considered medical contraindications for beginning or continuing one's work activities.

Given that, it is necessary to develop and implement innovative methods and instruments in order to provide stable conditions for protecting and promoting workers' health at workplaces, to prevent occupational and general somatic pathologies associated with occupational factors, to decrease scopes of persistent or temporary disability [9, 10]. Assessment and prediction of occupational health

[6] Ivanova A.M., Moruga A.S., Nikitina S.Yu., Fatyanova L.N., Eumarina V.Zh., Elefterova M.P. Zhenshchiny i muzhchiny Rossii. 2024: Statisticheskii sbornik [Women and men in Russia. 2024: Statistical data collection]. Moscow, Rosstat, 2024, 176 p. (in Russian).

[7] R 2.2.3969-23. Rukovodstvo po otsenke professional'nogo riska dlya zdorov'ya rabotnikov. Organizatsionno-metodicheskie osnovy, printsipy i kriterii otsenki; utv. Glavnym gosudarstvennym sanitarnym vrachom RF 07.09.2023 [Assessment of Occupational Health Risk for Workers. Organization and Methodical Essentials, Principles and Assessment Criteria; approved by the RF Chief Sanitary Inspector on September 07, 2023]. Moscow, 2023, 77 p. (in Russian).

risks based on a personalized approach is the key aspect of measures aimed at morbidity prevention since it determines their ultimate effectiveness. It is objectively necessary to use up-to-date technologies for profound examination of individual health, opportunities offered by the neural network artificial intelligence, which makes it possible to simulate complex relationships between effects produced by adverse occupational factors, individual indicators of workers' health and risks of occupational diseases. Scientific developments in the sphere such as conceptual foundations and creation of predictive digital neural network models trained on retrospective or actual data about working conditions, health, socioeconomic conditions and lifestyle factors provide information and analytical grounds for calculating and assessing evolution of personal and group (considering an occupation, age, and work records) occupational health risks for workers [21]. This approach has provided much wider possibilities offered by an instrumental base for assessment of personalized occupational health risks. At the same time, adverse exposures are multicomponent and negative health outcomes are multiple; they are described by qualitative and quantitative characteristics and this determines fuzziness and uncertainty of a simulated process (fuzzy input data). This requires using additional solution methods able to overcome limitations and to ensure accurate simulation of relationships, scenario analysis for image recognition, and minimization of errors[8] [23–26].

All the foregoing indicates the necessity to further develop scientific and methodical grounds for personalized prediction of likelihood of direct and indirect signs of health states pathogenetically associated with exposure to harmful and hazardous occupational and work-related factors. This development should be consistent with previously gained experience. Greater accuracy of personalized assessments will allow creating and implementing relevant algorithms and optimizing occupational health risk management as well as raising effectiveness of preventive activities aimed at prolonging occupational longevity.

The aim of this study was to develop and test a methodology and software for assessing and predicting personalized occupational health risks based on an adaptive neural fuzzy network for image recognition.

Materials and methods. The study design was based on an artificial intellect model as a mathematical structure trained to recognize regularities and establish whether an analyzed object belonged to a specific occupational health risk category per a system of indicators. Software necessary for the model functioning was developed in Python using Scikit-fuzzy library for creating and calculating membership functions for input data. A neural network that accepted input data was developed using TensorFlow, a library with high productivity and scalability for training and optimizing weights and parameters of membership functions [27–29]. Defuzzification was accomplished by the Centroid method in Python. At the output, a fuzzy result becomes quite an accurate value of a personalized occupational health risk.

When creating an input data sample, we have used a term-set (T), which describes levels of each indicator (for example, noise, vibration, chemical contents, physical loads, work records, etc.) as follows: T = {Negligible (N); Low (L); Medium (M); High (H); Very High (VH)}. A term-set for each indicator ($x_1 \ldots x_n$) is given as: N, L, M, H, VH. Occupational health risks for workers were assessed based on an obtained quantitative result (y_p). Occupational health risks for workers were assigned into various categories in conformity with the conventional scale based on establishing their membership within a specific range of scale values given in Table 1.

Matching a risk level with a specific scale range makes it possible to more accurately define this membership due to using sets with bounds, which are ± 20 % fuzzy. As a result,

[8] Jang J.-S.R. ANFIS: adaptive-network-based fuzzy inference system. *IEEE Transactions on Systems, Man, and Cybernetics*, 1993, vol. 23, no. 3, pp. 665–685. DOI: 10.1109/21.256541

values in neighboring scale ranges may overlap. If a risk level belongs simultaneously to two scale ranges, a more hazardous category is selected. If a personalized health risk falls into 'Low' category or higher, relevant measures should be taken to mitigate or eliminate it.

Table 1

Scale with ranges and categories of personalized occupational health risks for workers

Risk level range	Risk category *(R)* and respective color	
$R_1 \in [0; 0.25]$	Negligible	
$R_2 \in (0.15; 0.45]$	Low	
$R_3 \in (0.35; 0.65]$	Medium	
$R_4 \in (0.55; 0.85]$	High	
$R_5 \in [0.75; 1]$	Very high	

The obtained results were graphically visualized using a fuzzy inference surface representing a 3D-graph, where X and Y axis corresponded to input data (for example, risk factors) whereas Z axis corresponded to an output risk level after defuzzification.

Training and validation of the adaptive neural fuzzy network was accomplished on an example sample made of underground miners employed at a copper and nickel ore mine. The process relied on a detailed data array containing information about working conditions, exposure factors, exposure levels, age, and individual health indicators including those obtained by profound examinations (biochemical, immunologic, hematological, general clinical, chemical-analytical, functional and instrumental studies etc., and diagnosed diseases). Overall, 175,000 qualitative and quantitative parameters were covered. The training sample volume was 80 % and the validating sample volume was 20 %. The model was trained for 100 epochs. Accuracy of personalized health risk prediction was estimated by creating an Accuracy graph where the number of training epochs was shown on the horizontal axis and the vertical axis showed the level of accuracy varying between 0 and 1 (or between 0 and 100 % where 1 means 100 % accuracy). Conventionally, two lines are shown in such a graph; the first one to estimate accuracy for a training sample and the other one for a validating (test) sample. If both lines grow gradually and reach 100 %, this means a model is being trained with high quality. Losses within training were estimated by the model by creating a Loss graph where the horizontal axis shows the number of training epochs and the vertical axis shows the value of the loss function. Similarly, two lines are shown in this graph corresponding to losses in training and validating data sets. The lower is the value of the loss function the higher is quality of an estimated model.

Results and discussion. A methodology has been developed for assessing and predicting personalized occupational health risks for workers; its theoretical grounds are represented by an adaptive neural fuzzy network for image recognition, generalization of knowledge and a resulting inference. The network has a specific hybrid multilayer (5 layers overall) architecture. The prediction principles are formulated based on the network capabilities combining advantages of fuzzy systems with neural network training and structure. This ensures prediction accuracy and minimization of errors. Key aspects of these advantages include ability to learn and adapt to new data, which makes predictions more accurate; fuzzy logic, which makes it possible to analyze fuzzy and variable data; a neural component, which ensures non-linear approximation of complex relationships between risk factors and predicted indicators (for example, likelihood of a disease) as a basis for recognition of complex patterns (image-drafts); a combination encompassing fuzzy data analysis and capability to approximate neural networks, training with the use of data, which reflect complex cause-effect relations; interpretability of a resulting output, which makes it possible to get an insight into factors with the greatest impact on risk assessment; the latter is necessary for taking adequate regulatory actions.

A personalized digital model is built relying on a theoretical concept for calculating occupational health risks; the concept is implemented by using an adaptive neural fuzzy

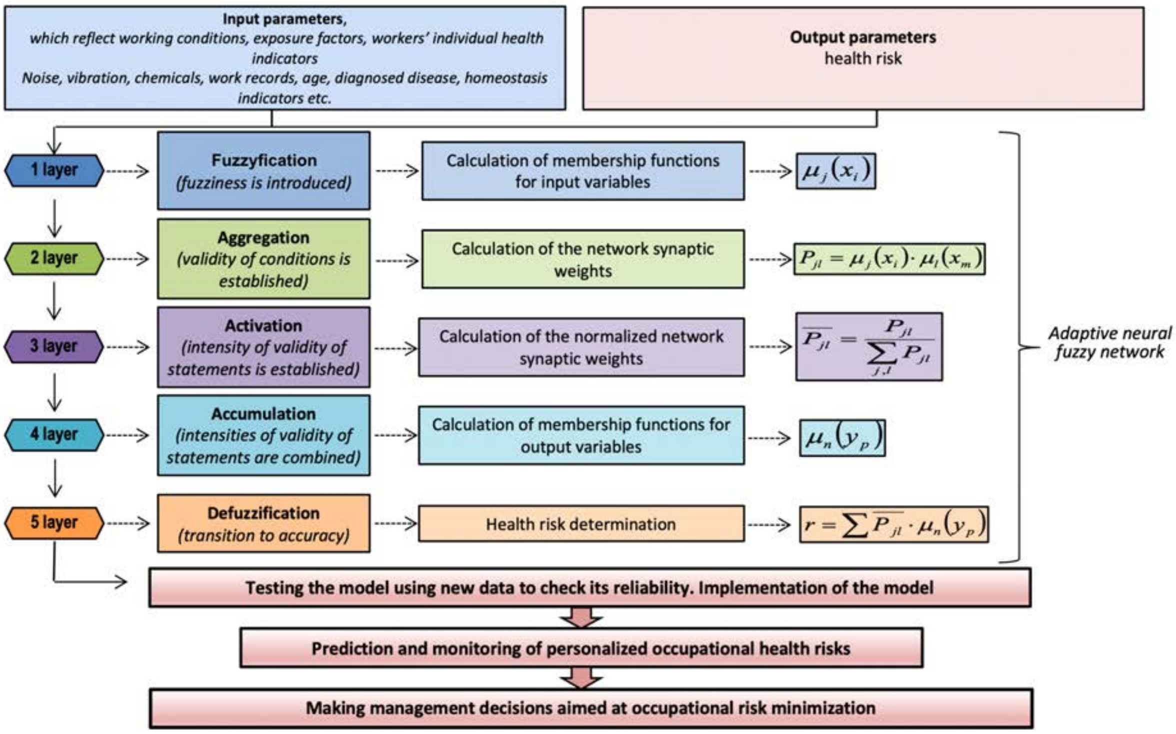

Figure 1. Multilayer hybrid architecture (5 layers) and operation stages of the adaptive neural fuzzy network for image recognition necessary for predicting personalized occupational health risks for workers

network for image recognition. Figure 1 provides basic components of the adaptive neural fuzzy network architecture.

The adaptive neural fuzzy network for image recognition includes the following subsequently linked operation stages:

– collecting and preparing analytical database about actual working conditions and exposure factors at workplaces combined with workers' personalized health indicators;

– selecting a set of input parameters (for example, noise, vibration, chemical levels in workplace air; work hardness and intensity; work records; age; individual levels of chemical in biological media; biochemical, hematological, and immunological indicator of homeostasis; functional indicators of organs and systems; a diagnosed diseases with ICD-10 code etc.) and an output resulting parameters (for example, a level of a personalized occupational health risk for a worker);

– fuzzyfication (introduction of fuzziness) using the membership functions for input variables: the first layer of network neurons (layer 1) is adaptive; it contains neurons that calculate values of membership functions for input variables: $\mu_j(x_i)$, where $x_i, i=\overline{1,n}$ are input variables. The layer ability to adapt is ensured by selecting a type of a membership function for input data, which determines level of membership of an element to a certain set. In this created system, three membership functions are employed simultaneously; they supplement each other considering their advantages, and this allows a substantial increase in prediction accuracy. They are the Gaussian function (ensures smooth transitions and accurate approximation), sigma function (implements smooth S-shaped transition of stated and a variable membership changes constantly), and trapezoid functions (represent values with complete membership at a certain interval of the membership function);

– aggregation (establishing intensity of validity of conditions) by analyzing a database of fuzzy linguistic rules: the second layer of network neurons (layer 2) is fixed; it contains neurons, which calculate the products of the membership function values established at the first layer:

$$P_{jl} = \mu_j(x_i) \cdot \mu_l(x_m), \quad (1)$$

where P_{jl} are synaptic network weights, $\mu_j(x_i)$, $\mu_l(x_m)$ are values of the membership function;

– activation (establishing intensity of validity of statements) by normalizing levels of fuzzy rule activation, which shows, how relevant a rule is for a specific actual set of input data values, and is given by a number between 0 and 1 where 0 means complete irrelevance and 1 complete relevance. This intensity is necessary for weighing influence of each rule when creating a resulting system inference: the third layer of the network neurons (layer 3) is fixed; it contains neurons for calculating normalized synaptic network weights:

$$\overline{P_{jl}} = \frac{P_{jl}}{P_{11} + P_{12} \ldots + P_{55}}, \quad (2)$$

where $\overline{P_{jl}}$ are normalized synaptic network weights, P_{jl} are synaptic network weights;

– accumulation (combining intensities of validity of statements) using the membership functions for output variables: the fourth layer of the network neurons (layer four 4) is adaptive; it contains neurons responsible for calculating values of the membership function for output variables and the product of values of the normalized synaptic network weights and the membership functions of output variables:

$$\overline{P_{jl}} \cdot \mu_n(y_p), \quad (3)$$

where $\overline{P_{jl}}$ are normalized synaptic network weights, $\mu_n(y_p)$ are values of the membership functions for output variables;

– defuzzification (transition to accuracy) involving determination of the accurate value of the output variable (y_p): the fifth layer of the network neurons (layer 5) is fixed; it contains the neuron that calculates a personalized occupational health risk based on the sum of products of values of the membership function for output variables and normalized network weights:

$$R = \sum\left(\overline{P_{jl}} \cdot \mu_n(y_p)\right), \quad (4)$$

where R is the value of a personalized occupational health risk;

– testing (validation and verification) the model using an independent set of new input data; this ensures proper tests of the model reliability, ability to generalize within image recognition and to adequately work with data not used in training;

– implementation of the model for personalized prediction, control and monitoring of occupational health risks involves use of the trained model in an actual system for health risk assessment and subsequent monitoring of changes in these risks over time.

Up to 96 % of the examined workers are simultaneously exposed to occupational noise, total vibration, chemicals (copper-nickel ore components, nitrogen oxides, carbon oxides, crystal silicon dioxide with its contents in dusts varying between 2 and 10 %, prop-2-en-1-al, ammonia, aliphatic saturated hydrocarbons C1-10 and other substances) in workplace air, work hardness and intensity. Duration of exposure varies between 2 and 22 years; the workers' age, between 43 and 63 years.

The results obtained by training the adaptive neural fuzzy network are given as the Accuracy and Loss graphs (Figure 2).

According to the Accuracy graph, the model is shown to remember training input data and to make personalized predictions of occupational health risks for the training sample (the blue line) with accuracy reaching 55 %; for the validating sample, (orange line), with accuracy reaching 72 %.

The Loss graph shows that the model error value tends to decline steadily over time for the training sample; the descending time-dependent trend is even more pronounced for the validating sample and its subsequent fluctuations are at the minimum, which means the model has been trained successfully and is quite stable.

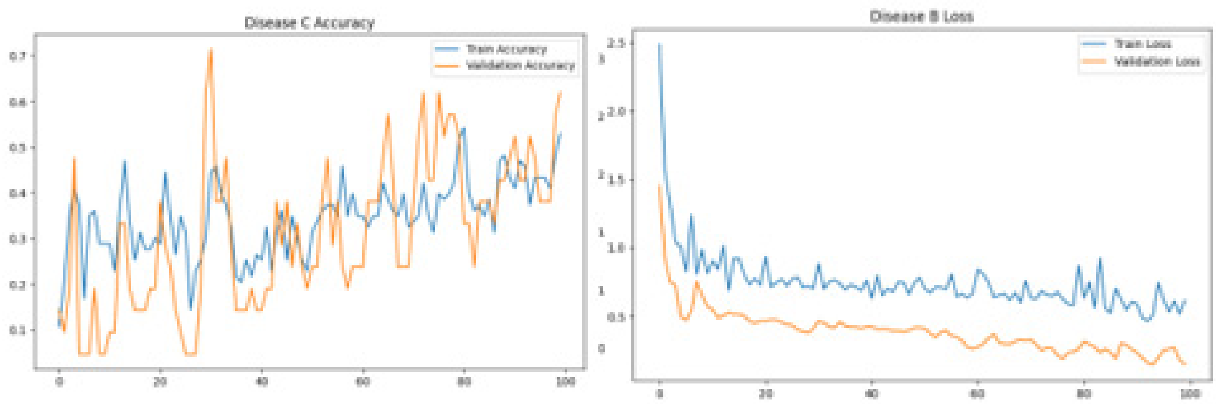

Figure 2. Accuracy and Loss graphs to describe the results obtained by training the adaptive neural fuzzy network

Table 2

Assessment and prediction of the group occupational health risk for all workers in thee validating sample

Disease	Predicted group occupational health risk for workers upon exposure to various combinations of occupational factors, work process, work records, etc.				
	Chemicals, work records	Noise, work records	Vibration, work records	Chemicals, vibration	Vibration, noise
Vibration disease	0.09	0.23	0.54	0.72	0.80
Sensorineural hearing loss	0.31	0.26	0.03	0.35	-
Dorsopathy	0.03	0.17	0.37	0.41	0.67

Our tests of the network involved assessing and predicting the group occupational health risks accomplished for the whole validating sample (Table 2). For example, simultaneous exposure to vibration and occupational noise was established to create the highest health risks caused by vibration disease (R = 0.80) and dorsopathy (R = 0.67). Combined exposure to occupational vibration and chemicals in workplace air created a group occupational risk (R = 0.35) caused by sensorineural hearing loss (SHL); it was assessed as 'medium'.

We established personalized occupational health risks for each worker from the validating sample; individual risks caused by vibration disease were assessed as 'high', for example, upon exposure to occupational noise 10–40 dBA higher than MPL over a work shift and total vibration at the level of 106–113 dB considering the whole set of biological and medical health measures and other affecting factors (Figure 3). Using one specific worker from this sample as an example, we showed that a personalized health risk caused by SHL was equal to 0.85 and assessed as 'very high' (Figure 3) upon simultaneous exposure to total occupational vibration at the level of 109 dB and occupational noise (11 dBA higher than MPL) considering his individual health indicators and against all other affecting factors.

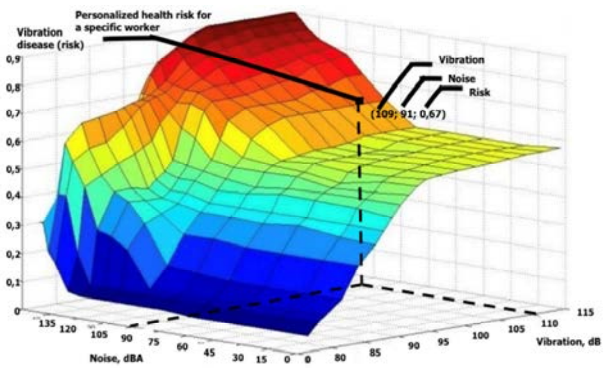

Figure 3. Graph which visualizes the surface of the fuzzy inference to assess personalized occupational health risk caused by vibration disease depending on exposure to occupational noise and work records against other affecting factors (a point at the surface denotes a specific worker with a personalized occupational health risk)

In another example, individual health risks are caused by SHL and are assessed as varying from medium to very high (Figure 4) upon combined exposure to occupational noise (5–30 dBA above MPL) and several chemicals (2.0–2.5 times higher than single maximum MPC) against other affecting factors. Using one specific worker from this sample as an example, an individual health risk caused by SHL was shown to equal 0.85 and assessed as 'very high' (Figure 4) upon simultaneous exposure to occupational noise 29 dB higher than MPL and chemicals in workplace air at the level 2.2 times higher than single maximum MPC considering his individual health indicators and against other affecting factors.

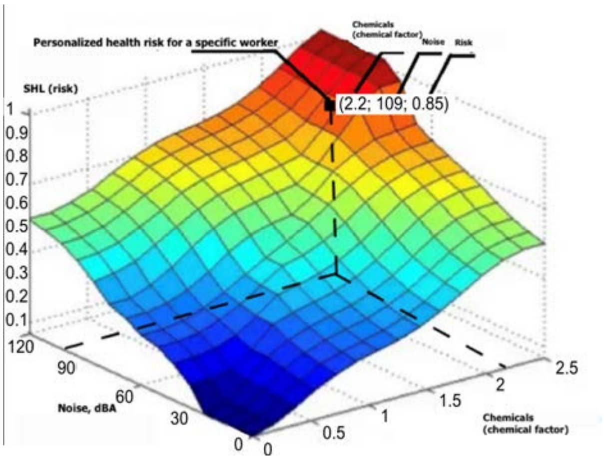

Figure 4. Graph which visualizes the surface of the fuzzy inference to assess personalized occupational health risk caused by SHL depending on combined exposure to physical (occupational noise) and chemical factors against other affecting factors (a point at the surface denotes a specific worker with a personalized occupational health risk)

We predicted personalized occupational health risks for workers from an independent sample (the model was tested using blast-hole drillers) using the adaptive neural fuzzy network. Our prediction showed that risks of expected occupational diseases and work-related diseases varied between low and high; of them, 75 % were caused by vibration disease; 48 %, polyneuropathy; 6 %, SHL; 75 % dorsopathy; 30 %, essential hypertension. Profound medical examinations of blast-hole drillers gave evidence that predicted health risks were actually realized in 87–89 % of the examined workers. The examinations established occupational diseases associated with simultaneous exposure to total vibration, occupational noise, work hardness and intensity including vibration disease, stage I, II and I-II (ICD-10 code T75.2), polyneuropathy (G62.8), and SHL (H90.6). Work-related diseases included neck and lumbar spine dorsopathy (M53.8) and essential hypertension (I11). Those workers were provided with individual recommendations to take medical and preventive measures aimed at reducing manifestations of negative health outcomes associated with working conditions. Rospotrebnadzor's territorial bodies were informed about predicted individual occupational health risks for workers employed at underground copper and nickel ore mining.

Therefore, this developed and software-supported methodology for assessing and predicting personalized occupational health risk is based on using the adaptive neural fuzzy network for image recognition. The methodology is quite effective, which is confirmed by the results of its testing on an independent dataset. The prediction accuracy is estimated to reach 89 ± 2 % and the prediction error trend comes to minimum ('flat bottom'). The system based on the suggested methodology has shown more accurate predictions, the results were easier to interpret; the system was more resistant to incomplete and variable data and errors in them and was able to optimize parameters on its own more effectively as compared to simplified statistical methods. The suggested scientific and methodical instruments based on image recognition provide a considerably wider opportunity to obtain prompt and accurate personalized prediction of occupational health risks for workers. This ensures making adequate decisions given multiple and uncertain cause-effect relations; allows developing more targeted, personalized and pathogenetically oriented preventive measures regulated by the valid legislation covering sanitary-epidemiological requirements to working conditions. The system is eligible for workers employed at variable productions and implements a transition from contact-based examinations to quantitative prediction without any information losses, which determines its scalability and possibility to replicate it.

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. Bukhtiyarov I.V. Current state and main directions of maintaining and strengthening the health of the working population of Russia. *Meditsina truda i promyshlennaya ekologiya*, 2019, vol. 59, no. 9, pp. 527–532. DOI: 10.31089/1026-9428-2019-59-9-527-532 (in Russian).

2. Panova T.V. Zdorov'e rabotayushchego naseleniya – vazhneishee uslovie kachestva proizvoditel'nosti truda [Health of the working population is the most important condition for quality and labor productivity]. *Ekonomicheskie nauki*, 2018, no. 4 (161), pp. 39–41 (in Russian).

3. Chebotarev A.G., Pfaf V.F., Gibadulina I.Yu. Current State of Labour Conditions, Occupational Morbidity and Improvement of Medical and Preventive Care for Employees of Mining Operations. *Gornaya promyshlennost'*, 2021, no. 3, pp. 139–143. DOI: 10.30686/1609-9192-2021-3-139-143 (in Russian).

4. Meltser A.V., Erastova N.V., Volochkova A.I. Hygienic aspects of preserving and strengthening the health of employees in corporate programs (scientific review). *Profilakticheskaya i klinicheskaya meditsina*, 2024, no. 4 (93), pp. 4–12 (in Russian).

5. Gurvich V.B., Gazimova V.G., Shastin A.S., Fedoruk A.A., Ruzakov V.O., Plotko E.G. The basic organizational problems of preventing morbidity of working population in modern conditions. *Meditsina truda i promyshlennaya ekologiya*, 2018, no. 11, pp. 32–35. DOI: 10.31089/1026-9428-2018-11-32-35 (in Russian).

6. Ustinova O.Yu., Zaitseva N.V., Vlasova E.M., Kostarev V.G. Corporate programs for preventing health disorders among workers employed at adverse productions as a tool for occupational risk management. *Health Risk Analysis*, 2020, no. 2, pp. 72–82. DOI: 10.21668/health.risk/2020.2.08.eng

7. Zheglova A.V., Yatsyna I.V., Gavrilchenko D.S. Corporate health preservation programs are the main element of the health-saving system of the working population. *Zdravookhranenie Rossiiskoi Federatsii*, 2022, vol. 66, no. 5, pp. 385–389. DOI: 10.47470/0044-197X-2022-66-5-385-389 (in Russian).

8. Nikanov A.N., Vinnikov D.V., Syurin S.A., Shitikova E.D. Occupational diseases in pyrometallurgical nickel refining workers of various specialties. *ZNiSO*, 2024, vol. 32, no. 5, pp. 66–75. DOI: 10.35627/2219-5238/2024-32-5-66-75 (in Russian).

9. Panev N.I., Korotenko O.Yu., Filimonov S.N., Semenova E.A., Panev R.N. Prevalence of cardiovascular pathology in workers of the aluminum industry. *Gigiena i sanitariya*, 2019, vol. 98, no. 3, pp. 276–279. DOI: 10.18821/0016-9900-2019-98-3-276-279 (in Russian).

10. Syurin S.A., Kovshov A.A. Working conditions and work-related pathologies at enterprises located in Chukotka autonomous area. *Health Risk Analysis*, 2020, no. 4, pp. 98–105. DOI: 10.21668/health.risk/2020.4.11.eng

11. Chebotarev A.G., Sementsova D.D. Comprehensive assessment of working conditions and occupational disease rates at mining and metallurgical enterprises. *Gornaya promyshlennost'*, 2021, no. 1, pp. 114–119. DOI: 10.30686/1609-9192-2021-1-114-119 (in Russian).

12. Bukhtiyarov I.V., Chebotarev A.G., Courierov N.N., Sokur O.V. Topical issues of improving working conditions and preserving the health of employees of mining enterprises. *Meditsina truda i promyshlennaya ekologiya*, 2019, vol. 59, no. 7, pp. 424–429. DOI: 10.31089/1026-9428-2019-59-7-424-429 (in Russian).

13. Shur P.Z., Goryaev D.V. Sovremennye korporativnye programmy snizheniya professional'nogo riska zdorov'yu kak sredstva sokhraneniya trudovykh resursov [Modern corporate programs for reducing occupational health risks as a means of preserving human resources]. *Gumanizatsiya ekonomiki: blagopoluchie cheloveka i kreativnyi kapital: IX Permskii ekonomicheskii kongress [Humanization of the economy: human well-being and creative capital: IX Perm Economic Congress]*. Perm, 2025. Available at: https://economcongress.ru/wp-content/uploads/2024/02/5-Shur-Goryaev.pdf (May 23, 2025) (in Russian).

14. Titova E.Ya., Golub' S.A. Contemporary problems of health protection for workers employed at a large industrial enterprise and working under occupational hazards. *Health Risk Analysis*, 2017, no. 4, pp. 83–90. DOI: 10.21668/health.risk/2017.4.09.eng

15. Shaikhlislamova E.R., Karimova L.K., Volgareva A.D., Muldasheva N.A. Occupational health of workers in underground occupations producing polymetalliccopperzinc ores. *Sanitarnyi vrach*, 2020, no. 5, pp. 9–22. DOI: 10.33920/med-08-2005-01 (in Russian).

16. Bukhtiyarov I.V., Chebotarev A.G., Prokhorov V.A. Problems of the promotion of healthy working environment, prevention of occupational diseases of the personnel of a mining and smelting complex. *Gornaya promyshlennost'*, 2015, no. 6 (124), pp. 14–17 (in Russian).

17. Denisenko M.B., Mkrtchyan N.V. Demograficheskie izmeneniya i predlozhenie rabochei sily v regionakh Rossii [Demographic changes and labor supply in the regions of Russia]. *Nauchnyi daidzhest*, 2022, no. 5 (10), 10 p. (in Russian).

18. Kontsevaya A.V. Korporativnye programmy ukrepleniya zdorov'ya v ramkakh Natsional'nogo proekta «Prodolzhitel'naya i aktivnaya zhizn'» [Corporate health promotion programs within the framework of the National Project «Long and Active Life»]. *Meditsinskii komponent korporativnykh programm ukrepleniya zdorov'ya na rabochem meste: vozmozhnosti 2025»: simpozium v ramkakh XX Vserossiiskogo foruma «Zdorov'e natsii – osnova protsvetaniya Rossii»* [*Medical Component of Corporate Health Promotion Programs at the Workplace: Possibilities for 2025: symposium within the framework of the XX All-Russian Forum "Health of the Nation – the Basis for Russia's Prosperity»*]. Moscow, May 14–16, 2025. Available at: https://docs.yandex.ru/docs/view?tm=1750087297&tld (June 10, 2025) (in Russian).

19. Fokin V.A., Zaitseva N.V., Shur P.Z., Redko S.V., Khrusheva E.V. Assessing and predicting individual occupational risk and determining its exact categories using probabilistic methods. *Health Risk Analysis*, 2021, no. 4, pp. 92–99. DOI: 10.21668/health.risk/2021.4.10.eng

20. Ponomareva T.A., Vorobeva A.A., Vlasova E.M., Ustinova O.Yu. Rehabilitation of employees of titanium-magnesium production with the installed disease of the upper respiratory ways. *Meditsina truda i ekologiya cheloveka*, 2019, no. 4 (20), pp. 39–49. DOI: 10.24411/2411-3794-2019-10046 (in Russian).

21. Zaitseva N.V., Kiryanov D.A., Zemlyanova M.A., Goryaev D.V., Ustinova O.Yu., Shur P.Z. Conceptual foundations of a corporate intelligent risk-based system for analysis, prediction and prevention of occupational and work-related health disorders of workers. *Health Risk Analysis*, 2023, no. 4, pp. 19–32. DOI: 10.21668/health.risk/2023.4.02.eng

22. Bukhriayrov I.V., Kuzmina L.P., Izmerova N.I., Golovkova N.P., Nepershina O.P. Improvement of mechanisms of detecting early signs of health disorders for preservation labor longevity. *Meditsina truda i promyshlennaya ekologiya*, 2022, vol. 62, no. 6, pp. 377–387. DOI: 10.31089/1026-9428-2022-62-6-377-387 (in Russian).

23. Gridina N.V., Evdokimov I.A., Solodovnikov V.I. Construction of hybrid neural networks using fuzzy logic elements. *Iskusstvennyi intellekt i prinyatie reshenii*, 2019, no. 2, pp. 91–97. DOI: 10.14357/20718594190209 (in Russian).

24. Teslenko A. Neuro-fuzzy methods in the management of technical systems and objects. Yazyk v sfere professional'noi kommunikatsii: sbornik materialov mezhdunarodnoi nauchno-prakticheskoi konferentsii prepodavatelei, aspirantov i studentov [Language in the Sphere of Professional Communication: Collection of Materials of the International Scientific and Practical Conference of Teachers, Postgraduates, and Students]. Ekaterinburg, Azhur Publ., 2020, pp. 698–703 (in Russian).

25. Andrievskaya N.V., Reznikov A.S., Cheranev A.A. Features of application of neuro fuzzy systems in systems of automatic control. *Fundamental'nye issledovaniya*, 2014, no. 11–7, pp. 1445–1449 (in Russian).

26. Abushariah M.A.M., Alqudah A.A.M., Adwan O.Y., Yousef R.M.M. Automatic heart disease diagnosis system based on artificial neural network (ANN) and adaptive neuro-fuzzy inference systems (ANFIS) approaches. *Journal of software engineering and applications*, 2014, vol. 7, no. 12, pp. 1055–1064. DOI: 10.4236/jsea.2014.712093

27. Glushenko S.A. An adaptive neuro-fuzzy inference system for assessment of risks to an organization's information security. *Business Informatics*, 2017, no. 1 (39), pp. 68–77. DOI: 10.17323/1998-0663.2017.1.68.77 (in Russian).

28. Alkhashedi A.A., Obadi A.A., Nuriev N.K., Pechenyy E.A. Development of a mathematical model for recognition task requests of communications services. *Fundamental'nye issledovaniya*, 2017, no. 6, pp. 9–14 (in Russian).
29. Yankovskaya A.E., Kitler S.V. Prinyatie reshenii na osnove parallel'nykh algoritmov testovogo raspoznavaniya obrazov [Decision-making based on parallel algorithms for test image recognition]. *Iskusstvennyi intellekt*, 2010, no. 3, pp. 151–152 (in Russian).

Zaitseva N.V., Savochkina A.A., Zemlyanova M.A., Goryaev D.V., Fadeev A.G. Methodology for assessing and predicting personalized occupational health risks based on adaptive neural fuzzy network for image recognition. Health Risk Analysis, 2025, no. 2, pp. 4–15. DOI: 10.21668/health.risk/2025.2.01.eng

Received: 11.06.2025
Approved: 20.06.2025
Accepted for publication: 28.06.2025

UDC 613;614
DOI: 10.21668/health.risk/2025.2.02.eng

Read online

Research article

SUBSTANTIATING THE MAXIMUM PERMISSIBLE CONCENTRATION OF CYLINDROSPERMOPSIN IN WATER FOR DRINKING AND HOUSEHOLD USE TO MINIMIZE HUMAN HEALTH RISKS

O.O. Sinitsyna, V.V. Turbinsky, M.V. Pushkareva, N.V. Kuz, M.A. Shiryaeva, G.V. Masaltsev, V.V. Safandeev

Federal Scientific Center of Hygiene named after F.F. Erisman, 2 Semashko St., Mytischi, Moscow region, 141014, Russian Federation

Increasing human-induced pollution of water objects, climate change, creation of reservoirs, and a decrease in water flow rates have accelerated saturation of water bodies with biogenic substances, which in turn has caused massive growth and spread of cyanobacteria. Intensification of "blooming" processes in water bodies is observed almost everywhere. In respect to human health hazards, the issue of massive growth of toxic cyanobacteria in surface water bodies used for drinking and recreational purposes is related to the possibility of various cyanotoxins forming in the water. This includes cylindrospermopsin (CYN), which is classified by the World Health Organization as a high-priority environmental pollutant, and this highlights high relevance of studying it. Assessing the experience gained by foreign researchers in studying the content of cyanobacteria in water bodies, it should be noted that there is a fairly wide species diversity of blue-green algae, depending on regional prevalence.

In many countries across the globe, the list of substances for drinking water quality control has been expanded to include not only the content of cyanobacteria metabolic products, but also, in some cases, the content of their specific species composition. A number of studies by foreign and Russian researchers have shown the need for timely measures aimed at raising drinking water safety by regulating metabolic products of cyanobacteria.

The aim of this study was to substantiate the maximum permissible concentration (MPC) of CYN content in water for household and drinking water use, as well as for recreational needs.

The study included analysis of the results obtained by research on the physicochemical properties and toxicity of CYN, as well as a subchronic experiment to investigate general toxic effects and specific ones including neurotoxic, embryotoxic, and teratogenic effects upon conditions intragastric administration to animals.

As a result, we established parameters of toxic effects produced by CYN and its hazard category and substantiated the MPC for the chemical in water equal to 1.0 µg/l, the sanitary-toxicological indicator of harm, and the hazard category.

Keywords: *cyanotoxins, cylindrospermopsin, general toxic effect, embryotoxic effect, teratogenic effect, drinking water, maximum permissible concentration.*

Oxana O. Sinitsyna – Corresponding Member of RAS, Doctor of Medical Sciences, Professor, Deputy Director for Science (e-mail: sinitsyna.oo@fncg.ru; tel.: +7 (926) 447-08-74; ORCID: http://orcid.org/0000-0002-0241-0690).

Viktor V. Turbinsky – Doctor of Medical Sciences, Head of Water Hygiene Department (e-mail: turbinskii.vv@fncg.ru; tel.: +7 (920) 666-72-73; ORCID: https://orcid.org/0000-0001-7668-9324).

Mariya V. Pushkareva – Doctor of Medical Sciences, Professor, Chief Researcher of Water Hygiene Department (e-mail: pushkareva.mv@fncg.ru; tel.: +7 (912) 980-92-74; ORCID: https://orcid.org/0000-0002-5932-6350).

Nadezhda V. Kuz – Candidate of Medical Sciences, Leading Researcher of Water Hygiene Department (e-mail: nadetzhda.v.k@gmail.com; tel.: +7 (903) 284-14-74; ORCID: http://orcid.org/0000-0002-7573-0185).

Margarita A. Shiryaeva – Junior Researcher of Water Hygiene Department (e-mail: Shiryaeva.MA@fncg.ru; tel.: +7 (903) 161-14-04; ORCID: https://orcid.org/0000-0001-8019-1203).

Gleb V. Masaltsev – Candidate of Biological Sciences, Head of Toxicology Department (e-mail: masalcev.gv@fncg.ru; tel.: +7 (916) 424-94-81; ORCID: https://orcid.org/0000-0003-1539-1633).

Vitaly V. Safandeev – Candidate of Biological Sciences, Head of Inhalation Toxicology Department (e-mail: Visa.doc@mail.ru; tel.: +7 (929) 583-26-41; ORCID: https://orcid.org/0000-0002-0073-1677).

Increasing human-induced pollution of water objects, climate change, creation of reservoirs, and a decrease in water flow rates have accelerated saturation of water bodies with biogenic substances. This, in its turn, has caused massive growth and spread of cyanobacteria (CB). The World Health Organization (WHO) ranks global development of toxic CB in surface water objects used for drinking water supply and recreational needs as a priority challenge for population health protection [1].

'Blooming' in water objects is being observed practically everywhere and the process is intensifying. A.V. Bakaev and others believe that rapid development of blue-green algae in water objects can be often considered an emergency [2].

The WHO classifies cyanotoxins per their predominant effects on the human body and primarily identifies hepatotoxins, nephrotoxins and neurotoxins. Cyanobacteria of the *Cylindrocpermopsis raciborskii* species are considered to produce toxic substances, including cylindrospermopsin (CYN), which primarily affects the liver, gastrointestinal tract and kidneys when entering the human body.

In Russia, CYN levels in drinking water, which are safe for human health, have not been established so far although many studies by Russian researchers give evidence of the issue being truly vital[1] [3–7].

The aim of this study was to substantiate the maximum permissible concentration (MPC) of CYN in water for household and drinking water use, as well as for recreational needs.

Materials and methods. Results reported in studies on CYN physical and chemical properties as well as its toxicity were searched in e-library, PubMed, Web of Science, Jstor, Open Access Button, Russian state Library, and MedLine; in addition, we analyzed regulatory and methodical documents valid in Russian and international legislation and devoted to regulating cyanotoxins in water.

The analytical CYN standard produced in Spain, CAS 143545-90-8, was used as the research object. The molecular formula was $C_{15}H_{21}N_5O_7S$; the molar mass, 399.42 g/mol.

Effects produced by CYN on self-purification in water objects were examined and the subsequent substantiation for CYN MPC in water was provided in conformity with the Methodical Guidelines MUK 2.1.5.720-98. Substantiation of safe standards for chemical levels in water supplied from water objects used for drinking and household purposes[2].

Concentrations of 1.0 μg/dm^3, 10.0 μg/dm^3, and 100.0 μg/dm^3 were selected to investigate CYN effects on water self-purification.

Bearing in mind the results reported by some foreign researchers in toxicological studies with their focus on CYN and available safe standards in other countries, we decided to shorten our experiment down to 2 months and to use prediction methods for establishing chronic MPC. Based on available literature data on CYN as well as guided by the MUK 2.1.5.720-98, the CYN doses of 0.1 – 1.0 – 10.0 μg/kg of body weight were selected for experimental studies to simulate subchronic intragastric administration into white rats' bodies. These doses amount to 1 / 14 000 000, 1 / 1 400 000 and 1 / 140 000 proportion of LD_{50} respectively.

General toxic CYN effects were examined using conventional white rats. All animal

---

[1] Sinitsyna O.O., Turbinsky V.V., Kuz N.V., Ryashentseva T.M., Pushkareva M.V., Masaltsev G.V., Veshchemova T.E., Vostrikova M.V. Toksichnost' anatoksina-a pri 3-kh mesyachnom vnutrizheludochnom vvedenii v organizm belykh krys: svidetel'stvo o registratsii bazy dannykh [Toxicity of anatoxin-a upon 3-month intragastric administration into white rats]: certificate of database registration, Registration Number: RU 2023625000; published on December 25, 2023. Application No. 2023624658 dated December 08, 2023.

[2] MU 2.1.5.720-98. Obosnovanie gigienicheskikh normativov khimicheskikh veshchestv v vode vodnykh ob"ektov khozyaistvenno-pit'evogo i kul'turno-bytovogo vodopol'zovaniya: metodicheskie ukazaniya, utv. i vved. v deistvie Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii 15 oktyabrya 1998 goda [MU 2.1.5.720-98. Substantiation of safe standards for chemical levels in water supplied from water objects used for drinking and household purposes: Methodical guidelines, approved and enacted by the RF Chief Sanitary Inspector on October 15, 1998]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200006903 (March 10, 2025) (in Russian).

experiments were accomplished in conformity with the principles fixed in the Guide R 1.2.3156-13 Assessment of Toxicity and Hazard of Chemicals and Their Mixtures for Human Health[3], and in conformity with the approval of the Biomedical Ethics Committee of the Federal Scientific Center of Hygiene named after F.F. Erisman dated November 21, 2022, the Meeting Report No. 03/22.

CYN was administered intragastrically into the experimental animals every day for 60 days; the control group was given distilled water in the same volume. The animals' health, water and food consumption was monitored throughout the experiment; any changes in body weight were registered in dynamics and any clinical signs of exposure to the tested substance were fixed on the 15th, 45th and 60th day in the experiment. On the same days, hematological and biochemical blood indicators were checked as well as physiological signs of the animals' health including behavioral aspects.

When the experiment was completed, the animals were euthanized; we performed post-mortem studies and macro-pathological studies of internal organs as well as histological analysis of specimens.

Blood serum was examined using ChemWell® 2910 biochemical analyzer to establish levels of alanine aminotransferase, albumin, alpha-amylase, aspartate aminotransferase, glucose, creatinine, lactate dehydrogenase, choline esterase, uric acid, urea, total protein, triglycerides, chlorides, cholesterol, and alkaline phosphatase.

Hematological studies were performed using Abacus Vet 5 Junior analyzer to establish counts of leucocytes, lymphocytes, monocytes, neutrophils, the leucocyte formula, erythrocytes, hemoglobin, PCV, MCV, MCHC, and RDW.

Morphofunctional studies of internal organs (thyroid, thymus, heart, lungs, stomach, liver, spleen, pancreas, ileum, large intestine, kidneys, adrenals, and testicles) were accomplished in conformity with [8] using morphological, morphometric and stereometric methods.

Effects on the animals' motor activity, investigative activity and cognitive functions were examined using the Open Field Test (the motor component) and the Burrowing test (orientation and investigative reactions)[4].

Hippocampus-dependent (explicit) and associative (implicit) memory was estimated in rats using the T-shaped labyrinth and the virtual labyrinth (What? Where? When? test) respectively.

In the T-shaped labyrinth test, the rats were trained in the experimental labyrinth for 3 days using food as reward. Next, the animals were tested 1 and 2 months later. Each testing session involved 10 runs made by the animals. Data per each 10 runs taken separately for each animal were averaged. The run was considered correct in case the animal went into a branch where food had been previously located.

To estimate associative (implicit) memory in the What? Where? When? test, each animal was placed into an individual automated chamber; containers with attractive and indifferent smells for rats were located in its two opposite areas.

Two hours later the animal was again placed in this individual automated chamber with different stimuli in its opposite corners,

---

[3] R 1.2.3156-13. Otsenka toksichnosti i opasnosti khimicheskikh veshchestv i ikh smesei dlya zdorov'ya cheloveka: rukovodstvo, utv. vrio Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 27 dekabrya 2013 g. [R 1.2.3156-13. Assessment of Toxicity and Hazard of Chemicals and Their Mixtures for Human Health: guidelines, approved by the acting RF Chief Sanitary Inspector on December 27, 2013]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200115595?ysclid=ma3l9lzp49885704016 (March 10, 2025) (in Russian).

[4] Metodicheskie rekomendatsii po ispol'zovaniyu povedencheskikh reaktsii zhivotnykh v toksikologicheskikh issledovaniyakh dlya tselei gigienicheskogo normirovaniya [Methodical guidelines on using animals' behavioral reactions in toxicological studies for establishing safe standards]. Kiev, Kievskii NII GT and PZ Publ., 1980, 43 p. (in Russian); Pavlenko S.M. Primenenie summatsionno-porogovogo pokazatelya v toksikologicheskom eksperimente na belykh krysakh [Use of the total threshold indicator in a toxicological experiment on white rats]. *Metodiki sanitarno-toksikologicheskogo eksperimenta [Methods of sanitary-epidemiological experimentation]: collection of research works by the Federal Scientific Center of Hygiene named after F.F. Erisman*, Moscow, 1975, pp. 5–7 (in Russian).

indifferent (area 1) and attractive (area 2) smells. The animals from all experimental groups were trained for 3 days in a raw together with registering time of a contact between the animals and an area where this or that container was located. The automated chambers were washed with 1:1 water-spirit solution after each testing to remove all smells.

Each training session lasted for 6 minutes. More frequent visits to the area 2 (attractive smell) indicated training was successful. On the 4th day, smell sources, which had been previously located in the opposite corners, were removed to estimate short-term memory. After that, we traced the rats' routes and the number of times they approached those corners where attractive and indifferent smell sources had been located previously.

Embryotoxic and teratogenic effects produced by ATC-a were examined using conventional white rats, totally 40 females and 20 males. All animal experiments were conducted in conformity with the principles fixed in the Guide R 1.2.3156-13[5].

Females were coupled with healthy males in the ratio 2:1 prior to exposure. Pregnancy was diagnosed in females when sperm cells were found in vagina smears. The females in the test group were orally exposed to CYN at doses equal to 0.1, 1.0 and 10.0 μg/kg of body weight every day starting from the beginning of pregnancy and up to its 20th day; the females in the control group were given distilled water.

The animals' health as well as food and water consumption was monitored during the whole experiment; we registered changes in body weight in dynamics and fixed any clinical signs of exposure outcomes. The animals were euthanized on the 20th day of pregnancy and postmortem studies were accomplished to identify scope of embryotoxic and teratogenic effects using the following indicators: the number of fetuses; the total weight of the litter; the number of yellow bodies; embryos' length and weight; placentas' weight and diameter. Complex indicators were calculated using conventional formulas by A.M. Malashenko and I.K. Egorov[6].

Two embryos were taken from each litter to identify absolute and relative masses of internal organs (thymus, heart, lungs, liver, and kidneys). The remaining embryos were divided into equal groups, which were used as experimental materials to study teratogenic effects according to the Wilson – Dyban method[7], which involved assessing general signs of fetus maturity (auricle sticking, eye closing, extremities and tail build); the second group was placed in 96 % ethanol for 7 days for subsequent examination of their skeletons per the Dawson method[8].

Primary data were analyzed using Microsoft Office Excel 2013; statistical data analysis was performed in SPSS Statistics v. 22.0. Significance of differences between the analyzed indicators was estimated using the Student's t-test at $p < 0.05$ ($t > 2$). The results were given as $M \pm m$ (where M is the mean value, m is the standards error of mean). We used the Shapiro – Wilk test to establish normalcy of distribution; Levene's test was used to check for the equality of dispersions. Occurrence of statistical

[5] R 1.2.3156-13. Otsenka toksichnosti i opasnosti khimicheskikh veshchestv i ikh smesei dlya zdorov'ya cheloveka: rukovodstvo, utv. vrio Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 27 dekabrya 2013 g. [R 1.2.3156-13. Assessment of Toxicity and Hazard of Chemicals and Their Mixtures for Human Health: guidelines, approved by the acting RF Chief Sanitary Inspector on December 27, 2013]. *KODEKS: electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/1200115595?ysclid=ma3l9lzp49885704016 (March 10, 2025) (in Russian).

[6] Malashenko A.M., Egorov I.E. Dominantnye letali u inbrednykh myshei pod deistviem etilenimina [Dominant lethals in inbred mice upon exposure to ethylenimine]. *Genetika*, 1967, no. 3, pp. 59–68 (in Russian).

[7] Dyban A.P., Puchkov V.F., Chebotar N.A. [et al.]. Metodicheskie ukazaniya po izucheniyu embriotoksicheskogo deistviya farmakologicheskikh veshchestv i vliyanie ikh na reproduktivnuyu funktsiyu [Methodical guidelines on examining embryotoxic effects of pharmaceutics and their influence on the reproductive function]. Moscow, USSR Ministry of Health Publ., 1986, 21 p. (in Russian).

[8] Dyban A.P., Baranov V.S., Akimova I.M. Osnovnye metodicheskie podkhody k testirovaniyu teratogennoi aktivnosti khimicheskikh veshchestv [Basic methodological approaches to testing teratogenic activity of chemicals]. *Arkhiv anatomii, gistologii i embriologii*, 1970, vol. 59, no. 10, pp. 89–100 (in Russian).

outliers was checked by the boxplot method[9]. Intergroup comparisons were accomplished by one-factor dispersion analysis with a posterior comparisons per Bonferroni (F-test, parametric indicators) or by using the Kruskal – Wallis non-parametric test[9,10]. We checked for trend occurrence in investigations (in case intergroup differences were significant) by using the Spearman's rank correlation method (two-side analysis)[11].

Results and discussion. We conducted a literature review, analyzed available research works using e-library, PubMed and Web of Science and studied regulatory and methodical documents valid in the Russian and international legislation with their focus on identifying cyanotoxins in water, assessing physical and chemical properties and toxicity of some cyanotoxins, investigating CYN toxicity upon intragastric administration into warm-blooded animals in acute, short-term experiments; we also analyzed data on occurrence of remote exposure effects. As a result we established the following:

CYN is an alkaloid produced by some CB species. The following structural variants can be found in natural conditions: 7-epi-CYN, 7-desoxi-CYN (Figure), 7-desoxidesulfo-CYN and 7-desoxidesulfo-12-acetyl-CYN. The molecular formula is $C_{15}H_{21}N_5O_7S$.

Upon entering water, CYN does not change its aesthetic properties; does not influence water color or smell; is not a volatile compound; is not decomposed by boiling; is relatively stable in dark and under the temperature between 4 and 50 °C for up to 5 weeks; is persistent to changes in pH and remains stable for a period up to 8 weeks when pH is equal to 4, 7 and 10 [1].

Inhalation exposure is possible only when the substance is sprayed, for example, during irrigation or a storm. In the environment, drinking water consumption is the basic way of human exposure to CYN in case this water comes from untreated or insufficiently treated surface water sources. Another exposure way is using water in lakes, rivers, or nearshore areas in the sea for recreation.

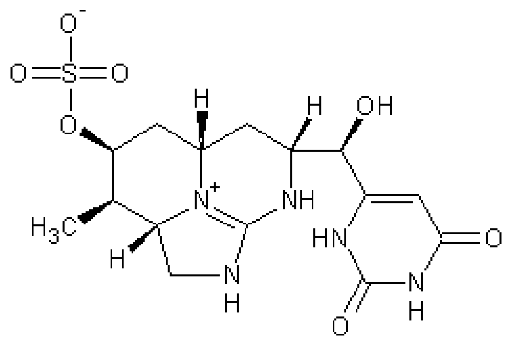

Figure. The structural formula of 7-desoxi-cylindrospermopsin

Acute CYN toxicity reported in foreign studies is described with the following values: intraperitoneal acute toxicity (DL_{50}), 20–65 mg/kg of body weight; acute toxicity upon oral administration is identified at 1400 mg/kg of body weight. [9–11]. Some authors reported lower lethal doses upon other exposure ways (intratracheal, for example) [12–14].

Several foreign researchers established the threshold dose in sub-acute experiments involving CYN administration in experimental animals with drinking water or using a gastric tube for 21 and 14 days, 66 µg/kg of body weight and 150 µg/kg of body weight respectively [15, 16].

According to available research publications, the liver and other gastrointestinal tract organs as well as the kidneys are primary targets upon CYN exposure under longer administration into animals with drinking water at doses ranging from 60 to 657 µg/kg of body weight [11, 17–19]. Intragastric CYN administration for 11 weeks in doses equal to 60–240 µg/kg of body weight led to an increase in the relative mass of the kidneys and liver. Necrotic or in-

[9] Khalafyan A.A. STATISTICA 6. Statisticheskii analiz dannykh [STATISTICA 6. Statistical data analysis]: manual, the 3rd ed. Moscow, OOO 'Binom-Press' Publ., 2007, 512 p. (in Russian).

[10] Abdi H. The Bonferroni and Šidák corrections for multiple comparisons. In book: *Encyclopedia of measurement and statistics*. USA, SAGE Publ., 2007, no. 3, pp. 103–107.

[11] Corder G.W., Foreman D.I. Nonparametric statistics: A step-by-step approach, 2nd ed. USA, Wiley Publ., 2014, 288 p.; Agresti A. Categorical data analysis, 2nd ed. USA, Wiley Publ., 2002, 734 p.

flammatory foci were identified in the liver of 60 % mice upon exposure to a dose equal to 120 μg/kg of body weight and in 90 % mice upon exposure to 240 μg/kg of body weight [11].

Fatal outcomes were detected in pregnant mice, which were given purified CYN by intraperitoneal injections thrice a day at a dose of 64 μg/kg of body weight. The relative weight of the liver was considerably increased in those mice that survived; however, the authors did not report any influence on fetuses' weight, death rate, or changes in skeletal or soft tissues [20]. Some researchers reported hemorrhagic lesions in certain tissues including the gastrointestinal tract [21].

The authors did not establish genotoxicity in ovary cells of Chinese hamsters (CHO-K1) exposed to CYN in concentrations equal to 0.5 and 1 μg/l in spite of induction of non-cytotoxic effects on cell morphology and microtubule structures [22].

Some foreign researchers (E. Bazin et al., 2010) established mutagenic and carcinogenic effects upon exposure to high CYN doses. Still, investigations aimed at determining bacterial mutagenicity using *Salmonella Typhimurium* TA98, TA100, TA1535, TA1537 strains and *Escherichia coli* WP2 uvrA and WP2 [pKM101] strains did not reveal any mutagenic CYN activity. Studies on genotoxicity for mammalian cells showed mutagenic effects, in particular, cellular lines of human hepatocytes showed elevated numbers of multinuclear and binucleated cells [23].

An experiment was performed on Swiss albino mice by foreign researchers who found neoplastic changes in mice exposed to CYN at doses of 2.75 or 8.25 mg/kg of body weight. However, those changes were not significant as compared to the control group. The authors believe that neoplastic changes detected in various target organs of the experimental animals are rather controversial evidence of CYN carcinogenicity at the moment [23, 24].

It is worth noting that pathways of toxic effects produced by long-term oral exposure to small CYN doses have not been examined to the full so far.

Many countries across the globe have extended the list of indicators used to control drinking water quality, both per CB levels in it and products of their life activity. In doing so, they usually emphasize the significance of research conducted within a country and show other countries the necessity to take relevant measures aimed at making drinking water safer by regulating levels of CB and cyanotoxins, CYN included. Our analysis has established that Australia, Brazil, and New Zealand employ their national research as criteria for establishing safe standards for drinking water quality per levels of CB and toxins (Table 1); they do not limit themselves to following only WHO guidelines[12] [1, 25–27].

Table 1

Permissible cylindrospermopsin concentrations in drinking water established in various countries

Country	Safe standard	Permissible concentration
Australia	*Cylindrospermopsin*	1 μg/l
Brazil	*Cylindrospermopsin*	15 μg/l
New Zealand	*Cylindrospermopsin*	1 μg/l

In Russia, the only MPC for cyanotoxins that has been scientifically substantiated and implemented in practical activities is MPC for Microcystin-LR in water objects used for drinking and household water supply as well as recreational needs. It is equal to 0.001 mg/l as the substance is assigned Hazard Category 1, sanitary-toxicological harm per SanPiN 2.1.3685-21[13].

[12] NHMRC, NRMMC. National Water Quality Management Strategy. Australian Drinking Water Guidelines 6. Australia, Canberra, Australian Government, 2011.

[13] SanPiN 1.2.3685-21. Gigienicheskie normativy i trebovaniya k obespecheniyu bezopasnosti i (ili) bezvrednosti dlya cheloveka faktorov sredy obitaniya: sanitarnye pravila i normy (s izmeneniyami na 30 dekabrya 2022 goda), utv. postanovleniem Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 28 yanvarya 2021 goda № 2 [SanPiN 1.2.3685-21. Hygienic standards and requirements to providing safety and (or) harmlessness of environmental factors for people: sanitary rules and norms (last edited as of December 30, 2022), approved by the Order of the RF Chief Sanitary Inspector dated January 28, 2021 No. 2]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573500115 (March 12, 2025).

Investigations with their focus on water self-purification in model water objects were conducted using three CYN concentrations: 1.0, 10.0 and 100.0 μg/l. They did not establish any influence on biochemical oxygen consumption during 5 days.

No spontaneous animal deaths were detected in a sub-chronic experiment that involved exposure to CYN at doses of 0.1; 1.0; and 10.0 μg/kg of body weight; we also did not establish any significant differences in body weight growth, any authentic differences in absolute or relative masses of internal organs between the tests and controls.

Hematological tests of animal blood were performed on the 30th, 45th, and 60th day in the experiment; they did not establish any significant differences between the analyzed indicators of the animals exposed to CYN at doses of 0.1; 1.0 and 10 μg/kg of body weight and the controls in all observation periods.

Biochemical blood serum tests were conducted on the 60th day in the experiment and established significant deviations in some indicators among those animals exposed to CYN at a dose of 10.0 μg/kg of body weight against the controls. We established authentic elevated levels of alanine aminotransferase, aspartate aminotransferase, glucose, creatinine, and triglycerides and an authentic decline in levels of albumin, uric acid, urea and total protein (Tables 2 and 3).

Increased motor activity in the Open Field test and investigative activity in the Burrowing test were established in the animals exposed to CYN at a dose of 10 μg/kg of body weight by the end of the experiment against the controls (Table 4).

The animals exposed to CYN at a dose of 10.0 μg/kg of body weight had fewer correct runs in the T-shaped labyrinth test; time spent on accomplishing them was also longer against the controls (Table 5).

We did not establish any effects produced by CYN on implicit (associative) memory in the What? Where? When? test using the indicator of the successfully completed training. This was evidenced by the most frequent visits to the area 2 (attractive smell) by the animals from all experimental groups prior to CYN administration (background).

Table 2

Changes in biochemical indicators of rats' blood serum, liver function indicators

CYN dose (μg/kg of body)	Mean ± standard error of mean				
	Alanine aminotransferase (U/l)	Albumin (g/l)	Aspartate aminotransferase (U/l)	Glucose (mol/l)	Creatinine (μmol/l)
15 days					
Control	72.74 ± 5.88	39.24 ± 0.43	158.53 ± 9.87	7.70 ± 0.26	109.15 ± 3.16
0.1	72.63 ± 5.48	38.68 ± 0.66	161.52 ± 10.84	7.23 ± 0.20	115.64 ± 2.75
1.0	64.10 ± 5.08	35.60 ± 0.65	143.27 ± 7.75	6.85 ± 0.26	108.10 ± 4.20
10.0	82.14 ± 3.49	39.42 ± 0.42	179.01 ± 9.97	7.56 ± 0.28	121.02 ± 4.71
45 days					
Control	60.79 ± 4.55	39.03 ± 0.68	222.15 ± 12.42	9.09 ± 0.47	116.46 ± 4.32
0.1	58.85 ± 2.76	39.29 ± 0.30	211.68 ± 8.51	9.15 ± 0.16	126.09 ± 3.90
1.0	59.68 ± 2.73	38.62 ± 0.64	229.07 ± 11.26	8.63 ± 0.25	118.06 ± 1.84
10.0	65.10 ± 4.23	40.44 ± 0.50	233.96 ± 15.67	9.40 ± 0.33	130.11 ± 5.89
60 days					
Control	59.23 ± 3.08	38.35 ± 0.68	123.83 ± 6.12	8.01 ± 0.23	108.06 ± 3.67
0.1	62.56 ± 3.83	38.63 ± 0.51	120.64 ± 4.67	7.94 ± 0.16	102.18 ± 3.25
1.0	57.81 ± 3.01	37.04 ± 0.70	121.82 ± 7.31	8.56 ± 0.27	106.23 ± 3.41
10.0	**↑75.03 ± 4.44***	**↓27.08 ± 0.22***	**↑158.97 ± 5.52***	**↑9.26 ± 0.25***	**↑140.48 ± 4.77***

Note: *significant at $p < 0.05$.

Table 3

Changes in biochemical indicators of rats' blood serum, kidney function indicators

CYN dose (μg/kg of body)	Mean ± standard error of mean			
	Uric acid (μmol/l)	Urea (mol/l)	Total protein (g/l)	Triglycerides (mol/l)
15 days				
Control	132.01 ± 17.97	7.24 ± 0.33	68.98 ± 1.08	0.57 ± 0.05
0.1	135.57 ± 20.86	6.66 ± 0.38	70.39 ± 0.98	0.43 ± 0.02
1.0	148.98 ± 17.32	7.71 ± 0.32	71.16 ± 1.27	0.47 ± 0.04
10.0	141.06 ± 5.63	7.82 ± 0.68	70.45 ± 0.77	0.48 ± 0.03
45 days				
Control	45.50 ± 5.57	7.11 ± 0.32	70.06 ± 0.68	0.78 ± 0.10
0.1	54.75 ± 9.37	7.47 ± 0.52	70.19 ± 0.63	0.68 ± 0.09
1.0	48.58 ± 10.16	7.44 ± 0.35	69.97 ± 0.93	0.62 ± 0.05
10.0	47.04 ± 11.63	6.81 ± 0.40	69.70 ± 0.76	0.63 ± 0.06
60 days				
Control	60.54 ± 10.13	6.30 ± 0.28	67.90 ± 1.05	0.69 ± 0.08
0.1	69.40 ± 9.86	6.04 ± 0.40	68.26 ± 0.89	0.54 ± 0.05
1.0	60.54 ± 7.20	7.30 ± 0.50	68.12 ± 0.86	0.65 ± 0.08
10.0	**↓39.28 ± 6.98***	**↓4.23 ± 0.28***	**↓48.19 ± 0.58***	**↑0.82 ± 0.06***

Note: *significant at $p < 0.05$.

Table 4

Levels of animals' motor and investigative activity established by Open Field and Burrowing tests

CYN dose (μg/kg of body)	Background	30 days	60 days
Open Field (distance covered, cm)			
Control	1648 ± 71.52	533.99 ± 150.30	578.93 ± 116.65
0.1	1472.38 ± 101.99	570.48 ± 112.03	686.33 ± 119.05
1.0	1453.09 ± 112.98	562.77 ± 177.51	959.73 ± 202.67
10.0	1638.84 ± 145.40	895.40 ± 235.10	1303.30 ± 67.07*
Burrowing test (number of events)			
Control	46.30 ± 7.22	8.10 ± 2.34	8.80 ± 2.20
0.1	43.20 ± 5.26	13.20 ± 3.65	11.40 ± 2.10
1.0	37.30 ± 3.34	9.90 ± 3.02	10.50 ± 3.63
10.0	41.70 ± 4.04	15.90 ± 3.83	20.60 ± 1.18*

Note:* significant at $p < 0.05$.

Table 5

Indicators of animals' hippocampus-dependent (explicit) memory in the T-shaped labyrinth test

CYN dose (μg/kg of body)	Background	30 days	60 days
Number of correct runs, %			
Control	26.67 ± 9.69	36.80 ± 10.52	33.33 ± 11.11
0.1	23.37 ± 7.14	23.33 ± 8.69	37.04 ± 10.31
1.0	26.73 ± 9.73	26.67 ± 10.89	40.00 ± 8.31
10.0	30.03 ± 9.27	13.27 ± 5.42*	36.67 ± 9.23
Time spent on a run, sec			
Control	42.05 ± 13.86	47.18 ± 9.06	59.22 ± 18.59
0.1	39.20 ± 12.38	35.86 ± 24.45	48.99 ± 11.54
1.0	64.80 ± 2.96	48.14 ± 16.65	27.06 ± 6.63
10.0	47.00 ± 11.72	102.00 ± 17.01*	25.87 ± 8.82

Note: * significant at $p < 0.05$.

Microscopic examination of histological specimens of various organs taken from the animals exposed to CYN at a dose of 10.0 µg/kg of body weight established authentic morphofunctional changes in the following organs. Fat dystrophy and micronecroses were increased in the liver; the glomerule alteration index and glomerule necrosis were higher in the kidney; gland hypersecretion was identified in the stomach and large intestine; more layers of spermatogenic cells moved away from the basement membrane of the testicular tubules in the testicle and we also observed rarer spermatids and sperm cells; thyrocyte desquamation was observed in the thyroid.

Experimental studies with their focus on embryotoxic and teratogenic CYN effects did not establish any spontaneous animal deaths in all experimental groups. We did not find authentic changes in body weight of pregnant female rats; average body weight growth during pregnancy was the same in the test groups and the control. We did not reveal any significant changes in the absolute or relative mass of embryos' internal organs as well as authentic changes in embryogenesis indicators in all test groups as compared to the control. Average numbers of yellow bodies, implantation sites, live fetuses, the overall weight of fetuses in a litter, crown-rump length of fetuses, placenta diameters and weights did not have significant differences in the tests against the controls. Pre-implantation, post-implantation and total death rates among offspring were the same in the test groups as in the parallel control without any significant differences between them.

Intravital microscopic examination of fetuses did not find any severe malformations. Subsequent micro-post mortem study of fixed fetus material and analysis of cross sections of the brain and other internal organs revealed that fetuses' internal organs had normal locations and structures in the test groups and their structure and topography did not have any differences from the control. Detected embryogenesis disorders were not diverse, were not numerous and were similarly rare in all animal groups; this did not allow suspecting any associations between them and CYN effects on offspring formation and intrauterine development.

Our assessment of fetus skeletons with clear soft tissues was performed in the test and control groups and did not find any osteogenesis disorders or desynchronized ossification of the cartilage anlages evidencing absence of any teratogenic CYN effects.

Discussion of the results and substantiation of CYN MPC in water. When assessing materials on regulation and control of CYN contents in water objects, we should remember that available foreign regulatory standards are based exclusively on studying toxic properties in experimental conditions. Russian practice of substantiating safe standards for chemicals in water is based not only on establishing concentrations with no observed toxic effects on laboratory animals but also, in conformity with the Methodical Guidelines 2.1.5.720-98[14], includes establishing threshold levels of chemicals' effects on water organoleptic properties and the overall sanitary condition of a water object; a safe standard is fixed per a limiting harm indicator with the lowest no-effect or threshold concentration.

When substantiating CYN MPC in water from water objects used for drinking and household water supply, we did not establish threshold concentrations per its effects produced on water organoleptic properties since available literature data give evidence that CYN does not influence water aesthetic properties. We also thought that involving volunteers in an experiment with a highly toxic substance with poorly studied potential carcinogenic effects could create substantial risks of adverse health impacts for them.

[14] MU 2.1.5.720-98. Obosnovanie gigienicheskikh normativov khimicheskikh veshchestv v vode vodnykh ob"ektov khozyaistvenno-pit'evogo i kul'turno-bytovogo vodopol'zovaniya: metodicheskie ukazaniya, utv. i vved. v deistvie Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii 15 oktyabrya 1998 goda [MU 2.1.5.720-98. Substantiation of safe standards for chemical levels in water supplied from water objects used for drinking and household purposes: Methodical guidelines, approved and enacted by the RF Chief Sanitary Inspector on October 15, 1998]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200006903 (March 10, 2025) (in Russian).

Investigations in model water objects showed no influence on water self-purification, which is consistent with opinions expressed by other Russian researchers. They consider that biocoenosis in 'blooming' water objects is more resistant to cyanometabolites and explain it by the result of co-evolutionary co-existence with toxic cyanobacteria in natural conditions[15].

Guided by the MUK 2.1.5.720-98[14], we substantiated exposure doses of 0.1 – 1.0 – 10.0 μg/kg of body weight when conducting our subchronic experiment aimed at establishing a threshold CYN concentration per its general toxic effects as well as at revealing potential remote effects (embryotoxic and teratogenic ones).

This sub-chronic experiment established several changes in biochemical blood serum indicators upon exposure to CYN at a dose of 10 μg/kg of body weight. These changes indicated impairments of enzyme-forming liver function, protein and carbohydrate metabolism; we also revealed some changes in kidney functional indicators, which is consistent with multiple studies by foreign researchers [15, 16, 18, 19, 28].

In addition, an increase in motor activity (Open Field test) and investigative activity (the Burrowing test) was established in the animals exposed to CYN at a dose of 10 μg/kg of body weight against the control by the end of the subchronic experiment.

These changes can be explained by the fact that the basic pathway of CYN effects is inhibiting protein synthesis, including synthesis of glutathione. This leads to oxidative stress, which, in its turn, enhances lipid peroxidation and induces DNA damage[16] [28–33]. CYN is able to penetrate through the blood-brain barrier [34, 35] and reduce the number of synapses; consequently, it is able to produce a neurotoxic effect. Still, the very neurotoxic effects produced by CYN have not been studied sufficiently so far [36]. Therefore, we made an effort in this study to estimate CYN influence on animals' cognitive functions in tests aimed at revealing disorders in ability to memorize, recognize and reproduce as well as in investigative activity.

Explicit memory disorders in animals were of the greatest interest to us since this memory is associated with activities of the brain hippocampus and is conscious. We revealed a decline in the number of correct runs and longer time spent on doing them in the T-shaped labyrinth test, which indicates disorders of hippocampus-dependent memory in rats exposed to CYN at a dose of 10 μg/kg of body weight. This disorder might result from changes in the body described in literature such as declining acetyl cholinesterase activity and growing lipid peroxidation levels with accompanying histopathological changes in the brain of animals under subchronic CYN exposure [34]. Recovery of indicators up to their levels identified in the controls by the end of the second months of CYN exposure might result from activation of compensatory mechanisms.

Implicit memory disorders can lead to 'accumulated forgetting' of visits to sites in the Open Field test, which is the reason why a covered distance becomes longer; this can also cause a growth in the number of time when rats peep in burrows in the Burrowing test after CYN administration due to the described damage to neurons at the synapsis level [35].

Examinations of histological specimens of organs taken from the animals exposed to CYN at a dose of 10 μg/kg of body weight found considerable changes in the liver such as fat dystrophy up to micro-necrosis, which is consistent with [11, 15, 16, 19, 28]; glomerule necrosis was established in the kidneys and gland hypersecretion in the stomach and large intestine, which is consistent with [18]. Established morphofunctional changes in animals' internal organs primarily give evidence that the liver, kidneys and the gastrointestinal tracts do not function correctly.

---

[15] Semenova A.S. Koevolyutsiya tsianobakterii i zooplanktona: zashchitnaya rol' tsianotoksinov [Co-evolution of cyanobacteria and zooplankton: protective role of cyanotoxins]: Grant No. 15-04-04030. Moscow, Russian Foundation for Basic Research (RFBR), 2015.

[16] Terao K., Ohmori S., Igarashi K., Ohtani I., Watanabe M.F., Harada K.I., Ito E., Watanabe M. Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green-alga Umezakia natans. *Toxicon*, 1994, vol. 32, no. 7, pp. 833–843. DOI: 10.1016/0041-0101(94)90008-6

Our experimental study of embryotoxic CYN effects did not find any authentic changes in the absolute and relative mass of fetuses' internal organs in the test groups against the control. Embryogenesis indicators established in the test groups did not reveal any authentic changes against the controls either, which means CYN does not have any embryotoxic effects.

We did not find any changes in the number of bones in fetuses or in their bone structure, or any significant malformations of bone tissues. Some sporadic changes were identified in some animals when we investigated pathology of internal organs; however, their dependence on an administered CYN dose was not confirmed ($p > 0.05$). Analysis of these deviations from the control group makes it possible to conclude that all CYN doses tested in the experiment do not have teratogenic effects on the body.

Neoplastic changes in target organs of various animals, which are reported by some researchers, provide some ambiguous evidence of carcinogenicity; this requires additional research [29, 30].

Considering authentic changes in biochemical blood indicators, changes in animals' motor and investigative activity, as well as morphofunctional lesions of internal organs, we estimate CYN dose of 10 µg/kg of body weight as an effective one per general toxicity in a sub-chronic experiment; doses of 0.1 and 1.0 µg/kg of body weight are considered as no-effect ones.

To substantiate CYN MPC in water for drinking and household water use, according to the Item 10.3 MUK 2.1.5.720-98 and Table 10.2, we selected the coefficient value *J* (*J1* = 20) to establish the threshold dose for chronic exposure depending on how well toxic effects of a substance are known and a structural group it belongs to. CYN is assigned the Hazard Category 1 and its MPC for chronic exposure is established at the level of 3.3 µg/kg of body weight per the ratio DL_{50} / MPC_{IEC}.

Bearing in mind that CYN is a stable substance, a safety factor 5 is introduced in accordance with Table 5.2, MUK 2.1.5.720-98, and the MPC for chronic exposure is 0.66 µg/kg of body weight.

According to the Item 11.7 in MUK 2.1.5.720-98, the maximum no-effect concentration (MEC) fir chronic exposures has been established in conformity with Table 11.1 using a safety factor equal to 10. Considering this safety factor, chronic CYN MEC upon oral administration to warm-blooded animals' bodies is 0.06 µg/kg of body weight.

Table 6 provides indicators used in complex assessment of hazards posed by CYN contents in water.

Table 6

Indicators used in complex assessment of hazards posed by CYN contents in water

Indicator	Values to be identified	Source	Result
Stability in water	Stability	[1]	stable
Effect on water organoleptic properties	Effects on color or smell	[1]	No effect
Effect on water self-purification*	MC_{san}		above 100.0 µg /l
Toxicological studies			
Acute experiment	LD_{50}	Ref. 6, 8, 9	1400 µg/kg of b.w.
Sub-acute experiment	MPC_{IEC}	[9, 10]	66.0 µg/kg of b.w.
Sub-chronic experiment*	Calculated chronic MPC Calculated chronic MEC Hazard Category	MUK 2.1.5.720-98	3,3 µg/kg of b.w. 0,06 µg/kg of b.w. hazard category 1
Remote effects			
Mutagenic		[11]	Upon exposure to high doses
Carcinogenic		[17, 18]	Upon exposure to high doses
Embryotoxic*	MPC_{sep}		above 10.0 µg/kg of b.w.
Teratogenic*	MPC_{sep}		above 10,0 µg/kg of b.w.

Note: *means our own research.

The maximum no-effect CYN concentration (MEC_{chr}) in water amounts to 1.2 µg/l per the sanitary-toxicological indicator of harm and in conformity with the recalculation formula, which considers an average human body weight (60 kg) and daily water intake (3 l).

Conclusion. Therefore, considering that there is no threshold effect on water organoleptic properties established for CYN and absence of any influence on self-purification in concentrations below 100 µg/l, we recommend CYN MPC at the level of 1.0 µg/l in water for drinking and household water use per the sanitary-toxicological indicator of harm, the Hazard Category 1.

Methodical Guidelines MUK Quantification of Cylindrospermopsin in Drinking and Natural Water by Immune Enzyme Analysis (the bottom limit of quantification is 0.00005 mg/l) have been developed for control of CYN contents in water.

Funding. The research has been accomplished within the State Program Provision of Chemical and Biological Safety in the Russian Federation for 2021–2024.

Competing interests. The authors declare no competing interests.

References

1. WHO. Guidelines for drinking-water quality, 4th ed. *WHO Documentation Centre*, 2011. Available at: https://iris.who.int/bitstream/handle/10665/44584/9789241548151_eng.pdf (January 11, 2025).

2. Bakaev A.V., Bakaeva E.N., Ignatova N.A. "Blooming" blue-green algae (cyanobacteria) a variety of emergency situations in reservoirs. *Inzhenernyi vestnik Dona*, 2012, vol. 23, no. 4–2, pp. 144 (in Russian).

3. Kuz' N.V., Zholdakova Z.I. The problem of the «flowering» of the water sources. Evaluation of the influence of water processing on the content of cyanobacterium in drinking water of economic-drinking water supply of Moscow. *ZNiSO*, 2017, no. 9 (294), pp. 35–39 (in Russian).

4. Egorova N.A., Kuz N.V., Sinitsyna O.O. Materials for the substantiation of the hygienic standard of microcystin-LR in water of water objects. *Gigiena i sanitariya*, 2018, vol. 97, no. 11, pp. 1046–1052. DOI: 10.18821/0016-9900-2018-97-11-1046-52 (in Russian).

5. Kuz N.V., Sinitsyna O.O., Turbinsky V.V. Flowering of water sources – new challenges to drinking water safety in the condition of climate change. *Zdorov'e – osnova chelovecheskogo potentsiala: problemy i puti resheniya*, 2022, vol. 17, no. 1, pp. 218–225 (in Russian).

6. Sinitsyna O.O., Turbinskii V.V., Pushkareva M.V., Kuz N.V., Masaltsev G.V., Shiryaeva M.A., Ryashentseva T.M., Valtseva E.A., Vostrikova M.V. Morphofunctional changes in the animal organisms under oral exposure to anatoxin-a in subchronic experimental settings. *Toksikologicheskii vestnik*, 2024, vol. 32, no. 6, pp. 336–347. DOI: 10.47470/0869-7922-2024-6-336-347 (in Russian).

7. Turbinsky V.V., Bragina I.V., Kuz N.V., Sinitsyna O.O., Pushkareva M.V. The problem of algal bloom in the source of drinking water supply for the population. *Gigiena i sanitariya*, 2024, vol. 103, no. 12, pp. 1466–1472. DOI: 10.47470/0016-9900-2024-103-12-1466-1472 (in Russian).

8. Belyaeva N.N., Rakitskii V.N., Nikolaeva N.I., Vostrikova M.V., Veshchemova T.E. Quantitative structural and functional assessment of various systems of the body of laboratory animals in hygienic studies. *Gigiena i sanitariya*, 2020, vol. 99, no. 12, pp. 1438–1445. DOI: 10.47470/0016-9900-2020-99-12-1438-1445 (in Russian).

9. Falconer I.R. An overview of problems caused by toxic blue–green algae (cyanobacteria) in drinking and recreational water. *Environ. Toxicol.*, 1999, vol. 14, no. 1, pp. 5–12.

10. Falconer I.R. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. *Phycologia*, 2001, vol. 40, no. 3, pp. 228–233. DOI: 10.2216/i0031-8884-40-3-228.1

11. Falconer I.R., Humpage A.R. Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins. *Environ. Toxicol.*, 2006, vol. 21, no. 4, pp. 299–304. DOI: 10.1002/tox.20194

12. Seawright J. Testing for necessary and/or sufficient causation: Which cases are relevant? *Political Analysis*, 2002, vol. 10, no. 2, pp. 178–193. DOI: 10.1093/pan/10.2.178

13. Norris R.L., Seawright A.A., Shaw G.R., Smith M.J., Chiswell R.K., Moore M.R. Distribution of 14C cylindrospermopsin in vivo in the mouse. *Environ. Toxicol.*, 2001, vol. 16, no. 6, pp. 498–505.

14. Cordeiro-Araújo M.K., Bittencourt-Oliveira M.C. Active release of microcystins controlled by an endogenous rhythm in the cyanobacterium *Microcystis aeruginosa*. *Phycological Research*, 2013, vol. 61, no. 1, pp. 1–6.

15. Reisner A.D. The common factors, empirically validated treatments, and recovery models of therapeutic change. *Psychol. Rec.*, 2005, vol. 55, pp. 377–399. DOI: 10.1007/BF03395517

16. Shaw G.R., Seawright A.A., Moore M.R., Lam P.K. Cylindrospermopsin, a cyanobacterial alkaloid: evaluation of its toxicologic activity. *Ther. Drug Monit.*, 2000, vol. 22, no. 1, pp. 89–92. DOI: 10.1097/00007691-200002000-00019

17. Humpage A.R., Falconer I.R. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.*, 2003, vol. 18, no. 2, pp. 94–103. DOI: 10.1002/tox.10104

18. Sukenik A., Reisner M., Carmeli S., Werman M. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in mice: Long-term exposure to low doses. *Environ. Toxicol.*, 2006, vol. 21, no. 6, pp. 575–582. DOI: 10.1002/tox.20220

19. Chernoff N., Hill D.J., Diggs D.L., Faison B.D., Francis B.M., Lang J.R., Larue M.M., Le T.-T. [et al.]. A critical review of the postulated role of the non-essential amino acid, β-N-methylamino-L-alanine, in neurodegenerative disease in humans. *J. Toxicol. Environ. Health B Crit. Rev.*, 2017, vol. 20, no. 4, pp. 1–47. DOI: 10.1080/10937404.2017.1297592

20. Merel S., Walker D., Chicana R., Snyder S., Baures E., Thomas O. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.*, 2013, vol. 59, pp. 303–327. DOI: 10.1016/j.envint.2013.06.013

21. Karlsson O., Roman E., Berg A.L., Brittebo E.B. Early hippocampal cell death, and late learning and memory deficits in rats exposed to the environmental toxin BMAA (beta-N-methylamino-L-alanine) during the neonatal period. *Behav. Brain Res.*, 2011, vol. 219, no. 2, pp. 310–320. DOI: 10.1016/j.bbr.2011.01.056

22. Geh E.N., Ghosh D., McKell M., de la Cruz A.A., Stelma G., Bernstein J.A. Identification of Microcystis aeruginosa Peptides Responsible for Allergic Sensitization and Characterization of Functional Interactions between Cyanobacterial Toxins and Immunogenic Peptides. *Environ. Health Perspect.*, 2015, vol. 123, no. 11, pp. 1159–1166. DOI: 10.1289/ehp.1409065

23. Bazin E., Mourot A., Humpage A.R., Fessard V. Genotoxicity of afreshwater cyanotoxin, cylindrospermopsin, in two human cell lines: Caco-2 and HepaRG. *Environ. Mol. Mutagen.*, 2010, vol. 51, no. 3, pp. 251–259. DOI: 10.1002/em.20539

24. Novak M., Hercog K., Žegura B. Assessment of the mutagenic and genotoxic activity of cyanobacterial toxin beta-N-methyl-amino-L-alanine in Salmonella typhimurium. *Toxicon*, 2016, vol. 118, pp. 134–140. DOI: 10.1016/j.toxicon.2016.04.047

25. Szlag D.C., Sinclair J.L., Southwell B., Westrick J.A. Cyanobacteria and Cyanotoxins Occurrence and Removal from Five High-Risk Conventional Treatment Drinking Water Plants. *Toxins (Basel)*, 2015, vol. 7, no. 6, pp. 2198–2220. DOI: 10.3390/toxins7062198

26. Kong Y., Lou I., Zhang Y., Lou C.U., Mok K.M. Using an online phycocyanin fluorescence probe for rapid monitoring of cyanobacteria in Macau freshwater reservoir. *Hydrobiologia*, vol. 741, pp. 33–49. DOI: 10.1007/s10750-013-1759-3

27. Hoagland P., Jin D., Beet A., Kirkpatrick B., Reich A., Ullmann S., Fleming L.E., Kirkpatrick G. The human health effects of Florida Red Tide (FRT) blooms: an expanded analysis. *Environ. Int.*, 2014, vol. 68, pp. 144–153. DOI: 10.1016/j.envint.2014.03.016

28. Chernoff N., Hill D.J., Chorus I., Diggs D.L., Huang H., King D., Lang J.R., Le T.-T. [et al.]. Cylindrospermopsin toxicity in mice following a 90-d oral exposure. *J. Toxicol. Environ. Health A*, 2018, vol. 81, no. 13, pp. 549–566. DOI: 10.1080/15287394.2018.1460787

29. Yang Y., Yu G., Chen Y., Jia N., Li R. Four decades of progress in cylindrospermopsin research: The ins and outs of a potent cyanotoxin. *J. Hazard. Mater.*, 2021, vol. 406, pp. 124653. DOI: 10.1016/j.jhazmat.2020.124653

30. Poniedziałek B., Rzymski P., Kokociński M. Cylindrospermopsin: water-linked potential threat to human health in Europe. *Environ. Toxicol. Pharmacol.*, 2012, vol. 34, no. 3, pp. 651–660. DOI: 10.1016/j.etap.2012.08.005

31. Poniedziałek B., Rzymski P., Kokociński M., Karczewski J. Toxic potencies of metabolite(s) of non-cylindrospermopsin producing Cylindrospermopsis raciborskii isolated from temperate zone in human white cells. *Chemosphere*, 2015, vol. 120, pp. 608–614. DOI: 10.1016/j.chemosphere.2014.09.067

32. Puerto M., Pichardo S., Jos Á., Gutiérrez-Praena D., Cameán A.M. Acute effects of pure Cylindrospermopsin on the activity and transcription of antioxidant enzymes in Tilapia (Oreochromis niloticus) exposed by gavage. *Ecotoxicology*, 2011, vol. 20, no. 8, pp. 1852–1860. DOI: 10.1007/s10646-011-0723-0

33. Žegura B., Gajski G., Štraser A., Garaj-Vrhovac V. Cylindrospermopsin induced DNA damage and alteration in the expression of genes involved in the response to DNA damage, apoptosis and oxidative stress. *Toxicon*, 2011, vol. 58, no. 6–7, pp. 471–479. DOI: 10.1016/j.toxicon.2011.08.005

34. Guzmán-Guillén R., Lomares I., Moreno I.M., Prieto A.I., Moyano R., Blanco A., Cameán A.M. Cylindrospermopsin induces neurotoxicity in tilapia fish (Oreochromis niloticus) exposed to Aphanizomenon ovalisporum. *Aquat. Toxicol.*, 2015, vol. 161, pp. 17–24. DOI: 10.1016/j.aquatox.2015.01.024

35. Rabelo J.C.S., Hanusch A.L., de Jesus L.W.O., Mesquita L.A., Franco F.C., Silva R.A., Sabóia-Morais S.M.T. DNA damage induced by cylindrospermopsin on different tissues of the biomonitor fish Poecilia reticulate. *Environ. Toxicol.*, 2021, vol. 36, no. 6, pp. 1125–1134. DOI: 10.1002/tox.23111

36. Hinojosa M.G., Prieto A.I., Muñoz-Castro C., Sánchez-Mico M.V., Vitorica J., Cameán A.M., Jos Á. Cytotoxicity and Effects on the Synapsis Induced by Pure Cylindrospermopsin in an E17 Embryonic Murine Primary Neuronal Culture in a Concentration- and Time-Dependent Manner. *Toxins (Basel)*, 2022, vol. 14, no. 3, pp. 175. DOI: 10.3390/toxins14030175

Sinitsyna O.O., Turbinsky V.V., Pushkareva M.V., Kuz N.V., Shiryaeva M.A., Masaltsev G.V., Safandeev V.V. Substantiating the maximum permissible concentration of cylindrospermopsin in water for drinking and household use to minimize human health risks. Health Risk Analysis, 2025, no. 2, pp. 16–29. DOI: 10.21668/health.risk/2025.2.02.eng

Received: 10.04.2025
Approved: 29.04.2025
Accepted for publication: 14.06.2025

UDC 612.014.43
DOI: 10.21668/health.risk/2025.2.03.eng

Read online

Research article

TEMPERATURE-RELATED MORTALITY RISKS: EFFECTS OF DIFFERENT SOURCES OF CLIMATIC DATA IN THE RF REGIONS IN 2004–2019

M.R. Maksimenko

National Research University Higher School of Economics, 11 Myasnitskaya St., Moscow, 101000, Russian Federation

Climate change and increasing thermal stress highlights the need to investigate the temperature-mortality relationship using long-term aggregated temperature data. Globally, two primary sources of temperature data are utilized: ground-based meteorological observations and raster datasets. Ground-based observations from meteorological stations offer precise local temperature measurements but lack comprehensive spatial coverage. In contrast, raster data provide complete spatial coverage but may not accurately represent local microclimatic conditions. This study aims to compare these data sources for analyzing temperature-related mortality across regions of Russia.

To assess the exposure-response relationship, a two-stage modeling approach was applied. At the first stage, region-specific estimates were derived using a distributed lag model. At the second stage, pooled estimates were computed through random-effects meta-regression.

The temperature-mortality relationship in Russia is characterized by a typical J-shaped curve, with cold temperatures posing a higher mortality risk. Heat-related risks were generally higher when estimated using raster data compared to in-situ observations. Minimum mortality risk temperatures typically fall between 15 and 20 °C, with higher thresholds observed in warmer regions.

This study suggests general comparability of raster and point-based temperature data for mortality analysis. However, in certain regions, particularly large and sparsely populated ones, estimates diverged due to multiple factors.

Keywords: *climate change, atmosphere reanalysis, air temperature, temperature stress, raster data, ground-based meteorological observations, mortality, regions of Russia.*

Climate change leads to more frequent and more intense heat waves as well as longer periods of stably high ambient air temperatures during which mortality risks grow disproportionally faster, especially for the most susceptible population groups [1]. Some studies report that a potential decrease in cold-related deaths does not balance a steep rise in heat-related excess mortality [2]. In particular, the number of temperature-related deaths is predicted to grow practically everywhere in Europe, even taking adaptation into account [3]. In this respect, studies that focus on temperature effects on population health and mortality are becoming more and more relevant and the issue requires more detailed analysis.

The greatest attention has been paid to the relationship between mortality risks and exposure to extremely high temperatures. Since drastic rises in mortality during heat periods have always been a serious challenge for public health, they have long been examined within epidemiological studies [4]. Accumulated data give evidence of a considerable health effect produced by heat waves including a significant growth in cardiorespiratory risks [5–7]. In addition, pollutant concentrations grow in ambient air during heat waves due to wildfires, air stagnation, occurring 'heat islands' and other factors; this is an additional health risk factor [8, 9].

At the same time, excess mortality in cold periods has been given much less attention by researchers and available data on causal relationships and physiological pathways are provided with a limited evidence base [10].

Mikhail R. Maksimenko – Junior Researcher (e-mail: mikemaksimenko@gmail.com; tel.: +7 (915) 476-60-68; ORCID: https://orcid.org/0000-0001-8441-6676).

Nevertheless, epidemiological studies show that it is cold-related risks that make the greatest contribution to the overall temperature-related mortality [11].

An accurate estimate of a temperature stress perceived by people is the most difficult task from the meteorological point of view. This is due to the fact that temperature effects depend on several concomitant factors such as humidity, wind speed, atmospheric pressure, etc. [12]. Various biometeorological indices are used to consider these factors; they are mostly calculated relaying on a combination of these parameters with air temperatures to estimate the integral heat stress value [13–15]. Nevertheless, basic results are quite similar in most cases when mean daily temperatures are employed to predict excess mortality risks [12].

Russian experience gained in investigating temperature-related mortality is, as a rule, based on studies accomplished in various cities across Russia. The first studies focused on analyzing cold-related risks and peculiarities of people's adaptation to low temperatures[1]. However, systemic epidemiological studies were first conducted only in mid-2000ties [16–18]. They revealed a significant rise in mortality in certain groups during the heat waves in 1999 and 2001 as well as the cold wave in 2006.

In particular, excess mortality has been evidenced during heat and cold waves in the southern regions [19–21], north regions [7, 14, 22], northwestern regions [23], Siberia [24], Far East [25, 26], and Moscow [9].

The considerable part of Russian studies focuses on estimating excess mortality during heat or cold waves per age groups or causes of death; another focus is determining associations between air temperatures and mortality risks within specific cities. Various biometeorological indices as mortality predictors have been compared as well [14, 21]. Special attention has been paid to interactions between air temperatures and ambient air pollution as an additional risk factor [9]. In addition, impacts exerted on mortality rates by duration of heat or cold waves have also been given some attention [20].

Common conclusions made in these studies give evidence of excess mortality both during heat and cold waves; however, specific risk levels depend on geographical conditions, analyzed population groups, heat waves criteria, meteorological parameters, etc. For example, using Volgograd, Rostov-on-Don and Astrakhan as examples, researchers have shown that mortality risks during heat waves are higher than similar risks during cold ones [19, 20]. On the contrary, cold-related risks have turned out to be higher in Murmansk, Arkhangelsk and Magadan [7]. Studies accomplished in Khabarovsk and Krasnoyarsk have established that the population in both cities faces excess mortality both during heat and cold waves. For Khabarovsk, the highest mortality risks were established during heat waves [26], whereas for Krasnoyarsk during cold waves [24].

There are comparatively few studies with their focus on estimating temperature effects on mortality at the regional level [27–29]. In general, cold-related risks are established to be higher in them; however, the analysis gets too complicated due to too wide confidence intervals in estimates.

Approaches to data analysis in environmental epidemiology. Data on the environmental conditions (temperatures, ambient air pollution, etc.) are usually obtained by ground-based observations or are raster (gridded) datasets. Ground-based observations provide highly accurate data directly at measuring points and are often considered the 'golden standard' due to it. However, they cannot provide the complete geographical coverage. Moreover, observation stations (especially meteorological ones) are often located in atypical places such as airports or outskirts

[1] Donaldson G.C., Tchernjavskii V.E., Ermakov S.P., Bucher K., Keatinge W.R. Winter mortality and cold stress in Yekaterinburg, Russia: interview survey. *BMJ*, 1998, vol. 316, no. 7130, pp. 514–518. DOI: 10.1136/bmj.316.7130.514; Donaldson G.C., Ermakov S.P., Komarov Y.M., McDonald C.P., Keatinge W.R. Cold related mortalities and protection against cold in Yakutsk, eastern Siberia: observation and interview study. *BMJ*, 1998, vol. 317, no. 7164, pp. 978–982. DOI: 10.1136/bmj.317.7164.978

of large cities; this makes them much less eligible for assessing health risks in densely populated residential areas [30, 31].

Gridded data on temperatures are created by using remote sensing or by interpolating and using various geostatistical methods. This approach provides complete coverage of the whole analyzed area. However, errors in measurements, calculations and aggregations limit their accuracy and applicability as a source of data on the environment [32]. Global gridded products, though having comparable time-specific detailing, often have too low spatial resolution and describe actual conditions only as averaged without considering local patterns [33] and turn out to be available only with a certain lag. Gridded datasets with extremely high resolution tend to have very limited coverage; therefore, they do not allow analysis within spacious regions.

Atmospheric reanalyses are a source able to provide gridded data on temperatures used in environmental epidemiology. They are based on remote sensing data, ground-based observations and atmospheric circulation models, which allows creating continuous time series and make retrospective forecasts. However, since these are model calculations, they are often unable to represent local weather conditions [34]. Nevertheless, multiple studies describe their mutual compatibility with other data sources for mortality analysis [35, 36].

Additional complications are associated with calculating an aggregated temperature level within a specific area since the choice of a concrete calculation method can turn out to be as crucial as the choice of a this or that data source [37]. Averaging of all available values seems to be the simplest and intuitively understandable aggregation method [38]. However, such estimates often turn out to be non-representative. For example, a situation in a border area can be reflected by observations made in other regions much more accurately; given that, sometimes it is advisable to expand a selection of meteorological stations by including a buffer area. Averaging also does not make it possible to consider differences related to population distribution variability. To overcome that, a possible solution might be to consider each meteorological station with a weight, which is inversely proportional to the distance between this station and the center of the region. In this case, the population center becomes the most representative central point, which reflects peculiarities of population distribution. The population center in a region is a point with the smallest distance from it to all other points in this region considering their weights per population numbers.

A similar issue related to the necessity to consider uneven population distribution arises when gridded data are used for aggregated estimation of heat stress [39]. Gridded surfaces of population density are used additionally to resolve it. Temperature values, which are weighted per population numbers, are much more accurate as aggregated heat stress estimates.

In Russian practice, most studies with their focus on temperature-related mortality have been conducted in specific cities; therefore, ground-based observations have been usually used as temperature data [40]. As a rule, information about temperatures beyond Moscow is available only from the station network of the Federal Service for Hydrometeorology and Environmental Monitoring (Rosgidromet); these data have been used by most Russian researchers. Since their analyses have been performed within boundaries of one specific city, they have not faced the issue of data aggregating and averaging. Studies that examine changes in temperature-related mortality at the regional level are rather scarce and they also rely, as a rule, on point data obtained at ground-based stations [28, 29]. Gridded data and reanalyses data have been used to investigate mortality rather rarely so far regardless of their considerable potential for analysis due to an opportunity to ensure full coverage of an analyzed area.

This study aimed to perform regional estimates of temperature-related mortality risks calculated using two data sources, ground-based observations and gridded reanalysis data. We assumed that considerable differences would not be found between these two data sources; however, small deviations would be observed for heat-related risks.

Materials and methods. The study covered the period between 2004 and 2019, which was characterized with a stable descending trend in the national-level mortality in Russia. The analysis included 80 RF regions with continuous time series. Crimea and Sevastopol were excluded from the analysis since data for these regions are available only starting from 2015. In addition, the Khanty-Mansi Autonomous Area and Yamal-Nenets Autonomous Area were included into the Tyumen region; the Nenets Autonomous area, the Arkhangelsk region.

All data on mortality and averaged temperatures in the analyzed regions were aggregated on a weekly basis. Although daily series can reflect impacts of short-term effects more accurately, use of weekly data shows the results, which are comparable per quality and representativeness [41].

The Russian Database on Short-Term Fluctuations in Mortality (RDSTFM) was used as a major source of demographic data. It contains weekly statistical data on mortality per RF regions over 2000–2021[2]. The database uses depersonalized micro-data provided by Rosstat and aggregated per regions on the weekly basis[3]. Weekly age-standardized death rates (ASDR) were taken as a research object using the Revision of the European Standard Population 2013[4]; it helped exclude effects produced by an age-specific structure on estimates of relationships between mortality and temperatures [42]. Data taken from the Russian Database on Birthrates and Mortality were used to estimate average annual population numbers; these data are calculated based on Rosstat data and the database itself was created by the Center for Demography Studies of the Russian School of Economics[5]. Data on population numbers were taken without recalculation considering the results of the 2021 Census.

Mean air temperatures were calculated for each region to estimate risks associated with heat stress; calculations were made for each region per weekly basis using two data sources, ground-based meteorological observations and gridded data of atmospheric reanalysis.

Mean weekly air temperatures were used as point data sources; they were calculated based on data collected at ground-based meteorological stations of the Aisori-M database provided by the Russian Scientific Research Institute for Hydrometeorological Information – Global Data Center[6]. This data array includes prompt meteorological observations from 600 stations that cover the whole territory of Russia and some countries of former Soviet Union. Since continuous data series were not available for all meteorological stations and not all of them were located near the Russian border, overall, 571 stations were included in the analysis. In addition, the Aisori-M database contains a reference book with description of possible changes in the measurement methodology for each station as well as their geographical coordinates, which were employed in further calculations.

Gridded data were obtained from EAC4 (ECMWF Atmospheric Composition Reanalysis 4) conducted by the European Centre for Medium-Range Weather Forecasts (ECMWF) on the global scale [43]. EAC4 Reanalysis has spatial resolution 0.75 per 0.75 degrees and is available at the Copernicus Atmosphere Data

---

[2] Rossiiskaya baza dannykh kratkosrochnykh kolebanii smertnosti [The Russian Database on Short-Term Fluctuations in Mortality (RDSTFM)]. *International Laboratory for Population and Health Studies, SRI HSE*. Available at: https://demogr.hse.ru/russtmf (April 17, 2025) (in Russian).

[3] Excluding weeks 9–13 in 2012 in the Pskov region.

[4] Eurostat. Revision of the European Standard Population. Report of Eurostat's task force: 2013 edition. *Publications Office of the European Union*. DOI: 10.2785/11470

[5] Tsentr demograficheskikh issledovanii [Center for Demography Studies]. *Russian School of Economics (RSE)*. Available at: https://www.nes.ru/demogr/ (April 17, 2025) (in Russian).

[6] Aisori-M: Spetsializirovannye massivy dlya klimaticheskikh issledovanii [Aisori-M: Specialized data arrays for climatic research]. Available at: http://aisori-m.meteo.ru (April 14, 2025) (in Russian).

Store[7]. The reanalysis data have been published since 2003 and are still renewed twice a year with a several months lag. Moreover, EAC4 has data on some other meteorological parameters as well as levels of various chemicals in ambient air. Initial data time resolution is 3 hours but they were aggregated per weeks within our analysis.

To allow for uneven population distribution within the analyzed regions, gridded data were taken from the Gridded Population of the World, Version 4 (GPWv4), one of the most commonly used data source on population numbers for making regional estimates of various square indicators. This data array is provided by the NASA Socioeconomic Data and Applications Center (SEDAC)[8] and has spatial resolution 0.5 per 0.5 degrees[9]. GPWv4 is based on the official demographic statistics; in particular, the municipal level is used in the analysis for Russia. Additionally, GPWv4 allows for peculiarities of the Earth surface; due to it, population density is more consistent with actual population distribution. The GPWv4 data set for 2010 was selected as a data source as reflecting population density in the middle of the analyzed period.

Three approaches were used to estimate mean weekly temperatures aggregated per regions; two of them were based on data collected at ground-base stations. For the first method, the population center was determined in each region based on population density taken from the GPWv4. Next, weights were assigned to all meteorological stations located within a given region and within a 200-km (in accordance with [29]) buffer area around it. These weights were inversely proportionate to distances between them and the regional population center. Mean weekly temperatures were calculated as a weighted sum of temperatures per all selected meteorological stations.

In addition, mean weekly temperatures were calculated per regions using an alternative way for additional verification of the estimates. It involved estimating weekly temperatures at the municipal level. Similarly, a population center was established for each municipality, meteorological stations were selected within a relevant buffer area, and weights were assigned in conformity with the distance between them and the municipality center. After that, a mean regional temperature was calculated as a weighted mean temperature per municipalities where weights were established based on a population number in each territorial unit.

Images from the EAC4 reanalysis averaged over weeks were used to calculate mean temperatures based on gridded data. These data were reduced to the spatial resolution of the population density grid to provide mutual compatibility. Therefore, mean temperature values were calculated for each region as mean temperature values in relevant grid cells weighted per the number of people living within each cell.

To estimate the relationship between mortality risks and temperatures, the two-level model with quasi-Poisson regression was used for each region at the first level and meta-analysis of regional estimates to obtain aggregated results at the second level. In particular, at the first stage, the dose-response curve was built for each region using the Distributed Lag Model (DLM) [44] to describe influence of temperatures on mortality considering remote temperature-related effects. Next, these regional estimates were aggregated to calculate this relationship for Russia as a whole and to subsequently adjust the regional results per these figures.

In this context, the dose-response curve reflected estimates of Relative Risks (RR) of mortality for all observed temperatures. Since it has a non-linear shape, as a rule, it is given as non-parametric functions. The Minimum Mortality Temperature (MMT) was taken as

---

[7] Atmosphere Data Store. Available at: https://ads.atmosphere.copernicus.eu (April 14, 2025).

[8] Gridded Population of the World (GPW), v4. *Socioeconomic Data and Applications Center (SEDAC)*. Available at: https://beta.sedac.ciesin.columbia.edu/data/set/gpw-v4-population-density-rev10 (April 17, 2025).

[9] Gridded Population of the World, Version 4 (GPWv4): Population Density Adjusted to Match 2015 Revision UN WPP Country Totals, Revision 11. *Center for International Earth Science Information Network, Columbia University (CIESIN)*, 2018.

the reference level, against which all temperature-related risks were calculated. Therefore, the minimum level of relative mortality risk was equal to 1 [45]. This methodology is described in greater detail in [46]. Time series were analyzed using quasi-Poisson regression; its formula can be written as:

$$\log\left(E\left(Y_{week}\right)\right) =$$
$$= intercept + ns\left(week,\ df\ = 7\ per\ year\right) +$$
$$+ cb\left(ns\left(T,\ \ knots = 3\right), lag = 0,1,2,3\right) +$$
$$+ offset\left(\log\left(Pop\right)\right),$$

where $E\left(Y_{week}\right)$ is expected value of weekly ASDR in the region;

$intercept$ is the free equation member reflecting mean ASDR in the region;

$\mathrm{ns}\left(week,\ df\ = 7\ per\ year\right)$ is the natural cubic spline with 7 degrees of freedom for each observation year included in the analysis to allow for seasonality and long-term trends in mortality.

The model $cb(ns\left(T,\ \ knots = 3\right),$ $lag = 0,1,2,3)$ is a distributed lag model (DLM), which is used for considering influence of temperatures on mortality risks allowing for remote effects.

The dose-response curve, which reflects the association between mortality risks and temperatures, was built using the natural cubic spline with three knots located evenly at the 25^{th}, 50^{th} and 75^{th} percentiles of temperature distribution in each region. This approach makes it possible to most accurately describe non-linear relationships between temperature and mortality and is common in similar research. Categorical variables were employed to take the lag structure into account. Therefore, influence exerted by temperatures on mortality was estimated not only at the current moment but also considering their effects over three previous weeks.

The parameter $offset\left(\log\left(Pop\right)\right)$ is used in the library *glm* of the machine language R when mortality ratios are employed as a dependent variable since both Poisson and quasi-Poisson regression requires enumerable data analysis. Therefore, the logarithm of the mean population number over the respective period was introduced as an additional model parameter to make mortality estimates in different regions compatible with each other.

The most optimal spline parameters as well as length and structure of lags were based on minimization of the Akaike information criterion (AIC) within modeling the dose-response curves.

A meta-analysis was performed to investigate regional dose-response relationship with its aim to obtain aggregated assessments of temperature-related risks [47, 48]. This approach makes it possible to combine results obtained for different regions allowing for variability between them due to using a meta-regression model with random effects.

The aggregated relationship between mortality and temperatures was calculated for the whole country on the basis of regional assessments; it was then used to adjust the latter. This involves using Best Linear Unbiased Predictors (BLUP), which consist of two components. Primarily, they include parameters of dose-response curves obtained for each region at the first level of the analysis. Adjusting these assessments per their deviation from aggregated results can raise accuracy of regional indicators. This BLUP component is random effects that have already been obtained by using meta-regression [47]. Regional variations of the MMT BLUP assessments were shown to present regional differences in the relationship between mortality and temperatures; these variations were calculated based on each of three methods for temperature data aggregation.

All data were statistically analyzed in the RStudio integrated development environment. In particular, such libraries as *glm, splines, dlnm, mvmeta* and *mixmeta* were used for building aggregated dose-response curves for analysis [44].

Results and discussion. On average**,** weekly ASDRs taken over the whole analyzed period (2004–2019) turned out to be the highest in Chukotka (28.6 ‰), Tyva (26.5 ‰), the

Jewish Autonomous Area (24.6 ‰), and the Amur region (24.1 ‰). The lowest values were observed in Ingushetia (11.6 ‰), Moscow (13.4 ‰), Dagestan (14.4 ‰), and Saint Petersburg (15.7 ‰). Mortality was declining quite rapidly in Russia over the whole analyzed period; in 2005, the mean weekly ASDR equaled 25.2 ‰ per all regions but it went down to 16.2 ‰ in 2019. It is noteworthy that these assessments are based on mean weekly mortality ratios; therefore, they can be rather different from annual mortality rates reported in other sources.

Over the analyzed period, an all-time low mean weekly temperature was detected in late December 2024 in Yakutia. It was equal to -43.15 °C as recorded at ground-based observation stations, -45.01 °C when using weighted mean temperatures in municipalities, and -44.66 °C according to gridded reanalysis data. Mean weekly temperatures reached their peak in the Volgograd region during a heat wave in August 2010 when the peak mortality was detected there; they equaled 31.53 °C, 32.18 °C and 32.34 °C for the same data sources respectively. All-time temperatures were registered both for extreme heat and extreme cold in the same period regardless of which data source was used; quantitative estimates also turned out to be quite similar.

The total number of weekly observations amounted to 66,640 in all regions over 2004–2019. Mean temperature values for regions, which were obtained using different methods, showed high consistency (Table 1). The correlation coefficient between temperature time series was 0.991 when calculated using ground-based observation data. The correlation coefficient between reanalysis data and aggregated regional data was 0.969 and 0.978 between reanalysis data and weighted mean temperatures per municipalities.

At the regional level, mean weekly temperatures established by using three different methods showed not only a close linear connection with each other but also absence of any shifts associated with systemic errors in most regions (Figures 1 and 2). However, estimates for temperatures above zero in general tended to be more consistent. Still, there were some exceptions, for example, Chukotka where data from meteorological stations obtained by using different aggregation methods were not consistent. Regional and municipal population centers in Chukotka do not allow obtaining a comparable picture and due to it estimates based on using different methods are not consistent with each other. Similar, although less apparent, differences were found in some other regions with low population density and uneven population distribution, in particular, in the Tyumen region, Krasnoyarskii Krai and the Altai Republic.

Table 1

Descriptive statistics of time series of weekly data over 2004–2019 per 80 regions used at the first level of analysis for three models, each of which relies on temperatures calculated by using diriment methods

Indicator	1st quartile	Median	Mean	3rd quartile
Dependent variable:				
Weekly ASDR, per 1 thousand people	17.20	19.50	20.08	22.40
Independent variables:				
Mean weekly temperatures detected at ground-based stations per municipalities, °C	-3.509	5.600	4.728	15.067
Mean weekly temperatures detected at ground-based stations per regions, °C	-3.468	5.586	4.727	15.064
Mean weekly temperatures based on reanalysis (EAC4), °C	-3.625	5.369	4.499	14.767

Note: results of the author's calculations using RDSTFM, Aisori-M, and EAC4 data

Figure 1. The graph to show spreads of mean weekly temperature estimates over 2004–2019 obtained by aggregating point data allowing for population centers in regions and EAC4 reanalysis data weighted per population density (RF regions are given with different colors; estimates for Chukotka are red; based on the author's calculations using Aisori-M, EAC4)

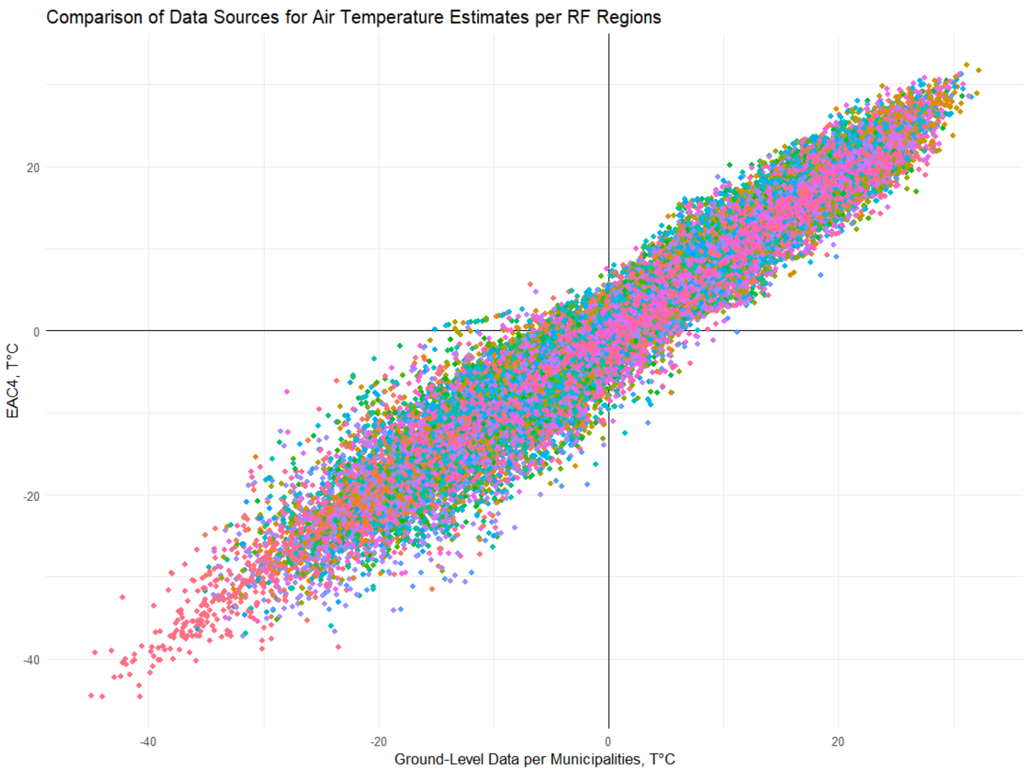

Figure 2. The graph to show spreads of mean weekly temperature estimates over 2004–2019 obtained by aggregating point data allowing for population centers in municipalities and EAC4 reanalysis data weighted per population density (RF regions are given with different colors; based on the author's calculations using Aisori-M, EAC4)

The dose-response curves that show the relationship between mortality and temperature were obtained for the whole country by using meta-regression; they have similar shape, which corresponds to the most common relationship between mortality and temperatures. Both cold and heat cause a rise in mortality risks; for most RF regions, MMT is within 15–20 °C, which is consistent with conclusions made in most other studies [3]. However, risks related to extreme cold turned out to be more statistically significant.

This is not consistent with the results reported in many studies where the highest risks are considered to be associated with heat [11] and might be due to two factors. First, a considerably long lag (3 weeks) was used in this study, which makes it possible to take remote cold-related effects into account more effectively. Heat-related risks usually turn out to be much higher when associations between temperatures and mortality are considered within a week and without allowing for lags. However, such a model based on statistical indicators tends to have weaker predictive ability. Secondly, our results are consistent with conclusions made in other Russian epidemiological studies where mortality risks are often higher during cold waves than heat ones[10] [22]. In any case, this estimate is only an averaged picture based on aggregated data from different regions with considerably different conditions.

The aggregated estimated minimum mortality temperature was found to be equal to 19.12 °C when using ground-based observation data aggregated per regions. The MMT was 19.34 °C for mean weighted temperatures per municipalities and 17.17 °C for reanalysis data. In terms of long-term temperature distribution, the MMT corresponded to the 88.4th, 88.8th and 83.0th percentile respectively. These relatively high percentile values can be explained by climatic peculiarities of Russia where winter season is very long in most regions; this is not observed in other regions where similar research has been accomplished [45, 49].

The shapes of the dose-response curves built relying on ground-based observations were very similar to each other for the whole range of the observed temperatures. Risks related to extreme temperatures turned out to be slightly higher for data based on regional estimates; the difference was insignificant though. More substantial inconsistencies were found between the results based on gridded data and aggregated ground-based observations. They were particularly substantial for extremely high temperatures (Figure 3).

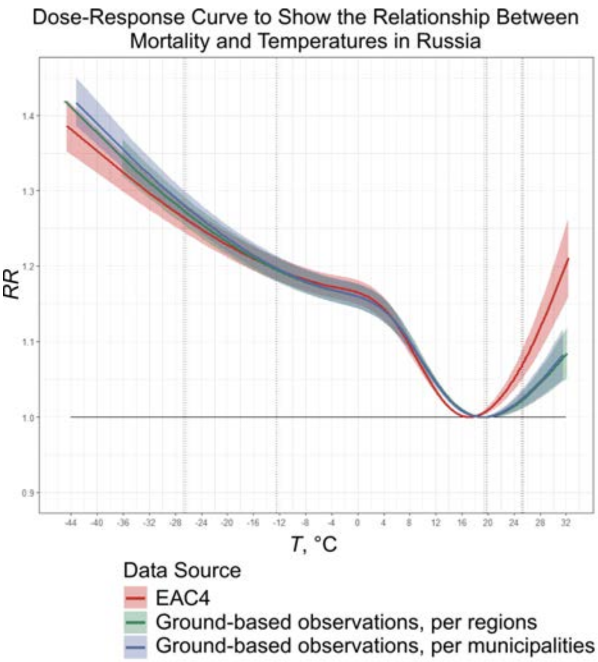

Figure 3. Assessments of temperature-related mortality risks obtained by using different methods: the dotted line show 1-th, 10-th, 90-th and 99th percentiles of temperature distribution in all Russian regions over the analyzed period (2004–2019) (based on the author's calculations using RDSTFM, Aisori-M, and EAC4)

Point estimates of relative risks at certain boundary values, for example, 95th or 99th percentiles of long-term temperature distribution for heat-related effects, can be used as indicators of mortality risks associated with extreme temperatures [50]. Thus, relative risks of deaths due to extreme cold based on temperature reanalysis data turned out to be lower than those established using ground-based observations.

[10] Donaldson G.C., Tchernjavskii V.E., Ermakov S.P., Bucher K., Keatinge W.R. Winter mortality and cold stress in Yekaterinburg, Russia: interview survey. *BMJ*, 1998, vol. 316, no. 7130, pp. 514–518. DOI: 10.1136/bmj.316.7130.514

Differences in risks for the upper percentiles of long-term mean weekly temperature distribution turned out to be much more significant. Relative mortality risks were equal to approximately 1.02 for the 99th percentile based on meteorological observations in the distributed lag model whereas they were 1.07 for gridded data and the difference turned out to be significant (Table 2). It should be noted that temperatures corresponding to these percentiles did not differ very much.

On average, BLUP values of MMT estimates per regions that were calculated using ground-based observations turned out to be 2–3 °C degrees higher than estimates based on using gridded data. In most cases, calculations based on using different methods yielded consistent results. Within federal districts, regions located further to the south with higher temperatures, as a rule, tended to have higher MMT values (the Belgorod Region in the Central Federal District, Kalmyk Republic in the Southern Federal District, the Saratov region in the Volga Federal District). However, it was not the case everywhere; for example, the Komi Republic in the North-Western Federal District was an exception.

We did not find any interrelations between ASDR and MMT levels. Although regions with higher mortality rates are, as a rule, located in colder climate, this did not have any influence on the ultimate regional differentiation. MMT corresponded to 75–85th percentiles of long-term temperature distribution in most regions when reanalysis data were used to estimate it. MMT established by using meteorological data was, as a rule, between the 80th and 90th percentiles. No values below the 70th percentile were detected whereas they were close to the 99th percentile in some regions (Kamchatka, Sakhalin, Komi, and Tyva), which is due to both cold climate and insufficient validity of BLUP estimates given small population numbers in them.

The greatest discrepancies between MMT estimates obtained by using different methods were found in some sparsely populated regions with low density of meteorological station coverage, for example, in Chukotka, where their representativeness turned out to be lower for aggregated regional temperature estimates. Meteorological networks have greater coverage in the Central, Volga and Southern Federal Districts and physical-geographic characteristics of regions in them do not differ greatly; given that, BLUP estimates of MMT turned out to be the most consistent. However, some specific regions, for example, Adygei, the Ivanovo region, the Arkhangelsk region, Primor'e, Yakutiya and Saint Petersburg showed abnormal spreads in MMT estimates between all three of them. This can be explained by data aggregation artifacts and failure to allow for local climatic peculiarities (Figure 4).

Table 1

Relative risks related to extreme temperatures, calculated using different methods for the 1st, 5th, 95th and 99th percentiles of temperature distribution in all Russian regions over the analyzed period (2004–2019)

Data aggregation method		1st percentile to MMT	5th percentile to MMT	95th percentile to MMT	99th percentile to MMT
Ground-based observations per municipalities	T, °C	-26.39	-17.65	21.8	25.43
	RR	1.272 (95 % CI: 1.253–1.291)	1.219 (95 % CI: 1.203–1.236)	1.004 (95 % CI: 1.000–1.008)	1.023 (95 % CI: 1.012–1.035)
Ground-based observations per regions	T, °C	-26.77	-17.63	21.74	25.31
	RR	1.282 (95 % CI: 1.262–1.302)	1.223 (95 % CI: 1.206–1.24)	1.005 (95 % CI: 1.001–1.009)	1.025 (95 % CI: 1.012–1.038)
Based on reanalysis (EAC4)	T, °C	-26.62	-17.89	21.41	25.05
	RR	1.264 (95 % CI: 1.245–1.283)	1.218 (95 % CI: 1.202–1.235)	1.021 (95 % CI: 1.013–1.028)	1.066 (95 % CI: 1.048–1.085)

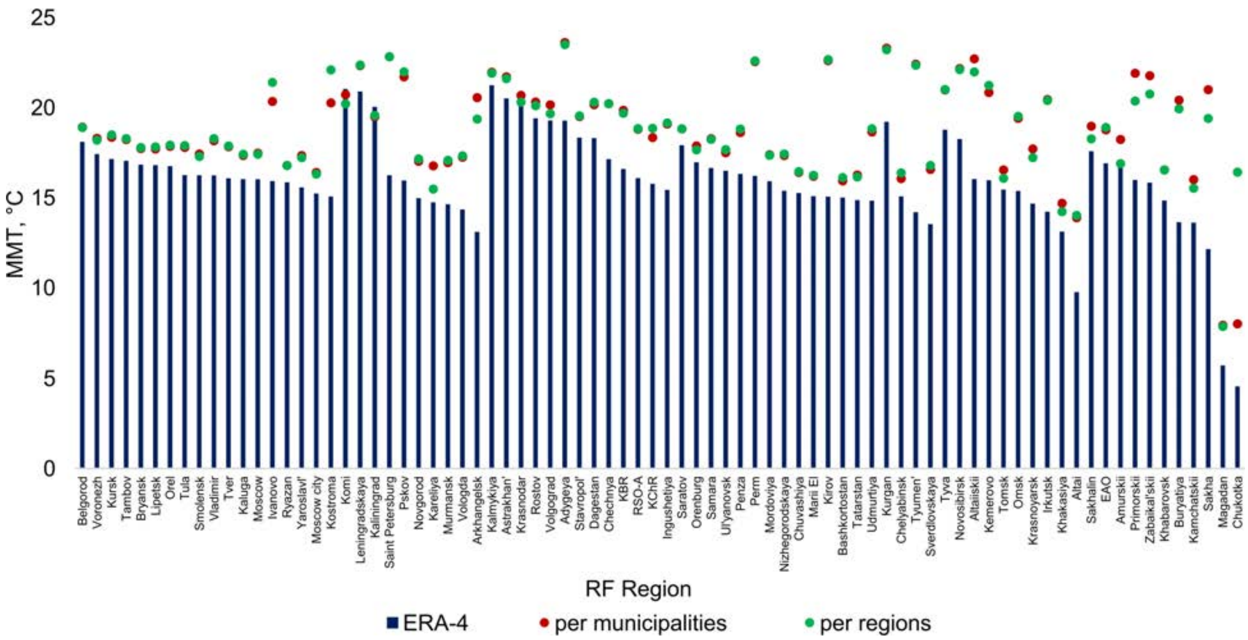

Figure 4. BLUP estimates of MMT for temperatures calculated using different methods, per RF regions over 2004–2019 (based on the author's calculations using RDSTFM, Aisori-M, and EAC4)

The resulting estimates of the relationship between mortality and temperatures have the J-like shape where cold-related risks prevail. These data allow calculating the temperature stress burden and predict population losses due to climate change as well as to develop regional measures for prevention and prophylaxis of negative outcomes caused by temperature exposures.

Experience gained by Russian researchers does not allow unambiguously identify what risks (cold- or heat-related ones) are more significant since conclusions depend on geographical coverage and a methodology [26]. Prevalence of cold-related risks in this study might be due to using a model with distributed lags, which considers temperature effects over three weeks. This method is eligible for analyzing annual dynamics but tends to underestimate mortality caused by heat waves [41]. Moreover, weekly data do not consider short-term effects produced by high temperatures, which are usually manifested several days after an exposure [44]. This might also make for underestimation of mortality related to high temperatures. It is also important to remember that all-cause mortality was analyzed in this study, including external causes that held a significant place in the beginning of the analyzed period. Clear relationships with temperatures have not been established for many external causes of death, which makes it difficult to identify them on the example of the employed data.

We did not find a significant relationship between initial ASDR values and temperature risks. A considerable proportion of deaths occur due to external causes and alcohol poisoning in regions with high mortality rates; these death causes have a very weak relationship with temperatures. Therefore, the overall mortality rate did not have any substantial influence on the relationship between temperature and mortality.

Regional differences in MMT reflect regional natural and climatic peculiarities. MMT tends to be higher in warmer regions, which is explained by people getting adapted to prevailing conditions [49]. MMT values similar to those established in most Russian regions (15–18 °C) were also observed in cities in Finland, Sweden, and Norway [45]. The best estimates are those based on reanalysis in accordance with percentiles of temperature distribution that correspond to MMT. As a rule, percentiles with minimum mortality risks are also between the 75th and 85th percentiles in similar climatic conditions [49]. A decline in

MMT at higher latitudes and lower mean annual temperatures reflects differences in adaptation potential of the Russian population.

Abnormally low MMT values were found in some regions, for example, in the Magadan region or Chukotka; this might be associated with high spreads in the data collected for these regions and low validity of the model. On the contrary, MMT turned out to be overestimated in Kamchatka, Komi, and the Zabaikal'skii Krai, where they were close to the maximum percentiles. In case when meteorological data are used, this can be related to low quality of initial data and peculiarities of BLUP-estimates.

In this study, assessments of temperature-related mortality risks obtained by various methods at the regional level turned out to be similar, which is consistent with the results reported in other studies [15, 30, 50–53]. Moreover, even a quite large aggregation level, both as regards time (per weeks) and space (per RF regions), in general, did not influence comparability of the obtained assessments.

Uneven distribution of meteorological stations was another difficulty. Most such stations were located in large settlements, which made them less representative for sparsely populated areas. The study [54] recommends considering stations located with the 50-km radius from the regional center to calculate temperatures. In [29] and in this study, a 200-km distance was used, which allowed increasing the sample size and make estimates more stable.

The dose-response curves based on various data sources were similar. However, heat-related risks turned out to be higher when reanalysis data were used. Other studies also reported the greatest discrepancies for high temperatures whereas cold-related risks remained comparable. In some studies, gridded data also overestimated heat-related risks [30], but an opposite trend was observed in some other cases [15, 52, 54].

Atmospheric reanalyses based on modeling do not always allow for local micro-geographical effects and extreme temperatures due to data averaging. However, meteorological observations are not representative either for such large areas due to uneven coverage.

The smallest discrepancies between data sources were found in regions with even population distribution and flat terrains. In other cases, certain problems occurred. The estimates were the most inconsistent in Chukotka; excessive number of meteorological stations that reflected local conditions rather poorly was used in Adygei; the reanalysis data were distorted in Saint Petersburg because the city is located so close to the sea [51].

Therefore, estimates of temperatures and related risks based on different data sources are consistent in the simplest cases only. Reanalyses are preferable in regions where a meteorological network has low density whereas meteorological data are more eligible for analysis within cities. Meteorological data are available in real time, which is very convenient for operative estimates; however, they can have some gaps or be fragmentary. Reanalysis data are more eligible for long-term series due to their wide coverage and completeness provided that reanalyses have been created following the same methodology.

When comparing approaches to aggregation of meteorological data, we found temperature estimates per municipalities to be more effective than those made per regions. The population center poorly reflects actual population density in large and sparsely populated areas thereby reducing data accuracy. Therefore, a more correct solution would be to estimate mean temperatures at a sub-regional level with subsequent aggregation of the results for a region as a whole.

Problems and limitations. In this study, temperature-related risks were estimated at the regional level in Russia and various sources of temperature data were compared as regards their eligibility. The study findings are consistent with conclusions made by other researchers, who used more detailed data and analyzed short-term fluctuations in mortality. However, several limitations should be considered since they may have influenced interpretation of the obtained results.

Firstly, despite overall stability of time series of mortality in most regions, the data were found to be highly volatile in some of them (for example, Chukotka and the Magadan region). This made it difficult to obtain authentic results, although use of meta-analysis was a partial solution to the problem. Nevertheless, we were not able to obtain adequate estimates of temperature-related mortality risks for some regions.

Secondly, specification of the dose-response function may have influenced the modeling results. Although we used the best values of information criteria in the selected model, location of spline knots and lag structure might have distorted the ultimate estimates.

Thirdly, the analysis was limited by absent data on ambient air pollution. In Russia, such data are available at the regional level only within reanalyses since the country does not have an extensive network for observation of ambient air quality with open and accessible data. Therefore, we were not able to include such data in our analysis.

Conclusions. The aim of this study was to analyze temperature-related mortality using data of atmospheric reanalysis EAC4 and meteorological observations. Regional temperatures were calculated using three different methods, which showed similar patterns of time series.

The relationship between mortality and temperatures in Russian regions had a J-like shape: risks were higher at low temperatures. Thus, relative risks reached 1.25 for the 1st percentile whereas they were 1.02 (according to meteorological data) and 1.07 (according to reanalysis) for the 99th percentile. This is due to considering lag effects on health produced by temperature exposures over several weeks, which makes it possible to allow for negative outcomes of cold exposure more effectively.

In most regions, MMT was within 15–20 °C corresponding to 75–85th percentiles of temperature distribution (per reanalysis data). Minimum mortality temperatures turned out to be several degrees higher when meteorological data were used. Optimal temperatures are often higher in warm regions and this indicates people's adaptation to prevailing conditions. Knowledge on threshold risk levels allows more effective assessment of threat for people's lives and health and helps develop relevant measures for preventing negative outcomes of temperature exposures. Such measures include systems for notifying, informing and warning people about a coming heat or cold wave.

Both data sources were found to be applicable for the task; however, meteorological data tended to become less authentic in sparsely populated regions with low density of meteorological observation networks and this influenced consistency of risk assessments. For example, the minimum mortality temperature tended to be overestimated per meteorological data relative to reanalysis data.

Funding. The research was not granted any sponsor support.

Competing interests. The author declares no competing interests.

References

1. Meehl G.A., Tebaldi C. More Intense, More Frequent, and Longer Lasting Heat Waves in the 21st Century. *Science*, 2004, vol. 305, no. 5686, pp. 994–997. DOI: 10.1126/science.1098704

2. Gasparrini A., Guo Y., Sera F., Vicedo-Cabrera A.M., Huber V., Tong S., de Sousa Zanotti Stagliorio Coelho M., Hilario Nascimento Saldiva P. [et al.]. Projections of temperature-related excess mortality under climate change scenarios. *Lancet Planet. Health*, 2017, vol. 1, no. 9, pp. e360–e367. DOI: 10.1016/S2542-5196(17)30156-0

3. Masselot P., Mistry M.N., Rao S., Huber V., Monteiro A., Samoli E., Stafoggia M., de'Donato F. [et al.]. Estimating future heat-related and cold-related mortality under climate change, demographic and adaptation scenarios in 854 European cities. *Nat. Med.*, 2025, vol. 31, no. 4, pp. 1294–1302. DOI: 10.1038/s41591-024-03452-2

4. Basu R. High ambient temperature and mortality: a review of epidemiologic studies from 2001 to 2008. *Environ. Health*, 2009, vol. 8, no. 1, pp. 40. DOI: 10.1186/1476-069X-8-40

5. Kovats R.S., Hajat S. Heat Stress and Public Health: A Critical Review. *Annu. Rev. Public Health*, 2008, vol. 29, pp. 41–55. DOI: 10.1146/annurev.publhealth.29.020907.090843

6. Cheng J., Xu Z., Bambrick H., Prescott V., Wang N., Zhang Y., Su H., Tong S., Hu W. Cardiorespiratory effects of heatwaves: A systematic review and meta-analysis of global epidemiological evidence. *Environ. Res.*, 2019, vol. 177, pp. 108610. DOI: 10.1016/j.envres.2019.108610

7. Revich B., Shaposhnikov D. The influence of heat and cold waves on mortality in Russian subarctic cities with varying climates. *Int. J. Biometeorol.*, 2022, vol. 66, no. 12, pp. 2501–2515. DOI: 10.1007/s00484-022-02375-2

8. Revich B.A., Shaposhnikov D.A. Climate change, heat waves, and cold spells as risk factors for increased mortality in some regions of Russia. *Studies on Russian economic development*, 2012, vol. 23, no. 2, pp. 195–207. DOI: 10.1134/S1075700712020116

9. Shaposhnikov D., Revich B., Bellander T., Bero Bedada G., Bottai M., Kharkova T., Kvasha E., Lezina E. [et al.]. Mortality Related to Air Pollution with the Moscow Heat Wave and Wildfire of 2010. *Epidemiology*, 2014, vol. 25, no. 3, pp. 359–364. DOI: 10.1097/EDE.0000000000000090

10. Arbuthnott K., Hajat S., Heaviside C., Vardoulakis S. What is cold-related mortality? A multi-disciplinary perspective to inform climate change impact assessments. *Environ. Int.*, 2018, vol. 121, pt 1, pp. 119–129. DOI: 10.1016/j.envint.2018.08.053

11. Gasparrini A., Guo Y., Hashizume M., Lavigne E., Zanobetti A., Schwartz J., Tobias A., Tong S. [et al.]. Mortality risk attributable to high and low ambient temperature: a multicountry observational study. *Lancet*, 2015, vol. 386, no. 9991, pp. 369–375. DOI: 10.1016/S0140-6736(14)62114-0

12. Barnett A.G., Tong S., Clements A.C.A. What measure of temperature is the best predictor of mortality? *Environ. Res.*, 2010, vol. 110, no. 6, pp. 604–611. DOI: 10.1016/j.envres.2010.05.006

13. Vaneckova P., Neville G., Tippett V., Aitken P., Fitzgerald G., Tong S. Do Biometeorological Indices Improve Modeling Outcomes of Heat-Related Mortality? *Journal of Applied Meteorology and Climatology*, 2011, vol. 50, no. 6, pp. 1165–1176. DOI: 10.1175/2011JAMC2632.1

14. Shartova N.V., Shaposhnikov D.A., Konstantinov P.I., Revich B.A. Universal thermal climate index (UTCI) applied to determine thresholds for temperature-related mortality. *Health Risk Analysis*, 2019, no. 3, pp. 83–93. DOI: 10.21668/health.risk/2019.3.10.eng

15. Urban A., Di Napoli C., Cloke H.L., Kyselý J., Pappenberger F., Sera F., Schneider R., Vicedo-Cabrera A.M. [et al.]. Evaluation of the ERA5 reanalysis-based Universal Thermal Climate Index on mortality data in Europe. *Environ. Res.*, 2021, vol. 198, pp. 111227. DOI: 10.1016/j.envres.2021.111227

16. Revich B., Shaposhnikov D. Temperature-induced excess mortality in Moscow, Russia. *Int. J. Biometeorol.*, 2008, vol. 52, no. 5, pp. 367–374. DOI: 10.1007/s00484-007-0131-6

17. Revich B.A., Shaposhnikov D.A., Galkin V.T., Krylov S.A., Chertkova A.B. Impact of high ambient air temperatures on human health in Tver. *Gigiena i sanitariya*, 2005, no. 2, pp. 20–24 (in Russian).

18. Revitch B.A., Shaposhnikov D.A., Semoutnikova E.G. Climate conditions and ambient air quality as risk factors for mortality in Moscow. *Meditsina truda i promyshlennaya ekologiya*, 2008, no. 7, pp. 29–35 (in Russian).

19. Revich B.A., Shaposhnikov D.A., Podol'naya M.A., Khor'kova T.L., Kvasha E.A. Heat waves in southern cities of European Russia as a risk factor for premature mortality. *Stud. Russ. Econ. Dev.*, 2015, vol. 26, pp. 142–150. DOI: 10.1134/S1075700715020100

20. Revich B.A., Shaposhnikov D.A. Cold waves in southern cities of European Russia and premature mortality. *Stud. Russ. Econ. Dev.*, 2016, vol. 27, no. 2, pp. 210–215. DOI: 10.1134/S107570071602012X

21. Shartova N.V., Shaposhnikov D.A., Konstantinov P.I., Revich B.A. Air temperature and mortality: heat thresholds and population vulnerability study in Rostov-on-Don. *Fundamental'naya i prikladnaya klimatologiya*, 2019, vol. 2, pp. 66–94. DOI: 10.21513/2410-8758-2019-2-66-94 (in Russian).

22. Revich B.A., Shaposhnikov D.A., Anisimov O.A., Belolutskaia M.A. Heat waves and cold spells in three arctic and subarctic cities as mortality risk factors. *Gigiena i sanitariya*, 2018, vol. 97, no. 9, pp. 791–798. DOI: 10.18821/0016-9900-2018-97-9-791-798 (in Russian).

23. Revich B.A., Shaposhnikov D.A., Anisimov O.A., Belolutskaya M.A. Impact of Temperature Waves on the Health of Residents in Cities of the Northwestern Region of Russia. *Stud. Russ. Econ. Dev.*, 2019, vol. 30, no. 3, pp. 327–333. DOI: 10.1134/S1075700719030158

24. Chernykh D.A., Taseiko O.V. Assessment of the risk mortality from thermal waves in Krasnoyarsk city. *Ekologiya cheloveka*, 2018, no. 2, pp. 3–8. DOI: 10.33396/1728-0869-2018-2-3-8 (in Russian).

25. Grigorieva E.A. Heat waves at the southern part of the Far East and human health. *ZNiSO*, 2017, no. 2 (287), pp. 11–14. DOI: 10.35627/2219-5238/2017-287-2-11-14 (in Russian).

26. Grigorieva E.A. Heat and cold waves at the South of the Russian Far East in 1999–2017. *IOP Conf. Ser.: Earth Environ. Sci.*, 2020, vol. 606, no. 1, pp. 012016. DOI: 10.1088/1755-1315/606/1/012016

27. Shaposhnikov D., Revich B. Toward meta-analysis of impacts of heat and cold waves on mortality in Russian North. *Urban Climate*, 2016, vol. 15, pp. 16–24. DOI: 10.1016/j.uclim.2015.11.007

28. Otrachshenko V., Popova O., Solomin P. Health Consequences of the Russian Weather. *Ecological Economics*, 2017, vol. 132, pp. 290–306. DOI: 10.1016/j.ecolecon.2016.10.021

29. Otrachshenko V., Popova O., Solomin P. Misfortunes never come singly: Consecutive weather shocks and mortality in Russia. *Econ. Hum. Biol.*, 2018, vol. 31, pp. 249–258. DOI: 10.1016/j.ehb.2018.08.008

30. Weinberger K.R., Spangler K.R., Zanobetti A., Schwartz J.D., Wellenius G.A. Comparison of temperature-mortality associations estimated with different exposure metrics. *Environ. Epidemiol.*, 2019, vol. 3, no. 5, pp. e072. DOI: 10.1097/EE9.0000000000000072

31. Clemens K.K., Ouédraogo A.M., Li L., Voogt J.A., Gilliland J., Scott Krayenhoff E., Leroyer S., Shariff S.Z. Evaluating the association between extreme heat and mortality in urban Southwestern Ontario using different temperature data sources. *Sci. Rep.*, 2021, vol. 11, no. 1, pp. 8153. DOI: 10.1038/s41598-021-87203-0

32. Daly C. Guidelines for assessing the suitability of spatial climate data sets. *Int. J. Clim.*, 2006, vol. 26, no. 6, pp. 707–721. DOI: 10.1002/joc.1322

33. Spangler K.R., Weinberger K.R., Wellenius G.A. Suitability of gridded climate datasets for use in environmental epidemiology. *J. Expo. Sci. Environ. Epidemiol.*, 2019, vol. 29, no. 6, pp. 777–789. DOI: 10.1038/s41370-018-0105-2

34. Donat M.G., Sillmann J., Wild S., Alexander L.V., Lippmann T., Zwiers F.W. Consistency of Temperature and Precipitation Extremes across Various Global Gridded In Situ and Reanalysis Datasets. *J. Climate*, 2014, vol. 27, no. 13, pp. 5019–5035. DOI: 10.1175/JCLI-D-13-00405.1

35. Mistry M.N., Schneider R., Masselot P., Royé D., Armstrong B., Kyselý J., Orru H., Sera F. [et al.]. Comparison of weather station and climate reanalysis data for modelling temperature-related mortality. *Sci. Rep.*, 2022, vol. 12, no. 1, pp. 5178. DOI: 10.1038/s41598-022-09049-4

36. Wu Y., Xu J., Liu Z., Han B., Yang W., Bai Z. Comparison of Population-Weighted Exposure Estimates of Air Pollutants Based on Multiple Geostatistical Models in Beijing, China. *Toxics*, 2024, vol. 12, no. 3, pp. 197. DOI: 10.3390/toxics12030197

37. Keller J.P., Peng R.D. Error in estimating area-level air pollution exposures for epidemiology. *Environmetrics*, 2019, vol. 30, no. 8, pp. e2573. DOI: 10.1002/env.2573

38. Jalaludin B., Morgan G., Lincoln D., Sheppeard V., Simpson R., Corbett S. Associations between ambient air pollution and daily emergency department attendances for cardiovascular disease in the elderly (65+ years), Sydney, Australia. *J. Expo. Sci. Environ. Epidemiol.*, 2006, vol. 16, no. 3, pp. 225–237. DOI: 10.1038/sj.jea.7500451

39. De Schrijver E., Folly C.L., Schneider R., Royé D., Franco O.H., Gasparrini A., Vicedo-Cabrera A.M. A Comparative Analysis of the Temperature-Mortality Risks Using Different Weather Datasets Across Heterogeneous Regions. *GeoHealth*, 2021, vol. 5, no. 5, pp. e2020GH000363. DOI: 10.1029/2020GH000363

40. Grigorieva E.A., Revich B.A. Health Risks to the Russian Population from Temperature Extremes at the Beginning of the XXI Century. *Atmosphere*, 2021, vol. 12, no. 10, pp. 1331. DOI: 10.3390/atmos12101331

41. Ballester J., van Daalen K.R., Chen Z.-Y., Achebak H., Antó J.M., Basagaña X., Robine J.-M., Herrmann F.R. [et al.]. The effect of temporal data aggregation to assess the impact of changing temperatures in Europe: an epidemiological modelling study. *Lancet Reg. Health Eur.*, 2023, vol. 36, pp. 100779. DOI: 10.1016/j.lanepe.2023.100779

42. Shchur A.E., Timonin S.A., Churilova E.V., Sergeev E.V., Sokolova V.V., Rodina O.A., Shamsutdinov B.A., Jdanov D.A., Shkolnikov V.M. Russian Short-Term Mortality Fluctuations Data Series. *Population and Economics*, 2023, vol. 7, no. 3, pp. 188–197. DOI: 10.3897/popecon.7.e114628

43. Inness A., Engelen R., Flemming J. The new CAMS global reanalysis of atmospheric composition: Newsletter. *ECMWF*, 2019, no. 158. Available at: https://www.ecmwf.int/en/newsletter/158/meteorology/new-cams-global-reanalysis-atmospheric-composition (April 11, 2025).

44. Gasparrini A. Distributed Lag Linear and Non-Linear Models in R: The Package dlnm. *J. Stat. Softw.*, 2011, vol. 43, no. 8, pp. 1–20.

45. Tobías A., Hashizume M., Honda Y., Sera F., Fook Sheng Ng C., Kim Y., Roye D., Chung Y. [et al.]. Geographical Variations of the Minimum Mortality Temperature at a Global Scale: A Multicountry Study. *Environ. Epidemiol.*, 2021, vol. 5, no. 5, pp. e169. DOI: 10.1097/EE9.0000000000000169

46. Gasparrini A. Modeling exposure-lag-response associations with distributed lag non-linear models. *Stat. Med.*, 2013, vol. 33, no. 5, pp. 881–899. DOI: 10.1002/sim.5963

47. Gasparrini A., Armstrong B., Kenward M.G. Multivariate meta-analysis for non-linear and other multi-parameter associations. *Stat. Med.*, 2012, vol. 31, no. 29, pp. 3821–3839. DOI: 10.1002/sim.5471

48. Sera F., Armstrong B., Blangiardo M., Gasparrini A. An extended mixed-effects framework for meta-analysis. *Stat. Med.*, 2019, vol. 38, no. 29, pp. 5429–5444. DOI: 10.1002/sim.8362

49. Yin Q., Wang J., Ren Z., Li J., Guo Y. Mapping the increased minimum mortality temperatures in the context of global climate change. *Nat. Commun.*, 2019, vol. 10, no. 1, pp. 4640. DOI: 10.1038/s41467-019-12663-y

50. Ruuhela R., Hyvärinen O., Jylhä K. Regional Assessment of Temperature-Related Mortality in Finland. *Int. J. Environ. Res. Public Health*, 2018, vol. 15, no. 3, pp. 406. DOI: 10.3390/ijerph15030406

51. Lee M., Shi L., Zanobetti A., Schwartz J.D. Study on the association between ambient temperature and mortality using spatially resolved exposure data. *Environ. Res.*, 2016, vol. 151, pp. 610–617. DOI: 10.1016/j.envres.2016.08.029

52. Roye D., Íñiguez C., Tobías A. Comparison of temperature–mortality associations using observed weather station and reanalysis data in 52 Spanish cities. *Environ. Res.*, 2020, vol. 183, pp. 109237. DOI: 10.1016/j.envres.2020.109237

53. Choi H.M., Bell M.L. Heat-mortality relationship in North Carolina: Comparison using different exposure methods. *J. Expo. Sci. Environ. Epidemiol.*, 2023, vol. 33, no. 4, pp. 637–645. DOI: 10.1038/s41370-023-00544-y

54. Hanigan I., Hall G., Dear K.B.G. A comparison of methods for calculating population exposure estimates of daily weather for health research. *Int. J. Health Geogr.*, 2006, vol. 5, pp. 38. DOI: 10.1186/1476-072X-5-38

Maksimenko M.R. Temperature-related mortality risks: effects of different sources of climatic data in the RF regions in 2004–2019. Health Risk Analysis, 2025, no. 2, pp. 30–45. DOI: 10.21668/health.risk/2025.2.03.eng

Received: 07.05.2025
Approved: 20.05.2025
Accepted for publication: 22.06.2025

UDC 614.2, 614.8, 612.014.43, 551.582.3
DOI: 10.21668/health.risk/2025.2.04.eng

Read online

Research article

ASSESSING ECONOMIC LOSSES FROM RISKS OF HEAT-RELATED PREMATURE MORTALITY USING SATELLITE MAPPING IN RUSSIAN MEGACITIES

V.I. Gornyy[1], S.G. Kritsuk[1], I.Sh. Latypov[1], A.A. Tronin[1], R.V. Buzinov[2], S.N. Noskov[2], G.B. Yeremin[2], D.S. Borisova[2]

[1]Saint Petersburg Federal Research Center of the Russian Academy of Sciences, 39, 14th Line V.O., St. Petersburg, 199178, Russian Federation
[2]North-West Public Health Research Center, 4, 2nd Sovetskaya St., St. Petersburg, 191036, Russian Federation

This research aims to address the challenges associated with utilizing satellite imagery to create maps of economic losses due to premature deaths caused by urban heat stress in the Russian cities of Omsk and Rostov-on-Don.

The study intends to provide quantitative data that can be utilized for economic decision-making processes in the areas of environmental safety and public health. It suggests a theoretically substantiated satellite-based approach to map potential risks, mortality rates, and economic losses associated with heat-related fatalities. This approach has the potential to translate long-term satellite observations of urban temperatures into actionable recommendations for urban planning and health management strategies. The relevance of this research stems from the ongoing global concern regarding climate change and its negative impact on the health of urban residents.

Our research team has created digital maps of two significant cities, Omsk and Rostov-on-Don, each with similar populations but differing climatic conditions. These maps show the number of deaths associated with heat-related events as well as the associated economic losses. Their spatial resolution is 100 × 100 m, which allows analyzing a situation in a city as whole and within its municipal districts. Having compared the results obtained by satellite mapping with medical statistics per some Omsk districts, we have assessed the accuracy of our methodology to be approximately 20 %. Our findings emphasize both the advisability to make strategic investments in mitigating risks of urban environment overheating in areas with a high potential for increased mortality from heat-related causes and the potential value of utilizing this information in urban planning initiatives aimed at adapting healthcare systems to global warming.

Keywords: *climate warming, urban heat islands, megacities, Omsk, Rostov-on-Don, overheating, risk, heat-related mortality, satellite, mapping, economic losses.*

In the context of climate warming [1] there is a significant concern regarding safety of urban residents due to increased mortality rates associated with urban heat island effects and the occurrence of ‘heat waves’ able to considerably increased normal temperatures [2, 3]. These events can lead to significant economic costs due to ad-

Victor I. Gornyy – Candidate of Geological and Mineralogical Sciences, Head of the Laboratory of Remote Methods of Geoecological Monitoring and Geoinformatics (e-mail: v.i.gornyy@mail.ru; tel.: +7 (812) 499-64-54; ORCID: http://orcid.org/0000-0001-9706-6919).

Sergey G. Kritsuk – Senior Researcher of the Laboratory of Remote Methods of Geoecological Monitoring and Geoinformatics (e-mail: sit.bloom@gmail.com; tel.: +7 (812) 499-64-54; ORCID: https://orcid.org/0000-0002-3781-322X).

Iskander Sh. Latypov – Candidate of Physical and Mathematical Sciences, Senior Researcher of the Laboratory of Remote Methods of Geoecological Monitoring and Geoinformatics (e-mail: liscander@mail.ru; tel.: +7 (812) 499-64-54; ORCID: http://orcid.org/0000-0001-8653-7308).

Andrei A. Tronin – Doctor of Geological and Mineralogical Sciences, Director of the Scientific Research Center of Ecosafety of the Russian Academy of Sciences (e-mail: a.a.tronin@gmail.com; tel.: +7 (812) 499-64-54; ORCID: http://orcid.org/0000-0002-7852-8396).

Roman V. Buzinov – Doctor of Medical Sciences, director (e-mail: info@s-znc.ru; tel.: +7 (812) 655-62-26; ORCID: https://orcid.org/0000-0002-8624-6452).

Sergey N. Noskov – Candidate of Medical Sciences, Senior Researcher (e-mail: info@s-znc.ru; tel.: +7 (812) 655-62-26; ORCID: http://orcid.org//0000-0001-7971-4062).

Gennadiy B. Yeremin – Candidate of Medical Sciences, Head of the Department of Hygiene, Leading Researcher (e-mail: info@s-znc.ru; tel.: +7 (812) 655-62-26; ORCID: https://orcid.org/0000-0002-1629-5435).

Daria S. Borisova – Junior Researcher (e-mail: info@s-znc.ru; tel.: +7 (812) 655-62-26; ORCID: https://orcid.org/0000-0002-0694-5334).

ditional deaths attributed to climate-related factors, including rising average temperatures worldwide; these costs are estimated to be between $6 billion and $88 billion annually[1]. In recent decades, large cities in Russia have experienced an unprecedented increase in air temperature compared to surrounding rural areas. This phenomenon, known as the urban heat island effect, has been attributed to several factors. The urban heat island is a result of high levels of anthropogenic heat production, which is generated by such activities as industry, transportation, and residential use of energy. Additionally, reduction of cooling effects from evaporation due to lack of green space and sealing of soil by road pavement contributes to the increased temperatures. Other factors that contribute to the urban heat island include concentrations of greenhouse gases and intensive heating of metal roofs by solar radiation, which have low thermal inertia. These factors lead to overheating of urban areas relative to rural areas. An optimal air temperature has been established to exist for each location, within which the lowest mortality rate is observed[2] [4–6]. As the average daily air temperature exceeds the threshold temperature for the lowest mortality, the annual death toll also increases. This conclusion is based on statistically supported epidemiological studies that have demonstrated a correlation between the relative risk of mortality and the average daily air temperature [4, 5, 7].

In light of climate change, an international team of scientists, based on a study of temperature trends between 1984 and 2015, has made several long-term forecasts regarding the estimated levels of additional deaths in various countries worldwide. The study reveals a negative outlook for all countries and calls for development of effective measures to protect and adapt populations to rising temperatures [8]. This requires development of efficient methods for monitoring heat-related mortality (HRM) in order to adapt public strategies accordingly. Extreme heat periods have a more significant impact on mortality from cerebrovascular diseases than from ischemic heart diseases [9]. Furthermore, prolonged hot weather leads to an increase in mortality from cardiovascular diseases, particularly among older individuals [10]. According to a meta-analysis of studies, ambient air temperature and its fluctuations have various implications for development of meteopathic reactions. A large-scale study conducted between 2004 and 2008 in the United States analyzed 7,758 cases and found that it was not possible to definitively determine impacts of seasonal variations in temperature, humidity, and atmospheric pressure on health. However, other studies have found correlations between atmospheric temperature and various health conditions. For example, a study [11] found a direct link between air temperature and prevalence of ischemic strokes. They identified a negative correlation between air humidity and stroke incidence and also found a correlation between increased emergency medical calls for circulatory diseases such as cardiac arrhythmia, arterial hypertension, and heart failure and higher air temperatures. It has also been observed that t_m (lowest mortality temperature) is influenced by climate. For individuals residing in southern latitudes, t_m is generally higher than for those in more northerly regions[1] [12, 13]. Even within a single city, there may be significant variations in average daily air tem-

---

[1] MR 2.1.10.0033-11. Otsenka riska, svyazannogo s vozdeistviem faktorov obraza zhizni na zdorov'e naseleniya: Metodicheskie rekomendatsii, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 31 iyulya 2011 g. [The Methodical Guidelines 2.1.10.0033-11. Assessment of health risks associated with impacts exerted on health by lifestyle-related factors: Methodical guidelines, approved by G.G. Onishchenko, Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on July 31, 2011]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200111974?ysclid=mbows1mjec956425907 (November 12, 2024) (in Russian).

[2] ISO 7730:2005. Ergonomics of the thermal environment – Analytical determination and interpretation of thermal comfort using calculation of the PMV and PPD indices and local thermal comfort criteria: International Standard, 3rd ed., 52 p.

peratures[3] [14–16]. Furthermore, *t* may differ by several degrees within different parts of a city. This can lead to an uneven distribution of mortality rates due to heat stress in various parts of the urban environment [16, 17]. In order for city authorities to make informed decisions regarding management of threats to population health posed by urban overheating, it is essential to have detailed information on spatial distribution of the average economic impact caused by premature deaths resulting from overheating. Bearing in mind that management decisions should be based on averaged long-term data, estimates should rely on a long-term analysis of time-series data obtained for each pixel in a map. From an economic standpoint, it is advantageous to utilize archival satellite data, as its analysis yields a map of the land surface temperature on the survey day. However, this approach has a drawback, as it may be difficult to obtain continuous time-series data due to cloud cover. This can prevent creation of a statistically representative distribution of averaged daily temperatures. Several methods could be employed to address this limitation. For example, at the time of data collection, an air temperature map is generated using the multiple correlation technique based on satellite imagery in combination with ground-based measurements of various characteristics of the urban environment. These measurements include, among others, the vegetation index and the height of surface relief. The distances from the coastline are also recorded [18–22]. A disadvantage of this approach is difficulty in generating a statistically significant number of maps needed to estimate a specific risk of overheating events.

Recent research has focused on developing methods to generate maps of deaths due to overheating on a specific date. For this purpose, maps of brightness temperatures acquired by thermal surveys conducted by Terra/Aqua(MODIS) satellites, ground-based measurements, and other characteristics of the urban environment were used, as well as statistical data on the number of urban deaths that occurred at various air temperatures [18–20]. This methodological approach to creating a digital map of mortality rates has some limitations. In order to make management decisions, we require more detailed spatial information than can be obtained from a grid with a resolution of 1×1 *km*. Moreover, this digital map is created at a particular point in time, so it is not possible to generate a statistically significant number of digital maps over a long period, and consequently, it is not possible to average potential data over a long time. In addition, collecting a large number of meteorological observations over several years using a representative ground-based observational network extends the timeframe for obtaining results.

Mathematical modeling of a large number of temperature measurements is possible but it would require significant computational resources and long-term validation of the model.

Recently, a new method for satellite-based mapping of air temperature has been developed [15]. This method combines the results of periodic satellite thermal surveys (spatial data), which were accomplished in cloudless periods, with long-term continuous air temperature monitoring data from standard weather stations (time-series data). This allows for the mapping of risk of population mortality and potential economic damage resulting from urban overheating [16]. Further development of this technique has led to the creation of mathematical expectation of economic losses due to premature heat-related mortality (HRM) caused by overheating events in urban areas [17]. As a result, based on the case study of Helsinki (Finland), it has been demonstrated that there is a spatial heterogeneity of heat-related economic losses that necessitates a differentiated approach to measures designed to mitigate the threats to public health posed by climate change. This technique has the potential to be used as a tool for forecasting and

[3] Bornstein R.D. Observations of the Urban Heat Island Effect in New York City. *Journal of Applied Meteorology*, 1968, vol. 7, no. 4, pp. 575–582.

mitigating economic damage associated with urban overheating in future. However, at present it is rather difficult to implement it in practice since it is very labor-intensive. Therefore, in this study, we **aim** to develop a simplified approach for satellite-assisted HRM mapping in two megacities located in various climatic regions and to explore the commonalities in the spatial occurrence of premature HRM incidents.

Theoretical grounds, materials and methods. The main principle behind our initial attempt to create maps of mortality rates and economic losses due to premature heat-related mortality (HRM) is to identify correlations between *T(x, y)* – the land surface brightness temperatures at each *(x, y)* – element of a given territory (corresponding to a pixel in a map) and *t(τ)* – urgent air temperatures recorded at weather stations over time. This approach, which combines spatial data from satellites with time-series data from weather stations, has been validated using available mortality data from Helsinki [16, 17]. However, it has also been identified as an extremely labor-intensive process, so in this study we have sought to develop a more streamlined methodology for analyzing aggregated data.

Epidemiological studies have demonstrated that in cities where *t* is an average daily air temperatures increase, the rate of mortality also increases in the entire population. The mortality rate, *‰/day* per day is calculated as $\mu_M(t) = M_M(t)/(N \cdot 10^{-3})$ the number of daily deaths due to all causes (*deaths/day*) divided by the number of residents in the city (*N*). The dependence of $\mu_M(t)$ on t – daily average air temperature can be represented by a second-order algebraic function. The annual mortality rate, $\overline{\mu}_M$, is defined as the annual number of deaths per 1000 residents.

Approximation of medical statistics data (Figure 1) suggests that when $t \geq t_m$, (where t_m – the temperature of minimum mortality, °*C*) the temperature dependence of $\mu_M^+(t)$ – the daily mortality rate due to overheating in a city, *‰/day* (where the subscript «*M*» denotes medical statistics), can be represented by a quadratic function:

$$\mu_M^+(t) = \mu_M(t) - \mu_M(t_m) = \\ = \gamma \cdot (t - t_m)^2, \quad t > t_m, \tag{1}$$

where $\mu_M(t_m)$ is all-cause mortality rate, ‰/day at t_m. It is possible to assume that over the 20-year period of repeated satellite surveys, the mortality rate for a particular city has remained consistent. γ is a coefficient.

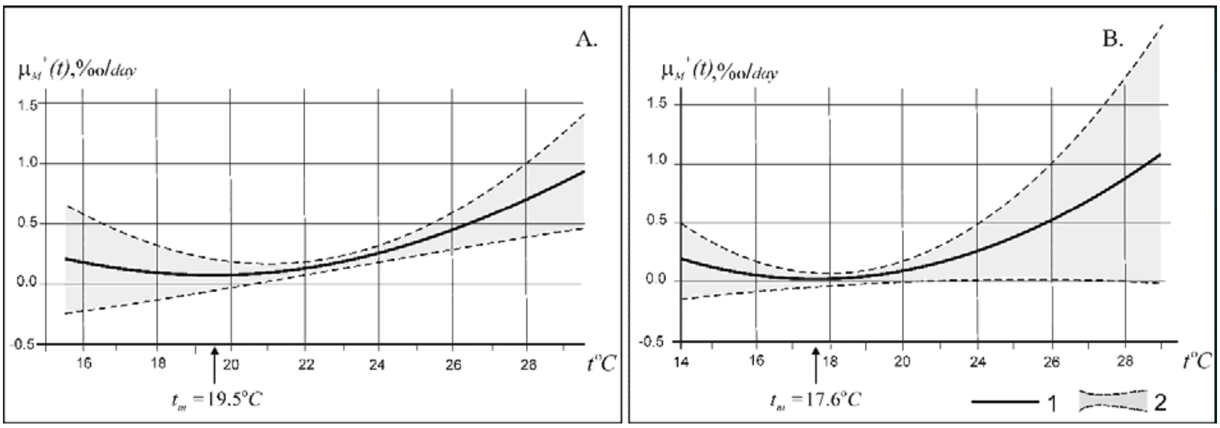

Figure 1. Approximation of dependence $\overline{\mu}_S^+(i)$ on *t*: A. Rostov-on-Don: $\mu_M^+(t) = 0.012t^2 - 0.4225t + 3.7482$; B. Omsk: $\mu_M^+(t) = 0.0082t^2 - 0.3255t + 3.2359$ (Legend: 1. Approximation. 2. Confidence intervals of approximation with a probability of 90 %)

In the context of the approximate equation (1), the task of satellite-based mapping of mortality rates can be reduced to the compilation of $\overline{\mu}_S^+(x,y)$ – maps of statistically averaged (potential) annual specific mortality rates due to overheating, based on satellite data for the (*x*, *y*) area of a pixel, $‰/(km^2 \cdot year)$. In other words, it is necessary to spatially distribute $\overline{\mu}_M^+$ – the annual mortality rates, *‰/year* due to heat exposure across the city.

The search for a solution to this issue is hampered by the fact that information on daily average air temperature is available only at weather stations. The only data that can be utilized to determine the mortality rate in a city is $T(x,y,i)$ – the brightness temperature, *K*, of the land surface, which is derived from Landsat satellite data at coordinates $x = 1,...,X;\, y = 1,...,Y$. This data is accessible at each point within the city. Based on this information, we can assume that $\overline{\mu}_M^+(x,y)$ – the specific mortality rate due to overheating in a pixel area with coordinates $x = 1,\ldots,X;\, y = 1,\ldots,Y,$ on a given *i*-th day can be approximated as:

$$\overline{\mu}_M^+(x,y,i) = \beta \cdot \left[n(x,y) \cdot T(x,y,i) - T_*\right]^2, \quad (2)$$

where β_i is a coefficient;

$n(x,y)$ is population density, residents (*x*, *y*) in a pixel, residents/km^2, which, similar to surface temperature, influences mortality;

T_* is a brightness temperature of the underlying surface (analogous t_m), K.

Next, we sum (2) over all the coordinates, which allows us to use the mortality data obtained from medical statistics:

$$\mu_M^+(i) = \beta \cdot S\,(i), \quad (3)$$

where $S\,(i) = \sum_{x=1;\,y=1}^{X,Y} n(x,y) \cdot \left[T(x,y,i) - T_*\right]^2,$

and T_* is a constant, determined on the base of analyses of satellite data (see Figures 2 and 3).

Let's assume that β does not depend on time. That is, $\mu_M^+(i)$ only depends on meteorological conditions on a day an image was made. To do this, we will develop a regression model of $\mu_M^+(i)$ based on all available data points for all *i*.

The parameter T_* is selected by minimizing the ratio of the standard deviation of β – the slope to its mean value (Figure 2), which corresponds to the maximum value of R^2 for the regression equation (Figure 3).

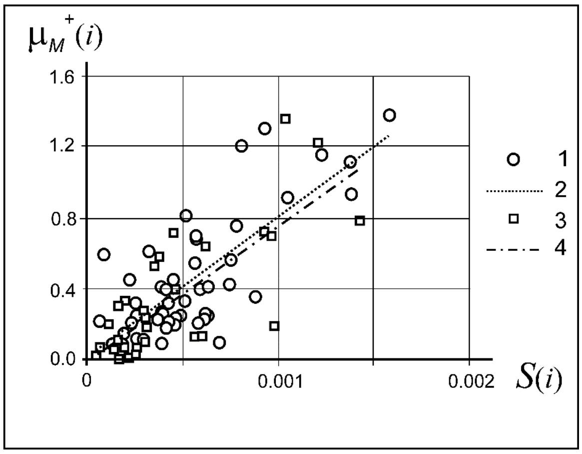

Figure 2. The dependence of $\mu_M^+(i)$ on $S(i)$. The value of T_* is shown to be optimal, providing the maximum value of R^2 (see fig. 3) (Legend: Rostov-on-Don: 1. $\overline{\mu}_M(i)$; 2. Regression for the all Landsat surveys, $i = 1,\ldots, I$; Omsk: 3. $\overline{\mu}_M(i)$; 4. Regression for the all Landsat surveys, $i = 1,\ldots, I$)

Therefore, the coefficient β is determined from the regression (Figure 2).

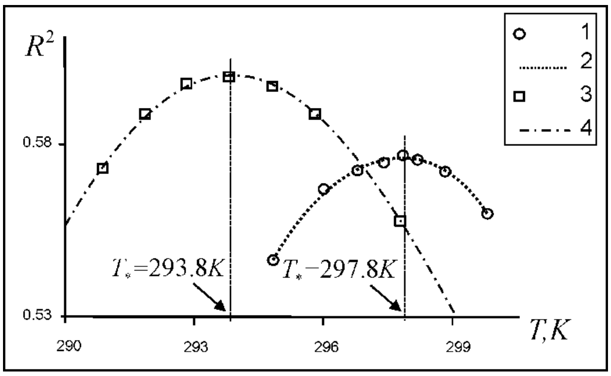

Figure 3. T_* – parameter selection (Legend: Rostov-on-Don – 1. $\overline{\mu}_M(i)$; 2. Regression for $i = 1,\ldots, I$; Omsk – 3. $\overline{\mu}_M(i)$; 4. Regression for $i = 1,\ldots, I$)

Next, $\hat{\mu}_M^+$ is the mortality rate, *‰/day:* due to heat stress, according to medical statistics, averaged over all surveys:

$$\hat{\mu}_M^+ = \left[\sum_{i=1}^{K} \mu_M^+(i)\right] / K. \qquad (4)$$

After that, $\hat{\mu}_S^+(x,y)$ is the specific mortality rate from overheating at (*x, y*) – an elementary site averaged over all days when the satellite imagery was carried out, ‰/day was calculated for each satellite survey day based on $T(x,y)$ – the land surface brightness temperature of the area corresponding to the pixel (*x, y*):

$$\hat{\mu}_S^+(x,y) = \\ = \{\beta \cdot \sum_{i=1}^{K} n(x,y) \cdot [T(x,y,i) - T_*]^2\} / K. \qquad (5)$$

The proportion of the mortality rate $\varphi(x,y)$ for a specific area, which corresponds to the pixel (*x, y*) in the total mortality rate of the city, can be calculated as:

$$\varphi(x,y) = \hat{\mu}_S^+(x,y) / \hat{\mu}_M^+ . \qquad (6)$$

Therefore, the annual specific mortality rate due to overheating in the pixel area (*x, y*):

$$\bar{\mu}_S^+(x,y) = \varphi(x,y) \cdot \bar{\mu}_M^+ , \qquad (7)$$

where $\bar{\mu}_S^+(x,y)$ and $\bar{\mu}_M^+$, $‰ / (year \cdot km^2)$ are the potential mortality rates resulting from overheating, which were obtained, respectively, through satellite imaging and medical statistics.

After that, $\bar{M}_S^+(x,y)$ or the specific annual number of deaths within the pixel's territory (*x,y*), $deaths / (year \cdot km^2)$, can be written as:

$$\bar{M}_S^+(x,y) = \bar{\mu}_S^+(x,y) \cdot n(x,y) \cdot 10^{-3}, \qquad (8)$$

where *n*(*x, y*) is the number of individuals in the elementary unit (*x, y*). Hence, $\bar{M}_S^+$ is the annual number of deaths, *deaths/year,* from overheating per year in area A, km^2 according to satellite data:

$$\bar{M}_S^+(A) = \sum_{x,y}^{A} \bar{M}_S^+(x,y). \qquad (9)$$

When analyzing the results, it is beneficial to have a representation of $r(x,y)$ as the risk (probability) of air temperature exceeding a threshold t_m. To achieve this, we employ a previously developed methodology [16] that involves identifying, for each individual pixel, the correlation between *T(x, y)* and *t* at designated weather station:

$$r(x,y) = \sum_{j \to t_m}^{i=J} p_j\left[t_{eq}(x,y)\right], \qquad (10)$$

where $p_j\left[t_{eq}(x,y)\right]$ is the frequency distribution of the equivalent temperature within *j*-th each bin of the histogram, with *j* = 1,..., *J*; $t_{eq}(x,y)$ is the equivalent temperature. This is the average daily air temperature, recorded at the weather station that corresponds to *T(x, y, i)*, the brightness temperature measured at the time of a satellite survey carried out on the *i*-th day.

The temperature interval of the histogram is 1 °C for the weather station, starting from t_{eq} = 0 °C and up to $t_{\max}$ as the maximum daily average air temperature historically recorded by the weather station. Due to this linear dependence, we can derive a regression equation:

$$t = t_{eq}(x,y,i) = a \cdot T(x,y,i) + b. \qquad (11)$$

Given this linear relationship, it is possible to linearly transform the statistical distribution:

$$p_j\left[t_{eq}(x,y)\right] = a(x,y) \cdot p_j(t) + b(x,y).$$

Based on (9), calculation of $l(x,y)$, specific economic damage due to premature heat-related mortality, *$/(km² · year)* takes into account ε – the cost of a single premature HRM, $\$ / death$ and is given as (12):

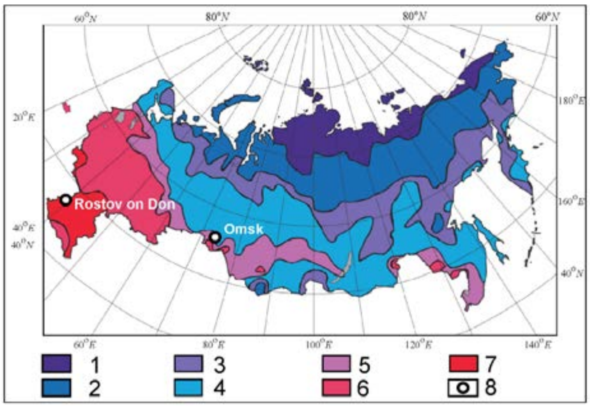

Figure 4. The map of the zoning of the Russian territory based on living conditions in the population [6]. Period: 2001–2010 (The legend: 1. Absolutely unfavorable zone; 2. Very unfavorable zone; 3. Unfavorable zone; 4. Conditionally unfavorable zone; 5. Conditionally favorable zone; 6. Favorable zone; 7. Most favorable zone)

$$l(x,y) = \varepsilon \cdot \bar{M}_S^{+}(x,y). \qquad (12)$$

Finally, $L(A)$ is the total average annual economic damage (potential economic damage) in locality or its district due to premature mortality, caused by heat stress, *$/year*:

$$L(A) = \varepsilon \cdot \bar{M}_S^{+}(A). \qquad (13)$$

Research objects. To develop a streamlined methodology for satellite-based mapping of economic losses due to HRM, two megacities with distinct living conditions and situated in various climatic zones of Russia were chosen: Rostov-on-Don and Omsk. Figure 4 provides a visual representation of the selected cities per living conditions. Table 1 presents basic information necessary for understanding the medical and climatic situation in these cities.

For satellite mapping of economic damages from premature HRM, data from summer surveys by Landsat series satellites was used (Table 2). The temperature sensitivity of the Landsat 7 satellite was no worse than 0.3 *K*, and the initial spatial resolution was 60 meters (converted to 30 meters during processing)[4] [24]. The temperature sensitivity for the Landsat 8 satellite is 0.4 *K* at a background temperature of 300 *K* with a spatial resolution of 100 *m*. In addition, we used urgent summer air temperature observations at meteorological stations in the World Meteorological Organization (WMO) network for the entire period during which satellite data (Table 2).

Table 1

City profiles

No.	Name	Population, people	City area / area of green spaces, km^2	Latitude, degrees	Average air temperature in July, °C
1	Rostov-on-Don	1,142,000	348 / 77	47°14’ N	24.0
2	Omsk	1,126,000	578 / 130	54°56’ N	19.6

Table 2

Satellite materials and meteorological data used for mapping

№	City	Satellite	Number of scenes	Period, dd.mm.yyyy	Data source	WMO weather station code
1	Rostov-on-Don	Landsat 7 Landsat 8	37 26	31.07.2000– 30.08.2022	https://earthexplorer.usgs.gov	34730
2	Omsk	Landsat 7 Landsat 8	10 34	01.06.2013– 25.08.2022	https://earthexplorer.usgs.gov	28698

[4] Landsat 7 (L7) Data Users Handbook. Version 2.0. South Dakota, Sioux Falls, EROS Publ., 2019, 139 p.

To analyze the results of the study, we used maps of functional zones[5].

Data on population density in these cities were taken from the digital World population density maps, created by the European Union[6]. In addition, we used data on economic losses from one premature HRM event in each of the studied cities. For Rostov-on-Don, the estimated loss was $14,400 per death, and in Omsk it was $15,599 per death [23].

Baseline data on daily mortality in selected cities were provided by the Institute for Economic Forecasting at the Russian Academy of Sciences and the Federal Service for Surveillance over Consumer Rights protection and Human Wellbeing.

Figure 5 demonstrates the method for mapping the risk of overheating and potential HRM using the results from multiple satellite surveys. This method is built on the fundamental principles discussed above.

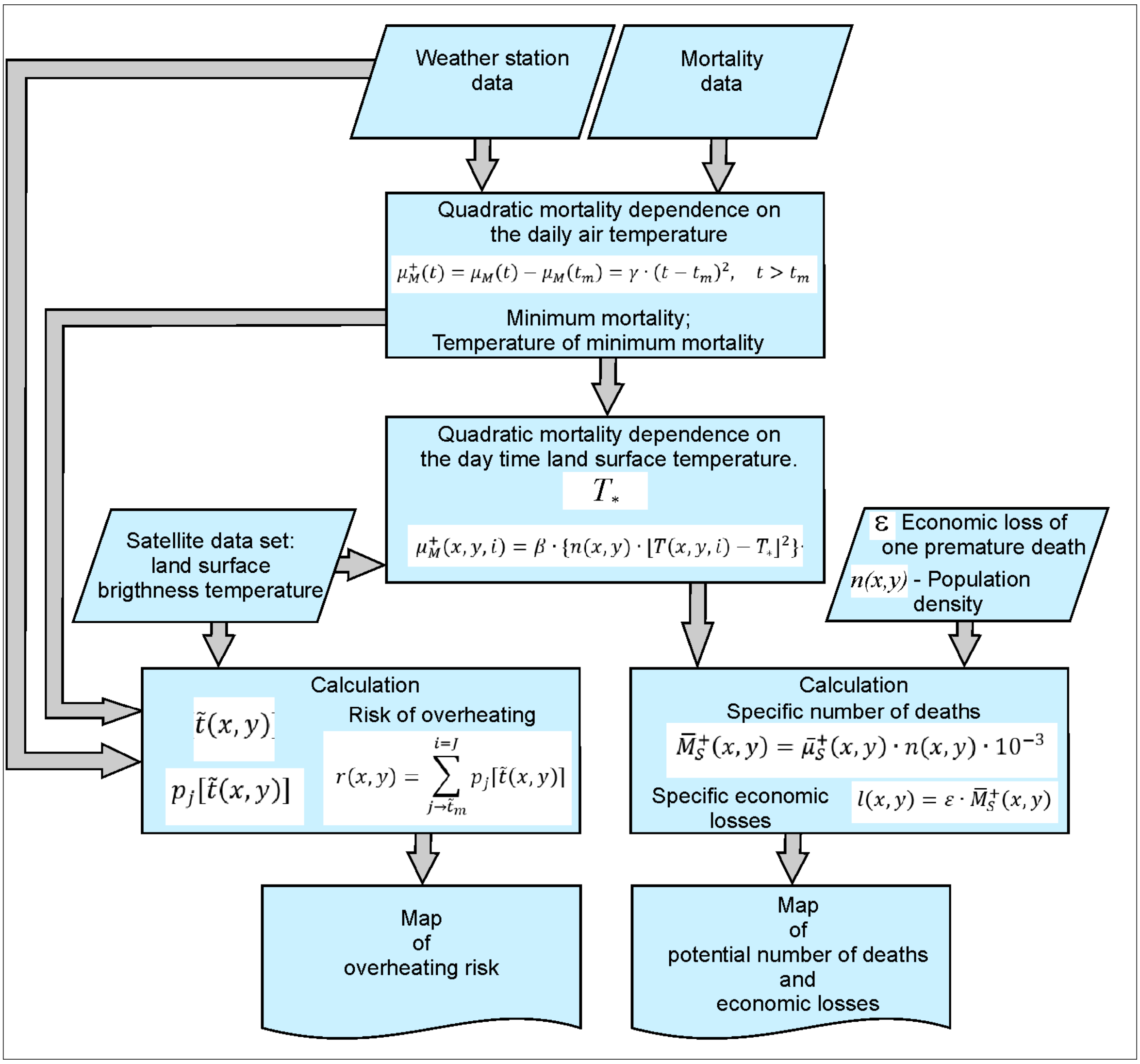

Figure 5. Information flow diagram for satellite mapping of risk of overheating and potential HRM

---

[5] Omsk. *World Population Density*. Available at: https://luminocity3d.org/WorldPopDen/#10/51.0280/34.8335 (November 16, 2024); General'nyi plan Rostova-na-Donu [The general Development Plan for Rostov-on-Don]. *Rostov-on-Don: City Duma and City Administration: official portal*. Available at: https://rostov-gorod.ru/administration/structure/departments/daig/action/01-grado-01-genplan/ (November 16, 2024) (in Russian).

[6] World Population Density. Available at: https://luminocity3d.org/WorldPopDen/ (November 16, 2024).

Results and discussion. The refined methodology for satellite-based mapping of risks and economic impacts due to HRM was tested using the cities of Omsk and Rostov-on-Don as case studies. Detailed maps were created showing the risk of air temperature exceeding the threshold for minimum mortality (Figures 6c and 7c).

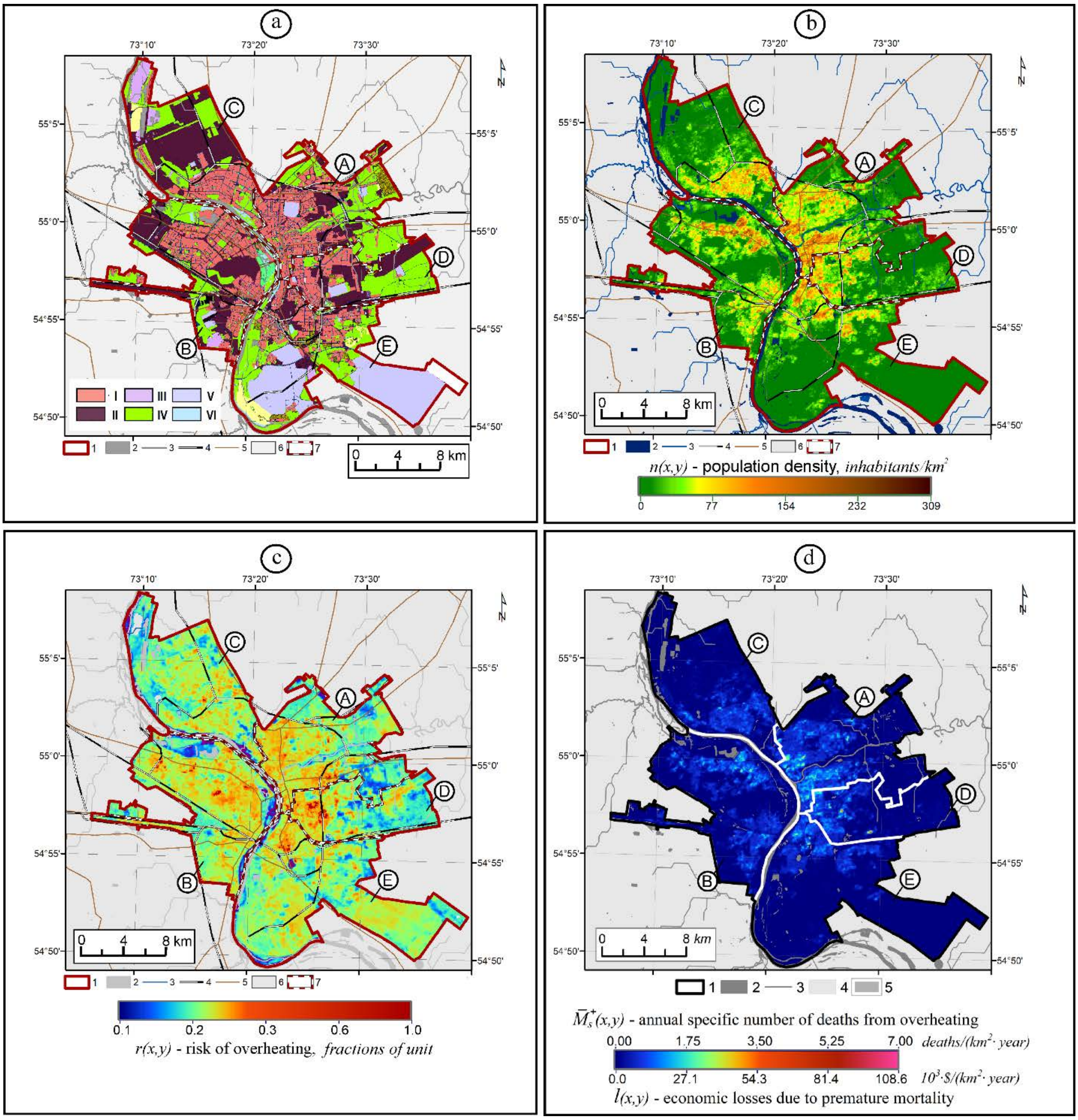

Figure 6. Satellite maps of city of Omsk (Russia) (Legend below maps: 1. City border. 2. Water areas. 3. Rivers. 4. Railways. 5. Car roads. 6. Suburb. 7. Borders of municipal districts. Capital letters in circles indicate municipal districts: A – Central. B – Kirovskiy. C – Sovetskiy. D – Oktyabrskiy. E – Leninskiy): (a) Simplified scheme of functional zones (Legend inside map: I. Buildings: residential low-rise, high-rise, public business, multifunctional, historical. II. Industrial and transport infrastructure. III. Waste storage and disposal. IV. Landscaped recreational, horticultural, cemeteries. V. Restricted territories. VI. Ponds); (b) Digital population density map (Period: 2020); (c) Digital map of $r(x,y)$ – risk of overheating; (d) Digital map of $\bar{M}_S^+(x,y)$ – specific number of deaths due to overheating and $l(x,y)$ – economic losses due to premature mortality caused by overheating

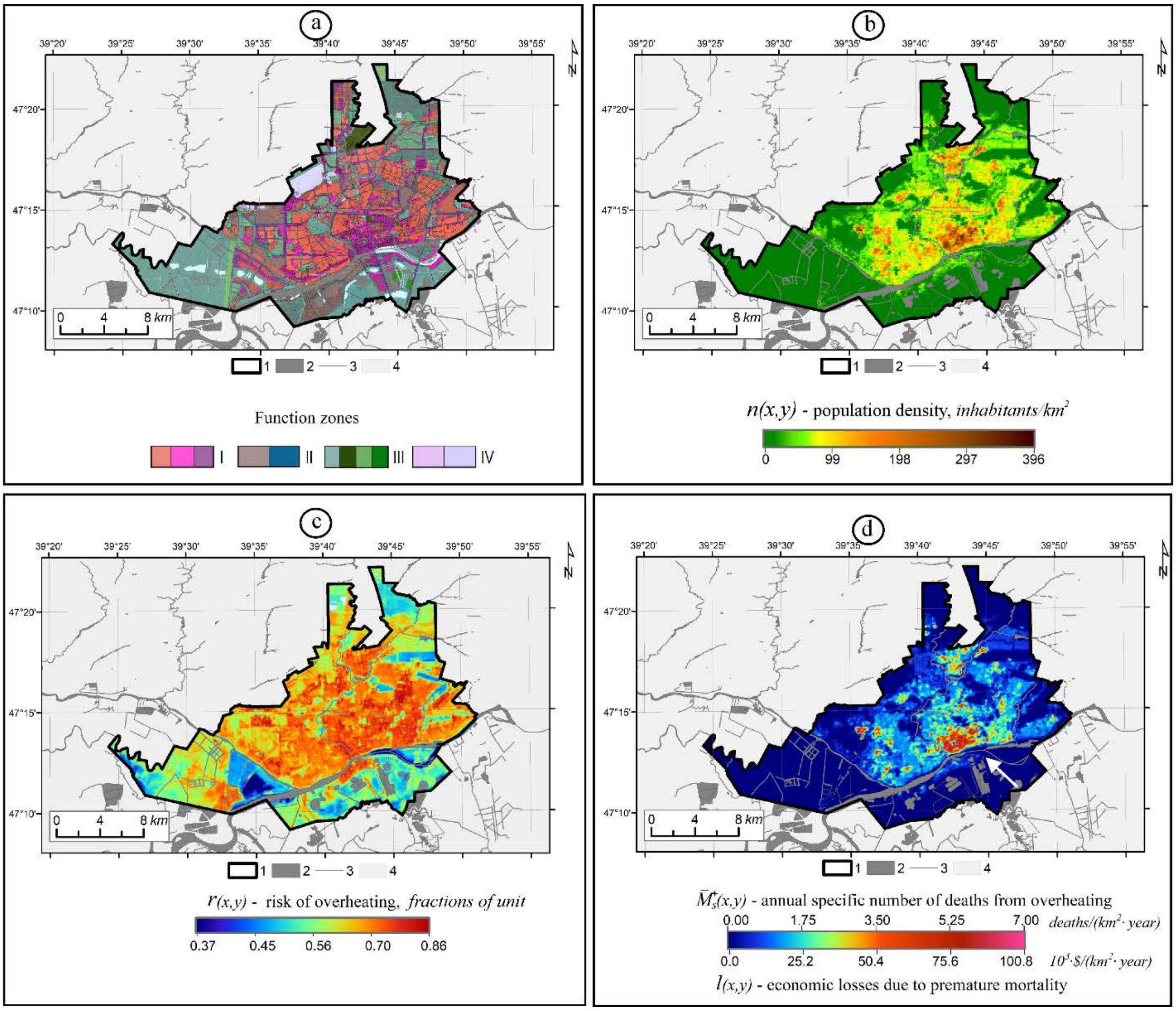

Figure 7. Satellite maps of city of Rostov-on-Don (Legend below maps: 1. City border. 2. Water areas. 3. Rivers. 4. Railways. 5. Car roads. 6. Suburb. 7. Borders of municipal districts): (a) Simplified scheme of functional zones (Legend below map inside Fig. (a): I. Buildings: residential low-rise, high-rise, public business, multifunctional, historical. II. Industrial and transport infrastructure. III. Landscaped recreational, horticultural, cemeteries. VI. Waste storage and disposal); (b) Map of $n(x, y)$ (Period: 2020); (c) Digital map of $r(x, y)$; (d) Digital map of $\bar{M}_S^{+}(x, y)$ and $l(x, y)$. The white arrow indicates an area with a high HRM

Table 3

Errors in Satellite Mapping of HRM in Omsk

Omsk municipal districts			Annual number of deaths due to HRM, deaths/year		Relative error of satellite technique, %
Letter designation in Fig. 6	Names of municipal districts	Population, people	Medicine statistics	Satellite mapping	
A	Central	245,578	17	20	+17
B	Kirovskii	248,209	19	16	-15
C	Sovetskii	204,001	11	10	-9
D	Oktyabrskii	271,440	14	8	-42
E	Leninskii	156,467	11	10	-9
Total City:		1,126,000	72	64	-11

Theoretical studies were validated using Omsk as an example, as statistical data on mortality were available not only for the entire city but also for its individual districts. This allowed us to assess the relative errors of our method for different districts of the city (see Table 3).

It is worth noting that in areas such as Sovetskii, Kirovskii, and Leninskii districts in Omsk, the relative errors did not exceed 15 %. This indicates a satisfactory result, given that these areas have a lower population density. In contrast, areas with a higher population density, such as Central (A at Fig. 6) and Oktyabrsky (D at Fig. 6), had relative errors ranging from 17 to 42 %. These initial results suggest that the relative error in the mapping of mortality and economic losses remains within 20 %, which is generally acceptable for economic assessments. An analysis of all satellite maps reveals that risks of heat stress, potential fatalities, and economic damage are not uniformly distributed within the studied cities. Increased potential mortality rates are concentrated in areas where high population density coincides with a higher risk of heat stress. This pattern is the most evident in urban centers such as the historical districts of Omsk and the core of Rostov-on-Don. Conversely, minimal mortality has been observed in areas with a high heat stress risk but low population density, for instance, in the Leninskii district of Omsk within areas for waste storage and disposal where there are no permanent inhabitants and therefore no heat-related mortality. It should be noted that obtaining more accurate mortality data presents a challenge due to difficulty in considering residents who may be present at workplaces that have different temperature conditions from their place of residence. In order to improve this technology, it may therefore be necessary to take into account daily patterns of population migration.

Satellite maps of mortality rates when compared with data collected through medical statistics reveal notable differences of approximately 10 % in Omsk and 4 % in Rostov-on-Don (see Table 4). These disparities are attributable to errors resulting from the statistical averaging process employed in the algorithm.

Comparison of Figures 4, 6, and 7 reveals an interesting result. Omsk is located on the border between the Conditionally Favorable and Conditionally Unfavorable zones, while Rostov-on-Don is situated within the Most Favored Zone. At the same time, HRM is higher in Rostov-on-Don compared to Omsk (see, Table 4 and Figures 6 and 7). This phenomenon can be explained by the fact that the Russian territory zoning map (Figure 4) was created based on an average of several zonal indicators, which were subsequently adjusted by azonal factors. The temperature indicator was only one of many factors, and therefore it was not the decisive factor in determining the zoning.

Further development of this technology may involve use of other relevant urban parameters. It is a known fact that over recent decades, the global temperature has grown leading to an increase in precipitation rates over land. With increased precipitation and temperature, absolute humidity levels of air

Table 4

Comparison of the main characteristics of annual HRM in the populations of Omsk and Rostov-on-Don

Annual characteristics	Omsk	Rostov-on-Don
Number of deaths according to medical statistics, deaths/year	72	152
The potential annual number of deaths due to satellite maps, deaths/year	64	146
HRM rate according to medical statistics, ‰/year	0.063	0.133
Potential HRM rate according to satellite maps, ‰/year	0.057	0.123
Economic losses from HRM according to medical statistics: Total city, \$/year: Specific average: \$/(year·km^2)	 $1.10 \cdot 10^6$ 1,903	 $2.19 \cdot 10^6$ 6,293
Potential economic losses from HRM according to satellite maps, \$/year	$0.99 \cdot 10^6$	$2.10 \cdot 10^6$

are likely to increase as well. This could have implications for healthcare since higher air humidity levels with high temperature may cause the mortality rising.

The practical significance of detailed mapping of economic losses from HRM is illustrated by the following example. Mitigating urban HRM under climate change could be achieved through implementation of various technological solutions such as creation of green spaces, use of reflective surfaces, and installation of ‘cold’ metal roofs. For instance, costs of converting conventional roofs to ‘cold’ roofs ranges between $60 and $150 per square meter at present [25]. Therefore, if one square kilometer of conventional rooftops were replaced with ‘cold’ ones, it would cost between $60 million and $150 million. This is a significant amount that exceeds estimated annual economic losses due to HRM in Rostov-on-Don, which stands at $2.19 million. Given that the lifespan of a typical roof is approximately 30 years, the total cost of implementing sustainable roofing solutions over a 30-year period would be comparable to that of the economic impact of ‘cold’ rooftop installations in Rostov on Don. It is worth noting that implementation of ‘cold’ roofs may also contribute to reducing heating and climate control system costs [25]. As cities strive to address health risks posed by climate change, it is advisable to invest in measures that minimize urban heat island effects, particularly in areas with higher mortality rates. In the case of Rostov-on-Don, this encompasses the central district indicated by the arrow in Figure 7d, covering approximately 8 square kilometers of the city (out of a total area of 350 square kilometers). Satellite maps of potential economic losses from heat-related mortality allow city officials to allocate budgets efficiently focusing on areas where the benefits of such measures would be most effective.

Furthermore, this information could assist in strengthening medical services in areas with a high risk of HRM, particularly for vulnerable populations such as older adults aged 60 or over and those with chronic conditions, especially cardiovascular issues. Such a targeted approach could help reduce the impact of HRM on these groups and improve their overall health measures. In short, for effective administrative decisions regarding climate-related risks, it is crucial to create maps illustrating potential mortality and economic losses from HRM. This is particularly pertinent for large cities with populations exceeding one million. By adopting this approach, city planners can proactively address the growing challenges posed by climate change, safeguarding population health and enhancing a city's resilience.

Conclusions:

1. The methodology for mapping risks of ambient air overheating, premature mortality and economic losses has been created based on combining satellite remote sensing technology, epidemiological data and mathematical modeling. This methodology can serve as an informational support tool within a decision-making framework aimed at mitigating potential harm to population health in major cities due to climate change-related impacts.

2. The results of the satellite mapping, based on the case studies of Omsk and Rostov-on-Don, indicate deviations of up to 20 % in the estimated annual numbers of deaths and economic losses attributable to premature heat-related mortality. These levels of errors are within the acceptable range for urban planning and management decisions related to protecting public health in the context of climate change.

3. The distribution of potential specific HRM and economic losses demonstrates a non-uniform pattern within the city. Higher figures are concentrated in areas with a higher population density, while areas with lower population densities, such as industrial zones, green spaces or recreational zones experience minimal impact. Focusing on areas with high population densities constitutes a viable strategy for addressing public health concerns related to climate change.

4. The growing significance of geospatial data for the economy, society, and environment is determined by its role as information support of decision-making processes that de-

pend on spatially distributed economic information. This is a promising direction for the satellite remote sensing technology.

Funding. The research was carried out within:

- the budget theme of the Ministry of Science and Education of the Russian Federation: "SCIENTIFIC BASES FOR ASSESSING THE HEALTH OF ECOSYSTEMS OF THE NORTH-WEST OF RUSSIA AND PREVENTION OF THREATS TO ENVIRONMENTAL SAFETY". Code FFZF-2022-0011;
- the implementation of the North-West Public Health Research Center research plan for 2021–2025, taking into account the national plan of measures for the second stage of adaptation to climate change up to 2025.

Competing interests. The authors declare no competing interests.

References

1. Allen M.R., Dube O.P., Solecki W., Aragón–Durand F., Cramer W., Humphreys S., Kainuma M., Kala J. [et al.]. Global warming of 1.5 °C: Special Report. *IPCC*, 2018, 616 p.

2. Koppe C., Kovats S., Jendritzky G., Menne B. Heat-waves: risks and responses. Copenhagen, WHO Regional Office for Europe, 2004, 123 p.

3. Shu C., Gaur A., Wang L., Lacasse M.A. Evolution of the local climate in Montreal and Ottawa before, during and after a heatwave and the effects on urban heat islands. *Sci. Total Environ.*, 2023, vol. 890, pp. 164497.

4. Ruuhela R., Jylha K., Lanki T., Tiittanen P., Matzarakis A. Biometeorological Assessment of Mortality Related to Extreme Temperatures in Helsinki Region, Finland 1972–2014. *Intern. J. Environ. Res. Public Health*, 2017, vol. 14, no. 8, pp. 944. DOI: 10.3390/ijerph14080944

5. Shartova N.V., Shaposhnikov D.A., Konstantinov P.I., Revich B.A. Air temperature and mortality: heat thresholds and population vulnerability study in Rostov-on-Don. *Fundamental'naya i prikladnaya klimatologiya*, 2019, no. 2, pp. 66–94. DOI: 10.21513/2410-8758-2019-2-66-94 (in Russian).

6. Vinogradova V.V., Zolotokrylin A.N. Current and Future Role of Climatic Factor in the Estimation of Natural Conditions of Life in Russia. *Izvestiya Rossiiskoi Akademii Nauk. Seriya Geograficheskaya*, 2014, no. 4, pp. 16–21. DOI: 10.15356/0373-2444-2014-4-16-21 (in Russian).

7. Andrade C. Understanding relative risk, odds ratio, and related terms: as simple as it can get. *J. Clin. Psychiatry*, 2015, vol. 76, no. 7, pp. e857-e861. DOI: 10.4088/JCP.15f10150

8. Guo Y., Gasparrini A., Li S., Sera F., Vicedo-Cabrera A.M., de Sousa Zanotti Stagliorio Coelho M., Nascimento Saldiva P.H., Lavigne E. [et al.]. Quantifying excess deaths related to heatwaves under climate change scenarios: A multicountry time series modelling study. *PLoS Med.*, 2018, vol. 15, no. 7, pp. e1002629. DOI: 10.1371/journal.pmed.1002629

9. Hanzlíková H., Plavcová E., Kynčl J., Kříž B., Kyselý J. Contrasting patterns of hot spell effects on morbidity and mortality for cardiovascular diseases in the Czech Republic, 1994–2009. *Int. J. Biometeorol.*, 2015, vol. 59, no. 11, pp. 1673–1684. DOI: 10.1007/s00484-015-0974-1

10. Urban A., Davídkovova H., Kysely J. Heat- and cold-stress effects on cardiovascular mortality and morbidity among urban and rural populations in the Czech Republic. *Int. J. Biometeorol.*, 2014, vol. 58, no. 6, pp. 1057–1068. DOI: 10.1007/s00484-013-0693-4

11. Salam A., Kamran S., Bibi R., Korashy H.M., Parray A., Mannai A.A., Ansari A.A., Kanikicharla K.K. [et al.]. Meteorological Factors and Seasonal Stroke Rates: A Four-year Comprehensive Study. *J. Stroke Cerebrovasc. Dis.*, 2019, vol. 28, no. 8, pp. 2324–2331. DOI: 10.1016/j.jstrokecerebrovasdis.2019.05.032

12. Zhao Q., Guo Y., Ye T., Gasparrini A., Tong S., Overcenco A., Urban A., Schneider A. [et al.]. Global, regional, and national burden of mortality associated with non-optimal ambient temperatures from 2000 to 2019: a three-stage modelling study. *Lancet Planet. Health*, 2021, vol. 5, no. 7, pp. e415–e425. DOI: 10.1016/S2542-5196(21)00081-4

13. Revich B.A., Grigorieva E.A. Health Risks to the Russian Population from Weather Extremes in the Beginning of the XXI Century. Part 1. Heat and Cold Waves. *Problemy analiza riska*, 2021, vol. 18, no. 2, pp. 12–33. DOI: 10.32686/1812-5220-2021-18-2-12-33 (in Russian).

14. Kritsuk S.G., Gornyy V.I., Latypov I.Sh., Pavlovskii A.A., Tronin A.A. Satellite Risk Mapping of Urban Surface Overheating (by the Example of Saint Petersburg). *Sovremennye problemy distantsionnogo zondirovaniya Zemli iz kosmosa*, 2019, vol. 16, no. 5, pp. 34–44. DOI: 10.21046/2070-7401-2019-16-5-34-44 (in Russian).

15. Kritsuk S., Gornyy V., Davidan T., Latypov I., Manvelova A., Konstantinov P., Tronin A., Varentsov M., Vasiliev M. Satellite mapping of air temperature under polar night conditions. *Geo-spatial Information Science*, 2022, vol. 25, no. 2, pp. 325–336. DOI: 10.1080/10095020.2021.2003166

16. Gornyy V.I., Kritsuk S.G., Latypov I.Sh., Manvelova A.B., Tronin A.A. Satellite Mapping of Urban Air Overheating Risk (Case Study of Helsinki, Finland). *Cosmic Res*., 2022, no. 60, suppl. 1, pp. S38–S45. DOI: 10.1134/S0010952522700058

17. Gornyy V.I., Kritsuk S.G., Latypov I.Sh., Tronin A.A. Satellite Mapping of Economic Damage from Urban Deaths Caused by Overheating (by Example of Helsinki, Finland). *Sovremennye problemy distantsionnogo zondirovaniya Zemli iz kosmosa*, 2022, vol. 19, no. 3, pp. 35–46. DOI: 10.21046/2070-7401-2022-19-3-35-46 (in Russian).

18. Srivanit M., Hokao K. Thermal Infrared Remote Sensing for Urban Climate and Environmental Studies: An Application for the City of Bangkok, Thailand. *JARS*, 2012, vol. 9, no. 1, pp. 83–100. DOI: 10.56261/jars.v9i1.168598

19. Smargiassi A., Goldberg M.S., Plante C., Fournier M., Baudouin Y., Kosatsky T. Variation of daily warm season mortality as a function of micro-urban heat islands. *J. Epidemiol. Community Health*, 2009, vol. 63, no. 8, pp. 659–664. DOI: 10.1136/jech.2008.078147

20. Ho H.C., Knudby A., Huang W. Spatial Framework to Map Heat Health Risks at Multiple Scales. *Int. J. Environ. Res. Public Health*, 2015, vol. 12, no. 12, pp. 16110–16123. DOI: 10.3390/ijerph121215046

21. Ho H.C., Knudby A., Xu Y., Hodul M., Aminipouri M. A comparison of urban heat islands mapped using skin temperature, air temperature, and apparent temperature (Humidex), for the greater Vancouver area. *Sci. Total Environ.*, 2016, vol. 544, pp. 929–938. DOI: 10.1016/j.scitotenv.2015.12.021

22. Ho H.C., Knudby A., Walker B.B., Henderson S.B. Delineation of Spatial Variability in the Temperature-Mortality Relationship on Extremely Hot Days in Greater Vancouver, Canada. *Environ. Health Perspect.*, 2017, vol. 125, no. 1, pp. 66–75. DOI: 10.1289/EHP224

23. Zubets A.N., Novikov A.V. Quantitative assessment of the value of human life in Russia and in the world. *Finance: Theory and Practice*, 2018, vol. 22, no. 4, pp. 52–75. DOI: 10.26794/2587-5671-2018-22-4-52-75 (in Russian).

24. Barsi J.A., Schott J.R., Palluconi F.D., Helder D.L., Hook S.J., Markham B.L., Chander G., O'Donnell E.M. Landsat TM and ETM+ thermal band calibration. *Can. J. Remote Sensing*, 2003, vol. 29, no. 2, pp. 141–153. DOI: 10.5589/m02-087

25. Dominguez-Delgado A., Domínguez-Torres H., Domínguez-Torres C.-A. Energy and Economic Life Cycle Assessment of Cool Roofs Applied to the Refurbishment of Social Housing in Southern Spain. *Sustainability*, 2020, vol. 12, no. 14, pp. 5602. DOI: 10.3390/su12145602

Gornyy V.I., Kritsuk S.G., Latypov I.Sh., Tronin A.A., Buzinov R.V., Noskov S.N., Yeremin G.B., Borisova D.S. Assessing economic losses from risks of heat-related premature mortality using satellite mapping in Russian megacities. Health Risk Analysis, 2025, no. 2, pp. 46–59. DOI: 10.21668/health.risk/2025.2.04.eng

Received: 23.05.2025
Approved: 09.06.2025
Accepted for publication: 26.06.2025

UDC 614+316.622
DOI: 10.21668/health.risk/2025.2.05.eng

Read online

Research article

FACTORS OF SELF-PRESERVATION AND RISKY BEHAVIOR OF EMPLOYABLE POPULATION IN AN INDUSTRIAL MEGALOPOLIS

S.S. Gordeeva, S.Yu. Sharypova, A.S. Shlyapina

Perm State University, 15 Bukireva St., Perm, 614990, Russian Federation

Socio-economic stability of any state is determined by the health of employable population, formed by an individual's behavior, which can be aimed both at preserving health (self-preservation) and at its destruction (risky behavior).

Based on literature analysis, three groups of factors have been identified that determine an individual's behavior in the field of health: socio-biological, socio-economic, and socio-psychological. These factors gave grounds for developing a mathematical model of multifactorial causation of self-preservation and risky behavior pursued by employable population living in an industrial megacity. The empirical base was formed by data from a formalized survey conducted in 2025 among residents of Perm city (n = 582). The data were analyzed using the SPSS Statistics software. The model was built using the neural network procedure of the multilayer perceptron (MLP).

The study has found that health behavior of employable population living in Perm is characterized by a moderate risk profile. This is reflected in a moderate level of commitment to physical activity and medical practices, as well as relatively low manifestation of addictions. The factors identified in the study have a complex interrelationship. At the same time, there is a lack of an unambiguous link between health attitudes and actual implementation of consistent health-preserving behavior. Social support functions as a factor contributing to health preservation, but only with moderate intensity; its excessive manifestation can negate an individual's personal responsibility. In addition, influence of social connections has a bilateral effect: they can both provide support and contribute to formation of unhealthy behaviors through normative pressure. A high level of subjective security perception contributes to increased inclination for self-preservation behavior, while a decrease in this perception initiates the opposite process.

The study highlights complex mechanisms of interaction between factors and self-preservation behavior pointing out that practices aimed at preserving health do not always manifest themselves in a systematic and sustainable form. The results substantiate the necessity to conduct investigative search for additional components forming the structure of self-preservation behavior in addition to behavioral practices. Preventive approaches to reducing risky behavior among employable population should take into account specific factors, which determine this behavior in various social groups, and adapt to their specific needs.

Keywords: *risky behavior, self-preservation behavior, health, employable population, multifactorial causation, neural network modeling.*

Given the ongoing social and economic transformation and unfavorable demographic trends, health of working age population becomes a key element of the human capital, which provides the most vital grounds for reproduction of social structures, functioning of social institutions and competitiveness of the society in general. Sustainable Development Agenda up to 2030[1], which was developed with the WHO participation, aims to decrease the global disease burden and reduce likelihood of untimely deaths of employable population due to diseases predominantly caused by behavioral risk factors by one third [1]. These factors do not only affect population health, in particular, that of working age population but

Svetlana S. Gordeeva – Candidate of Sociological Sciences, Associate Professor at the Department of Sociology (e-mail: SSGordeyeva@mail.ru; tel.: +7 (342) 239-63-29; ORCID: https://orcid.org/0000-0002-5309-8318).

Sofya Yu. Sharypova – Candidate of Sociological Sciences, Associate Professor at the Department of Sociology (e-mail: sonia.eliseeva@bk.ru; tel.: +7 (342) 239-63-29; ORCID: https://orcid.org/0000-0003-3519-8531).

Anastasiya S. Shlyapina – assistant at the Department of Sociology, sociologist of the Center for Sociological Research (e-mail: shlyapina.psu@mail.ru; tel.: +7 (342) 239-66-93; ORCID: https://orcid.org/0000-0002-5398-2891).

[1] Sustainable Development Goals. *UN: official web-site.* Available at: https://www.un.org/sustainabledevelopment/ (April 29, 2025).

also endanger employment stability, especially given workforce ageing [2]. Thus, in many cases, chronic diseases result in sick leaves, lower workforce productivity, and an increase in allowances given to disabled people. All this creates substantial financial burdens for employers, state budgets and national economies [3]. People who live in industrial megacities can be additionally exposed to unfavorable sanitary-epidemiological factors, including working conditions that do not conform to safe standards, low quality of drinking water and ambient air [4]. Under negative environmental exposures, risky behaviors lead to more serious health impairments in working age population. Therefore, social and behavioral aspects of protecting working age population's health in industrial regions play a crucial role in labor market formation, acceleration of GDP growth and rise in the welfare of the society as a whole.

Self-preservation as a behavioral practice is closely connected with 'health' as a category acting as its system-forming element. Positive self-preservation behavior is usually called health promotion or health protection [5], whereas negative self-preservation behavior is risky or self-destructive [6]. In scientific discourse, health behavior is described from various theoretical positions including medical, psychological, and sociodemographic approaches [7, 8]. In sociology, this category is mostly described within the behavioral approach through a system of actions taken by an individual and aimed at protecting or destructing one's health during the whole lifespan [9], as well as choices made by an individual as regards their health [10]. It includes various behavioral practices such as eating habits, physical and medical activity, addictions, etc. [11]. Self-preservation is not considered as a simple individual phenomenon; it is built in a wider social context and acts as a fundamental norm of a social order in various societies being 'formed genetically and supported socially' [12].

Health behavior is determined by many factors; they act at macro- and micro-levels and have both positive and negative effects. Macro-factors are social-political, socioeconomic and sociocultural contexts. For example, smoking can be associated with socializing, sharing, and male identity in some South Asian cultures; this illustrates how sociocultural norms can make a norm out of behavioral practices, which are potentially hazardous for health [13]. Micro-factors of self-preservation are a set of individual traits, which determine a person's health behavior. These traits differ depending on a study and cover the following: sex, age, education, income, social environment [14], employment, religion, health [15], marital status, health attitudes [16] etc. This study focuses on micro-factors, which can promote preservation of physical health or hamper it.

Health Belief Model (HBM)[2] can give theoretical grounds for analyzing how *sex and age* influence health behavior. For example, some researchers give evidence that female sex correlates with lower risks of using psychoactive substances, tobacco in particular; refusal from such practices becomes more common in older age groups [17]. However, if we look at some other self-preservation practices, for example physical activity, then we can see that the study by M. Helgesson and others [18] shows that a considerable part of people aged older than 50 years have lower adherence to such practices against younger age groups due to *poor physical health* and limited mobility.

According to the so called Marriage Protection Theory[3], *marital status* is a significant factor of self-preservation since married people tend to have better health due to mutual social and financial support provided by a partner as well as due to taking care of each

---

[2] Rosenstock I.M. The Health Belief Model and Preventive Health Behavior. *Health Education Monographs*, 1974, vol. 2, no. 4, pp. 354–386. DOI: 10.1177/109019817400200405

[3] Ross C.E., Mirowsky J., Goldsteen K. The Impact of the Family on Health: The Decade in Review. *Journal of Marriage and the Family*, 1990, vol. 52, no. 4, pp. 1059–1078. DOI: 10.2307/353319

other's health. According to several studies, living with a partner correlates with more likely refusal from tobacco smoking, which can manifest itself both through less frequent smoking and through giving up smoking completely [19].

Material well-being as a health behavior factor has a rather complicated effect: a higher income provides better access to sport infrastructure, variable food products and healthcare services including prevention [20]. Limited financial resources lead to a situation when a person can't afford individual medical procedures or more expensive drugs [21]. When financial resources are limited, taking care of one's health often ceases to be a priority and only serious health issues can return it into the focus of attention.

According to the Human Capital Theory[4], *education* improves knowledge, skills, and other mental abilities that can be employed to promote health. As a rule, educated people have better understanding how important it is to protect health and this may result in more active involvement in prevention and overall health improvement [22]. As reported by some American researchers, higher health literacy allows people to reconsider a 'conventional' lifestyle characterized with unhealthy diets and low physical activity [23].

Analysis of work practices, their organization and peculiarities provides a theoretical and methodological framework for getting an insight into interrelations between an occupational environment and health behavior. *Occupational activities*, on one hand, give more opportunities to certain groups of working age population to pursue self-preservation; for example, some employers provide better access to qualitative healthcare services for their employees. In addition, providing workers employed at industrial enterprises with optimal diets at workplace is a highly effective measure for preventing occupational diseases and promoting health and work capacity [24]. On the other hand, long and intensive work hours can result in higher stress levels and this affects a person's health [25]. Some researchers [26] believe that workers who have to deal with intensive physical labor face higher risks of unhealthy behaviors against other occupational groups.

Another significant factor of self-preservation is *health protection intention*, which includes both assessment of current health and an intention to make it better acting as a direct determinant of behavioral intentions aimed at health protection and promotion [27]. As some researchers put it [28], people who take good care of their health usually tend to have more healthy eating habits and are less prone to stick to unhealthy diets.

Social support provided by family and friends also promotes commitment to health protection. For example, in diabetes treatment, high family support promotes more affective adherence to self-assistance [29]. Moreover, peer emotional support correlates with keeping recommended levels of physical activity in free time [30]. Interactions at workplace can also create unique contexts able to form both individual and team decisions as regards health protection behaviors. This research [31] suggests that social / organizational characteristics of the workplace environment, particularly feeling the company values the workers' health and seeing co-workers engaging in healthy behaviors, may be related to nutrition and PA behaviors and obesity.

We should remember that individual health behavior is also shaped by effects of a person's perception of health threats and their severity as well as by estimating potential advantages and barriers associated with taking health protection measures[5] [18]. *Subjective perception of safety* is an individual process, through which people identify risks and make decisions regarding them. Some studies [32] show that using safety belts in the front seat to protect health is, as a rule, less frequent inside

[4] Becker G.S. Human capital: a theoretical and empirical analysis, with special reference to education. Cambridge, MA, National Bureau of Economic Research Publ., 1964.

[5] Rosenstock I.M. The Health Belief Model and Preventive Health Behavior. *Health Education Monographs*, 1974, vol. 2, no. 4, pp. 354–386. DOI: 10.1177/109019817400200405

the city than that outside it. This is probably due to lower risk perception inside the city.

Following the research literature review, factors associated with health protection or destruction were systematized as follows for further empirical check: a) social and biological (sex and age); b) socioeconomic (marital status, having children, education, employment, and income); c) social-psychological (health intentions, social support, subjective perception of safety).

The aim of this study was to develop a mathematical model to describe multifactorial causation of self-preservation and risky behavior of working age population living in an industrial megacity.

Materials and methods. The empirical base of the study was the data obtained by a formalized survey, which was conducted in March 2025 on a sample made of Perm city residents[6]. This city is among the key industrial megacities in Russia where several huge enterprises are located including LUKOIL-Permnefteorgsintez (petrochemical production), Kamteks-Khimprom JSC (chemical production), ODK-Permskie Motory (production of aircraft engines) and others.

Working age men aged 18–62 years and women aged 18–57 years were selected from the total sample for further analysis. Bounds of the working age range were identified using the approach by the Federal Statistics Service, Perm regional office[7]. Officially, working age starts from 16 years in the Perm region just as in the country as a whole. The lower bound of the range was moved to 18 years due to absence of any people below this age among the survey participants.

Working age population was selected as the research object due to its key role in providing economic and social stability of the country; another factor is its numerical superiority, which makes it possible to spread behavioral models typical for this group, including health-related ones, onto other population groups through social interactions. According to PernStat data, as of January 01, 2024, the number of working age population in the Perm region equaled 1412.8 thousand people (for comparison: the age group younger than this age included 499.5 thousand people; older, 583.0 thousand people).

The analyzed sample included 582 people. For further computations, the sample was adjusted per sex to reach conformity with the age structure of the working age population in Perm where men accounted for 49.8 % and women for 50.2 %. The prevailing sample characteristics were as follows: 53 % of the respondents were married or lived with a partner, 59 % stated they didn't have children, 56 % had higher education, and 66 % were employed.

The behavioral concept [33] suggests the most common approach for identifying self-preservation behavior (both its positive and negative orientation). Empirically, it is interpreted as a set of behavioral practices aimed at preserving or destructing physical health and is operationalized through the following components: a) physical activity (*"how often do you do exercises or sports?"*); b) diets (*"Can you consider your diet healthy?"*); c) addictions (smoking – *"Do you smoke, even if just from time to time, cigarettes, electronic cigarettes, tobacco heating systems, vapes, hookahs, etc.?")*; alcohol intake – "*Do you drink alcohol? If yes, then how often?"*); d) medical activity *("How often do you have check-ups or periodical health examination?"*). An aggregated index S, which described individual self-preservation behavior, was created based on the consistency of these variables (confirmed with the Cronbach's alpha) and considering their transformation. Each of the component value was assigned relevant scores, the sum of which (between 5 and 25 scores) made it possible to identify a respondent's individual level of self-preservation. Higher scores indicated a respondent adhered to health protection; lower

---

[6] Conducted by the authors of the present study.

[7] Statisticheskii ezhegodnik Permskogo kraya. 2024: Statisticheskii sbornik [Statistical annual bulletin of the Perm region. 2024: Statistical data collection]. Perm, Federal Statistics Service, Perm regional office (PermStat), 2024, 337 p. (in Russian).

scores meant a respondent tended to pursue risky behavior. When working with the variables, we transformed the S index data into percentage descriptions of intensity of specific behavioral practices in the respondents from 0 to 100 to make the results more perceivable.

The following questions were used to measure self-preservation factors established by the literature review; for social-biological factors, *"What is your sex?", "How old are you?"*; for socioeconomic factors, *"What is your marital status?", "Do you have children younger than 18 years?", "What is your education?", "What do you do for a living?", "Please, tell, what description fits your financial position the best"*. In addition, some other questions were used in the analysis to establish whether a respondent had sufficient financial resources for health protection: *"Can you afford to apply for chargeable healthcare services in case of necessity?"*. To investigate social-psychological factors, the following questions were selected: *How would you describe your attitudes towards your health?"*, *"How well do you feel support (emotional, financial, or informational) provided by your family members and friends as regards health and safety issues?"* and *"Can you say you feel yourself completely safe?"*.

All data were analyzed with SPSS Statistics software package. We employed descriptive statistics methods, correlation and regression analysis to describe self-preservation and its relationships with the identified factors. The neural network procedure of the multilayer perceptron (MLP) was used to create a mathematical model, which reflected multifactorial causation of self-preservation behavior. In this procedure, hidden neurons were formed based on the linear combinations of categorical inputs, which were automatically transformed into dummy variables by dummy coding. Attributes were normalized for variables in the form of an absolute scale, for example, age.

Results and discussion. Our investigation results obtained for some specific health behaviors among working age population in Perm (hereinafter Perm residents) indicate that positive trends are prevailing. The half of the respondents does sports every day (12 %) or several times a week (38 %). In addition, 43 % of the respondents have annual check-ups or periodical medical examinations. Those who do not visit a doctor even when something is obviously wrong account for not more than 6 %. When estimating diets, most Perm residents (48 %) give it the medium score of 3, but the odds are to the positive, that is, 31 % choose the score of 4 per the scale where 1 means the diet is totally unhealthy and 5 means completely healthy. In addition, addictions are not so prevalent among Perm residents since smoking is reported by less than a half of the respondents (42 %) and the average frequency of alcohol intake is once a month or even rarer; 16 % report they do not drink alcohol at all.

The mean value of the *S* index, which describes an individual level of self-preservation behavior based on the sum of the above given components, equals 67 % out of 100 %. This means that working age Perm residents tend to pursue moderately risky health behavior. Based on this index, the respondents were divided into groups; some of them were mostly oriented at self-preservation while the others were prone to having risky health behavior (Table 1).

The next research task was to empirically test the theoretical factor structure and its feasibility as regards self-preservation. The identified social-biological factors confirmed their influence on self-preservation intensity in the sample made of Perm residents. Sex appeared to have stronger influence (Pearson's $r = 0.256$ at $p < 0.001$) than age (Pearson's $r = 0.082$ at $p < 0.05$). Only education remained a significant predictor among socioeconomic factors (Spearman's $\rho = 0.173$ at $p < 0.001$). The income level did not appear to have an expected significant effect; therefore, having financial resources for health protection was used as an indicator for the correlation (Pearson's $r = -0.139$ at $p < 0.001$). The social-psychological factors also confirmed their significance for self-preservation behavior: social support (Pearson's $r = 0.139$ at $p < 0.001$) and subjective perception of safety (Pearson's $r = 0.105$

Table 1

Description of groups with different levels of health preservation behavior

	Critically risky	Risky	Moderately risky	Health-preserving	Maximum self-preservation
Frequency of physical activity*	The group is empty, No data on mean values available	4 (rarer than once a week)	3 (once a week)	2 (several times a week)	2 (several times a week), 35 % do it every day
Estimating one's diet as healthy[8]		2.7	3.2	3.4	4.4
Smoking (%)		96 % smoke	62 % smoke	5 % smoke	100 % do not smoke
Alcohol intake*		2 (once a week)	4 (several times a months)	6 (rarer than once a month)	7 (do not drink alcohol)
Frequency of medical check-ups*		4 (when something is wrong)	2 (once every 2–3 years)	1 (every year)	1 (every year)
S index value (%)	0–10	11–50	51–70	71–90	91–100
Proportion of the respondents (%)	0	14	38	43	5

Note: * median values of the characteristics were used.

at $p < 0.001$). Health protection intention turned out to be the strongest predictor in the whole factor structure (Spearman's $\rho = -0.405$ at $p < 0.001$). The status of dependent and independent variables in the 'Factor – Self-Preservation' pair was confirmed using the Somers' D at $p < 0.001$. We also established a significant correlation between social-psychological and socioeconomic factors (Spearman's $\rho > 0.2$ at $p < 0{,}001$).

Correlation analysis became the preliminary stage in selecting relevant predictors in neural network modeling with the aim to exclude variables without significant linear relations with the dependent variable. Despite non-linearity of models built using a multilayer perceptron, preliminary filtration per correlation signs makes a model easier to interpret, mitigates a risk of over-learning, and makes further network training simpler. To minimize limitations in multi-collinearity, regression analysis was additionally performed; it confirmed its absence and allowed estimating significance of the chosen predictors.

The dependent variable was created based on the *S* index; it was represented by belonging to one of two groups: 1) the group that predominantly demonstrated risky health behavior and combined risky and moderately risky levels (see Table 1); 2) the group pursuing health preservation behavior (it combined health-preserving level and maximum health preservation level from Table 1). These groups were tentatively called 'risky' and 'self-preserving'. When estimating the explanatory power of the model, we divided the data into the training sample (70 % of the valid data[9]) and testing sample (30 % of the valid data).

The neural network model includes six hidden neurons; each of them is activated based on the linear combination of predictors with relevant weights and shift. Weights reflect a contribution made by a predictor into neuron activation. Interpretation covers the variables that are the most significant per weight; however, it is noteworthy that effects produced by the predictors on the model output are non-linear and depend on activation of other neurons and their weights in subsequent layers (Table 2).

[8] The respondents estimated their diets as 'healthy' using the scale from 1 to 5, where 1 means a diet is totally unhealthy, 5 means a diet is completely healthy (the Table provides the simple mean value).

[9] Ninety observations were excluded from computation of the multilayer perceptron; therefore, valid data are represented by 85 % of the respondents (493 people) and not the whole sample.

Table 2

Assessment of neural network model parameters

		Predicted							
Predictor		Hidden layer 1						Output layer	
Input layer		H(1:1)	H(1:2)	H(1:3)	H(1:4)	H(1:5)	H(1:6)	[O12=1]	[O12=2]
	Shift	0.229	0.408	-0.359	0.422	-0.185	-0.131		
Sex	[Q3=1]	-0.335	-0.024	-0.240	-0.342	0.637	0.601		
	[Q3=2]	-0.066	-0.316	0.088	-0.132	-0.081	-0.407		
Education	[Q8=1]	0.296	-0.188	-0.541	0.413	-0.087	0.105		
	[Q8=2]	0.362	-0.077	0.419	0.264	0.205	0.388		
	[Q8=3]	0.312	0.218	-0.281	0.339	-0.058	-0.069		
	[Q8=4]	0.236	-0.163	0.250	0.175	0.252	-0.103		
Health preservation intention	[Q13=1]	-0.126	0.299	0.156	0.411	-0.344	-0.470		
	[Q13=2]	-0.049	-0.444	0.426	-0.406	-0.353	0.308		
	[Q13=3]	0.134	0.520	-0.353	-0.415	0.311	0.352		
	[Q13=4]	-0.307	0.505	-0.512	0.034	0.395	0.515		
	[Q13=5]	0.219	0.463	0.313	-0.126	0.275	-0.166		
Perception of safety	[Q51=1]	0.083	-0.345	-0.189	-0.112	-0.042	0.301		
	[Q51=2]	-0.276	0.111	0.304	-0.011	-0.080	-0.053		
	[Q51=3]	-0.212	0.085	0.106	-0.195	0.329	-0.290		
	[Q51=4]	-0.142	0.187	-0.265	-0.349	-0.617	-0.033		
	[Q51=5]	0.413	0.454	-0.021	0.104	-0.203	-0.049		
Social support	[Q54=1]	0.223	0.465	0.337	0.241	-0.171	-0.372		
	[Q54=2]	0.008	-0.075	-0.322	0.499	-0.163	0.511		
	[Q54=3]	-0.005	-0.459	-0.177	-0.205	-0.099	0.199		
	[Q54=4]	0.308	0.249	0.179	-0.040	-0.208	-0.547		
	[Q54=5]	-0.462	0.174	0.248	-0.452	-0.452	-0.446		
Financial possibilities	[Q59=1]	-0.200	0.272	0.447	-0.412	-0.556	0.089		
	[Q59=2]	-0.468	0.193	0.203	-0.371	0.167	-0.143		
Age	Q4	-0.483	0.148	0.189	0.036	-0.707	-0.110		
Hidden layer	Shift							0.382	-0.086
	H(1:1)							-0.407	-0.340
	H(1:2)							0.418	-0.173
	H(1:3)							-0.359	0.383
	H(1:4)							0.201	-0.138
	H(1:5)							0.743	-0.357
	H(1:6)							0.364	0.050

Activation of the *first neuron (H1:1)* is predominantly determined by education (Q8): the maximum contribution is made by 'vocational education' (0.362) and 'unfinished higher education' (0.312). Activation is suppressed by such variables as male sex (Q3 = 1; -0.335), age (Q4; -0.483) and absence of financial resources to pay for chargeable healthcare services (Q59 = 2; -0.468). Social-psychological factors demonstrate rather controversial influence thereby reflecting non-linearity of the model: feeling completely safe (Q51 = 5; 0.413) and moderate social support (Q54 = 4; 0.308) strengthen activation but absolute support provided by one's family and close friends (Q54 = 5; -0.462) reduces it. Activations is also reduced by absence of health preservation intention (Q13 = 4; -0.307). As a result, the combination of the above given predictors, considering other hidden layers, reduces the contribution made by the first neuron into classification of the respondents: there is declining likelihood of being assigned into the 'risky' group (-0.407) or 'self-preserving' group (-0.340).

Activation of the *second neuron (H1:2)* is enhanced by negative social attitudes towards health (Q13 = 3, 4, 5), feeling completely safe (Q51 = 5; 0.454) and absence of social support (Q54 = 1; 0.465). The opposite effect is produced by health protection intention (Q13 = 2; -0.444), feeling unsafe (Q51 = 1; -0.345), moderate support provided by close friends and family (Q54 = 3; -0.459), and female sex (Q3 = 2; -0.316). Analysis of the output layer shows that this neuron increases likelihood of belonging to the 'risky' group (0.418) but declines activation of the 'self-preserving' group (-0.173).

The third neuron (H1:3) is characterized with polar distribution of weight coefficients for mutually exclusive variables. In particular, activation of the third neuron is enhanced by completed vocational (Q8 = 2; 0.419)[10] or higher (Q8 = 4; 0.250) education and weakened by secondary (Q8 = 1; -0.542) and incomplete higher (Q8 = 3; -0.281) education. A similar picture is observed as regards health intentions where a pronounced intention to take care of one's health makes a substantial contribution to the neuron activation (Q13 = 2; 0.426), whereas its decline occurs due to such attributed as a reactive attitude towards health, that is, attention is paid only in case of health issues (Q13 = 3; -0.353) and absence of any care about health (Q13 = 4; -0.512). In addition, the neuron activation is influenced considerably by available financial resources (Q59 = 1; 0.447), feeling unsafe (Q51 = 2; 0.304) and total absence of social support (Q54 = 1; 0.337). The neuron has a double effect on the output layer as it increases likelihood of belonging to the 'self-preserving' group (0.383) and declines it as regards the 'risky' one (-0.359).

The fourth neuron (H1:4) is activated by a responsible attitude towards health (Q13 = 1; 0.411), secondary (Q8 = 1; 0.413) or incomplete higher (Q8 = 3; 0.339) education as well as low support provided by close friends and family (Q54 = 2; 0.499). This neuron is inhibited by male sex (Q3 = 1; -0.342), an attentive (Q13 = 2; -0.406) or reactive (Q13 = 3; -0.415) attitude towards health, elevated feeling of safety (Q51 = 4; -0.349) and absence of social support (Q54 = 5; -0.452). In comparison to other neurons, output weights of this one have a very weak effect on the ultimate classification reaching 0.201 for the 'risky' group and -0.138 for the 'self-preserving' one.

Activation of the *fifth neuron (H1:5)* is enhanced by male sex (Q3 = 1; 0.637), negative health intentions (Q13 = 3; 0.311, Q13 = 4; 0.395, Q13 = 5; 0.275), higher education (Q8 = 4; 0.252) and medium safety perception (Q51 = 3; 0.329). The neuron activity is weakened by an attentive (Q13 = 2; -0.353) and responsible (Q13 = 1; -0.344) attitude towards health, elevated feeling of safety (Q51 = 4; -0.617), social support (Q54 = 5; -0.452); age (Q4; -0.707) and available financial resources (Q59 = 1; -0.556). This neuron increases likelihood of belonging to the 'risky' group (0.743) and declines it as regards the 'self-preserving' one (-0.357).

The sixth neuron (H1:6) has elevated activation for men (Q3 = 1; 0.601) and weakened for women (Q3 = 2; -0.407). Among various health intentions, the highest weights are identified for an unconcerned (Q13 = 3; 0.515), reactive (Q13 = 2; 0.352) and attentive (Q13 = 1; 0.308) attitude towards health. In addition, enhanced neuron activation is associated with low level of feeling safe (Q51 = 1; 0.301) and moderate social support (Q54 = 2; 0.511). In addition to female sex, the neuron activation is weakened by the following attributes: a responsible attitude towards health (Q13 = 1; -0.470), medium level of feeling safe (Q51 = 3; -0.290) and strong social support (Q54 = 5; -0.446, Q54 = 4; -0.547). The sixth neuron makes a positive contribution to predicting belonging to the 'risky' group (0.364) and a minimal contribution to the 'self-preserving' group (0.050).

Following the analysis results, the mathematical model that describes multifactorial causation of self-preservation behavior adopted by working age population living in an industrial megacity (exemplified by Perm) can be given as an equation for input signals of output neurons corresponding to the groups:

[10] It is worth noting that it is enhanced due to the respondents being unable to define their attitude towards health (Q13 = 5; 0.313). This is likely to be associated with the limited sample.

- 'risky': $O1 = F$ (0.382 – 0.407 · H1:1 + 0.418 · H1:2 – 0.359 · H1:3 + 0.201 · H1:4 + 0.743 · H1:5 + 0.364 · H1:6);
- 'self-preserving': $O2 = F$ (-0.086 – 0.340 · H1:1 – 0.173 · H1:2 + 0.383 · H1:3 – 0.138 · H1:4 – 0.357 · H1:5 + 0.050 · H1:6).

The type of the function F in this model is a sigma (logistic) function, which is employed to transform the combination of all signals and weight coefficients into an output value determining likelihood of belonging to one of the groups.

The analysis conducted with using the multilayer perceptron revealed a set of interrelated factors that determine people's proneness to risky or self-preservation behavior. Based on significance of each attribute, social health intentions were found to be the most significant within the created model (99 %); they were followed by sex (61.6 %), social support (56 %), financial resources (37.2 %), education (28.2 %), feeling safe (27.8 %), and age (27 %).

The model showed its capability to classify initial attributes with accuracy reaching 70 %; its predictive capability equaled 83 % for the 'self-preservation' group and 45 % for the 'risky' one.

According to our findings, a health behavior adopted by working age people living in Perm can be described as moderately risky. This is consistent with data obtained by other researchers, in particular, using a sample of workers employed at industrial enterprises in the Perm region [6]. This study found that health behaviors were moderately risky in a large proportion of the respondents (53 %).

Our analysis of self-preservation behavior established the greatest influence exerted by such factors as health protection intention, sex, social support and financial resources a person can use to get chargeable healthcare services. These conclusions are also identified in public conscience. Thus, in a study conducted in 2023 [34], healthcare workers in Moscow mentioned responsibility for one's health (22 %) and availability of healthcare services, chargeable ones included (20 %), as basic determinants of adherence to self-preservation. In the respondents' opinion, absence of support provided by close friends or family reduces motivation to pursue a healthy lifestyle.

The architecture made of six hidden neurons revealed non-linear interactions between social-biological, socioeconomic and social-psychological variables, which create latent behavioral profiles. At the same time, social-psychological factors turned out to have a dualistic effect. First of all, despite high significance of health intention as a self-preservation factor, we should remember that intensity of this factor does not always correlate with actual implementation of persistent health protection behavior. A. Brügger and others established that the respondents who took great care of their health were less prone to search for information about health after they had implemented health protection practices [35]. This is due to the fact that previously taken health protection efforts can justify the following decline in health protection activities.

Second, family and peer support acts as a health protection factor only if it is moderately intensive; maximum intensity can level off personal responsibility. In addition, a person's social relations do not always provide support; in some cases, they have a negative effect promoting unhealthy behaviors. For example, many men consider smoking a good way to maintain social relationships (with friends or colleagues) [13].

Thirdly, high subjective perception of safety increases proneness to self-preservation but any decline in the level of perception induces the inverse process. Researchers explain this regularity by the fact that outer threats can be perceived by a person as uncontrollable as regards their health; therefore, a person does nothing in relation to the existing health risk factors, that is, continues to pursue passive health protection behavior [36].

The factors described in this study are interrelated. For example, social support correlates with financial resources as regards health protection. This is due to the fact that family or peer social support is a multi-dimensional construction, which includes several types; one of them is instrumental support, in particular, granting access to financial resources [37].

Although material welfare and education are separate variables, they, as a rule, are interrelated when influencing self-preservation [38]. Thus, the role played by education in determining such behavior grows especially in case when financial resources are limited since many health protection practices involve only minimal or even zero financial costs [39].

Conclusion. Health and self-preservation issues are of special importance since they influence a person's social interaction and individual priorities and occupy a significant place in the hierarchy of social norms and values [40]. Significance of research on health behavior is becoming more and more obvious in modern industrial megacities where various stress situations and changes in the urban space create additional psychoemotional burdens requiring adaptive reactions of the body and social institutions. Although urban environments offer such advantages as closeness to work, possibility to get better education and easier access to a developed social infrastructure, Russian researchers [41] report that other conditions of an industrial environment increase health risks substantially [42]. Given that, it is quite relevant to analyze factors of self-preservation and risky behavior pursued by working age population in industrial megacities. It will help develop effective strategies aimed at resolving socioeconomic issues, in particular, raising the quality of life and protecting people's health. The created model describing multifactorial causation of self-preservation emphasizes that it is important to find a balance between outer support and internal autonomy when forming such behavior. It helps optimize preventive and self-preservation programs for various sociodemographic groups.

The study highlights complex mechanisms of interaction between factors and self-preservation behavior pointing out that behavioral practices aimed at health protection do not always manifest themselves in a systematic and sustainable form. The results substantiate the necessity to conduct investigative search for additional components forming the structure of self-preservation behavior in addition to behavioral practices.

Funding. The study was funded from the grant of the Russian Science Foundation No. 23-18-00480 'Self-Preservation Strategies of Russians in New Normal'.

Competing interests. The authors declare no competing interests.

References

1. Staykova A.P., Titopoulou M. Study on the Behavioral Health Risk Factors of Sewing Industry Workers in Bulgaria. *European Journal of Economics and Business Studies*, 2017, vol. 3, no. 2, pp. 200–211. DOI: 10.26417/ejes.v8i1.p200-211

2. Park J., Kim Y. From Workers to the Working-Age Population: A New Paradigm for the Occupational Health Service. *Saf. Health Work*, 2019, vol. 10, no. 3, pp. 251–253. DOI: 10.1016/j.shaw.2019.07.002

3. Vasilieva T.P., Larionov A.V. A Conceptual Approach to Managing the Behavioral Determinant of Public Health. *ZNiSO*, 2025, vol. 33, no. 1, pp. 7–17. DOI: 10.35627/2219-5238/202533-1-7-17 (in Russian).

4. Kleyn S.V., Onishchenko G.G., Zaitseva N.V., Glukhikh M.V. Life expectancy at birth in rf regions with diriment sanitary-epidemiological wellbeing and different lifestyles. management reserves. *Health Risk Analysis*, 2022, no. 4, pp. 18–32. DOI: 10.21668/health.risk/2022.4.02.eng

5. Zubok Yu.A., Prokazina N.V. The attitude of young people to the new coronavirus infection: the dynamics of ideas and practices of health-saving behavior. *Problemy sotsial'noi gigieny, zdravookhraneniya i istorii meditsiny*, 2021, vol. 29, no. s1, pp. 752–757. DOI: 10.32687/0869-866X-2021-29-s1-752-757 (in Russian).

6. Ryazanova E.A. To the problem of typology of the riskogenic behaviour (analysis on the example of an industrial enterprise of Perm region). *Health Risk Analysis*, 2016, no. 2, pp. 68–75. DOI: 10.21668/health.risk/2016.2.08.eng

7. Kislitsyna O.A. Social inequality in the health status of Russians: the role of material, psychosocial and behavioral factors. *Sotsial'nye aspekty zdorov'ya naseleniya*, 2023, vol. 69, no. 3, pp. 7. DOI: 10.21045/2071-5021-2023-69-3-7 (in Russian).

8. Korolenko A.V. Patterns of population's self-preservation behavior: research approaches and building experience. *Economic and Social Changes: Facts, Trends, Forecast*, 2018, vol. 11, no. 3, pp. 248–263. DOI: 10.15838/esc.2018.3.57.16

9. Kovaleva A.A. Samosokhranitel'noe povedenie v sisteme faktorov, okazyvayushchikh vliyanie na sostoyanie zdorov'ya [Self-preservation behavior in the system of factors influencing health]. *Zhurnal sotsiologii i sotsial'noi antropologii*, 2008, vol. XI, no. 2, pp. 179–191 (in Russian).

10. Nazarova I.B. Monitoring of the population health and health risk factors (research methodology). *Vestnik RUDN. Seriya: Sotsiologiya*, 2022, vol. 22, no. 3, pp. 616–629. DOI: 10.22363/2313-2272-2022-22-3-616-629 (in Russian).

11. Rostovskaya T.K., Ivanova A.K., Kniazkova E.A. The practice of self-preserving behavior of the citizen of the Russian Federation: the results of the All-Russian sociological study "The demographic self-feeling of Russia". *Problemy sotsial'noi gigieny, zdravookhraneniya i istorii meditsiny*, 2021, vol. 29, no. 1, pp. 66–75. DOI: 10.32687/0869-866X-2021-29-1-66-75 (in Russian).

12. Yanitsky O. The reasons and consequences of modern war: a sociological view. *Institute of Sociology of Russian Academy of Sciences*, 2015, 172 p. Available at: http://www.isras.ru/publ.html?id=3298 (April 29, 2025) (in Russian).

13. Bush J., White M., Kai J., Rankin J., Bhopa R. Understanding influences on smoking in Bangladeshi and Pakistani adults: community based, qualitative study. *BMJ*, 2003, vol. 326, no. 7396, pp. 962. DOI: 10.1136/bmj.326.7396.962

14. Barakhoeva R.B. Factors Influencing the Level of Formation of Self-Preservation Behaviour. *Mezhdunarodnyi nauchno-issledovatel'skii zhurnal*, 2023, no. 12 (138). DOI: 10.23670/IRJ.2023.138.67 (in Russian).

15. Danilova A.A., Zabelina E.V., Telitsyna A.Y., Chestyunina Y.V. Factors of Subjective Health: Structural Model in the Period of Late Socialization. *Monitoring obshchestvennogo mneniya: ekonomicheskie i sotsial'nye peremeny*, 2023, no. 6, pp. 144–170. DOI: 10.14515/monitoring.2023.6.2399 (in Russian).

16. Vangorodskaya S.A. The factors of self-preservation behavior of the population in the region (based on empirical studies). *Nauchnyi rezul'tat. Sotsiologiya i upravlenie*, 2018, vol. 4, no. 2, pp. 13–26. DOI: 10.18413/2408-9338-2018-4-2-0-2 (in Russian).

17. Ryu H., Moon J., Jung J. Sex Differences in Cardiovascular Disease Risk by Socioeconomic Status (SES) of Workers Using National Health Information Database. *Int. J. Environ. Res. Public Health*, 2020, vol. 17, no. 6, pp. 2047. DOI: 10.3390/ijerph17062047

18. Helgesson M., Marklund S., Gustafsson K., Aronsson G., Leineweber C. Favorable Working Conditions Related to Health Behavior Among Nurses and Care Assistants in Sweden – A Population-Based Cohort Study. *Front. Public Health*, 2021, vol. 9, pp. 681971. DOI: 10.3389/fpubh.2021.681971

19. Bagisheva N.V., Mordyk A.V., Nesterova K.I., Goltyapin V.V., Aroyan A.R., Rudenko S.A., Shirinskaya N.V. Factor analysis of socio-demographic preconditions for tuberculosis development in patients with chronic obstructive lung disease. *Vestnik sovremennoi klinicheskoi meditsiny*, 2021, vol. 14, no. 1, pp. 7–15. DOI: 10.20969/VSKM.2021.14(1).7-15 (in Russian).

20. Pokida A.N., Zybunovskaya N.V. Health in the Perception of Russians and Real Medical Practices. *ZNiSO*, 2021, vol. 29, no. 7, pp. 19–27. DOI: 10.35627/2219-5238/2021-29-7-19-27 (in Russian).

21. Basova E. Regional Inequality in the Context of the Availability of Socially Significant Benefits: Comparative Assessment. *The Journal of Social Policy Studies*, 2022, vol. 20, no. 1, pp. 83–98. DOI: 10.17323/727-0634-2022-20-1-83-98 (in Russian).

22. Ryazantsev S.V., Khramova M.N., Smirnov A.V. The social demographic aspects of vaccination of population of Russia in the context of COVID-19 pandemic. *Problemy sotsial'noi gigieny, zdravookhraneniya i istorii meditsiny*, 2021, vol. 29, no. 5, pp. 1047–1056. DOI: 10.32687/0869-866X-2021-29-5-1047-1056 (in Russian).

23. Cook W.K., Libo L., Tam C.C., Mulia N., Kerr W.C. Associations of clustered health risk behaviors with diabetes and hypertension in White, Black, Hispanic, and Asian American adults. *BMC Public Health*, 2022, vol. 22, no. 1, pp. 773 DOI: 10.1186/s12889-022-12938-y

24. Zaitseva N.V., Alekseev V.B., May I.V., Kiryanov D.A., Zemlyanova M.A., Khismatullin D.R., Peskova E.V. Problem Searching approaches to nutrition optimization to increase the potential of life expectancy taking into account bioinformatic signals of the human regulatory system. *Gigiena i sanitariya*, 2024, vol. 103, no. 12, pp. 1494–1503. DOI: 10.47470/0016-9900-2024-103-12-1494-1503 (in Russian).

25. Kozina I.M., Serezhkina E.V. Measuring Psychosocial Risks and Quality of Work Life Among Russian Professionals. *Monitoring obshchestvennogo mneniya: ekonomicheskie i sotsial'nye peremeny*, 2021, no. 3, pp. 21–40. DOI: 10.14515/monitoring.2021.3.1891 (in Russian).

26. Pokida A.N., Zybunovskaya N.V. Differences in Behavioral Practices of Health Maintenance and Promotion between Knowledge and Manual Workers. *ZNiSO*, 2022, no. 9, pp. 18–28. DOI: 10.35627/2219-5238/2022-30-9-18-28 (in Russian).

27. Wang Y., Wen X., Zhu Y., Xiong Y., Liu X. Chinese Residents' Healthy Eating Intentions and Behaviors: Based on an Extended Health Belief Model. *Int. J. Environ. Res. Public Health*, 2022, vol. 19, no. 15, pp. 9037. DOI: 10.3390/ijerph19159037

28. Pokida A.N., Zybunovskaya N.V. Food Culture of the Russian Population: Results of a Sociological Survey. *ZNiSO*, 2022, vol. 30, no. 2, pp. 13–22. DOI: 10.35627/2219-5238/2022-30-2-13-22 (in Russian).

29. Huang H.-T., Kuo Y.-M., Wang S.-R., Wang C.-F., Tsai C.-H. Structural Factors Affecting Health Examination Behavioral Intention. *Int. J. Environ. Res. Public Health*, 2016, vol. 13, no. 4, pp. 395. DOI: 10.3390/ijerph13040395

30. Haidar A., Ranjit N., Archer N.P., Hoelscher D.M. Parental and peer social support is associated with healthier physical activity behaviors in adolescents: a cross-sectional analysis of Texas School Physical Activity and Nutrition (TX SPAN) data. *BMC Public Health*, 2019, vol. 19, pp. 640. DOI: 10.1186/s12889-019-7001-0

31. Tabak R.G., Hipp J.A., Marx C.M., Brownson R.C. Workplace Social and Organizational Environments and Healthy-Weight Behaviors. *PLoS One*, 2015, vol. 10, no. 4, pp. e0125424. DOI: 10.1371/journal.pone.0125424

32. Malekpour F., Moeini B., Tapak L., Sadeghi-Bazargani H., Rezapur-Shahkolai F. Prediction of Seat Belt Use Behavior among Adolescents Based on the Theory of Planned Behavior. *J. Res. Health Sci.*, 2021, vol. 21, no. 4, pp. e00536. DOI: 10.34172/jrhs.2021.71

33. Popova L.A., Milaeva T.V., Zorina E.N. Self-preservation behavior: generational aspect. *Economic and Social Changes: Facts, Trends, Forecast*, 2021, vol. 14, no. 5, pp. 261–276. DOI: 10.15838/esc.2021.5.77.15

34. Lokosov V.V., Yarasheva A.V., Aleksandrova O.A. Factors forming the foundations for self-preservation behavior of the population. *Narodonaselenie*, 2024, vol. 27, no. 1, pp. 192–205. DOI: 10.24412/1561-7785-2024-1-192-205 (in Russian).

35. Brügger A., Höchli B. The Role of Attitude Strength in Behavioral Spillover: Attitude Matters – But Not Necessarily as a Moderator. *Front. Psychol.*, 2019, vol. 10, pp. 1018. DOI: 10.3389/fpsyg.2019.01018

36. Shlykova E.V. Otnoshenie k risku kak differentsiruyushchii faktor vybora sposoba vynuzhdennoi adaptatsii [Attitude to risk as a differentiating factor in selecting a method of forced adaptation]. *Sputnik ezhegodnika «Rossiya reformiruyushchayasya»*, 2016, iss. 2, pp. 1–15 (in Russian).

37. Lakon C.M., Zheng Y., Pechmann C. Social network tie functions of social support and social influence and adult smoking abstinence. *PLoS One*, 2024, vol. 19, no. 3, pp. e0296458. DOI: 10.1371/journal.pone.0296458

38. Kawachi I., Adler N.E., Dow W.H. Money, schooling, and health: Mechanisms and causal evidence. *Ann. N Y Acad. Sci.*, 2010, vol. 1186, pp. 56–68. DOI: 10.1111/j.1749-6632.2009.05340.x

39. Kraft P., Kraft B., Hagen T., Espeseth T. Subjective Socioeconomic Status, Cognitive Abilities, and Personal Control: Associations With Health Behaviours. *Front. Psychol.*, 2022, vol. 12, pp. 784758. DOI: 10.3389/fpsyg.2021.784758

40. Galkina E.P., Kadnichanskaya M.I., Vlasova N.V. Typology of self-preservation behavior of the population in a pandemic. *Logos et Praxis*, 2022, vol. 21, no. 3, pp. 144–155. DOI: 10.15688/lp.jvolsu.2022.3.15 (in Russian).

41. Aksenova E.I., Kamynina N.N., Turzin P.S. Urban stress of a megalopolis resident: stress factors and correction. *Problemy sotsial'noi gigieny, zdravookhraneniya i istorii meditsiny*, 2023, no. 31 (s2), pp. 1087–1091. DOI: 10.32687/0869-866X-2023-31-s2-1087-1091 (in Russian).

42. Zaitseva N.V., Kostarev V.G., Kleyn S.V., Glukhikh M.V., Kiryanov D.A. Reserves and limitations of the gain in life expectancy at birth for people in an industrially developed region associated with losses due to circulatory diseases. *Zdravookhranenie Rossiiskoi Federatsii*, 2024, vol. 68, no. 6, pp. 480–487. DOI: 10.47470/0044-197X-2024-68-6-480-487 (in Russian).

Gordeeva S.S., Sharypova S.Yu., Shlyapina A.S. Factors of self-preservation and risky behavior of employable population in an industrial megalopolis. Health Risk Analysis, 2025, no. 2, pp. 60–72. DOI: 10.21668/health.risk/2025.2.05.eng

Received: 10.03.2025
Approved: 06.05.2025
Accepted for publication: 14.06.2025

UDC 613; 614
DOI: 10.21668/health.risk/2025.2.06.eng

Read online

Review

FACTORS AND STRATEGIES FOR REDUCING HEALTH RISKS FOR FOREIGN STUDENTS ATTENDING RUSSIAN UNIVERSITIES

L.V. Kirichenko[1], M.V. Kilanova[1], M.V. Glukhikh[1,2]

[1]Perm State Medical University named after Academician E.A. Wagner, 26 Petropavlovskaya St., Perm, 614000, Russian Federation
[2]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation

At present, investigating influence exerted by factors of the intra-university environment on foreign students' health is a relevant research trend. The aim of this study was to summarize and analyze literary data devoted to investigating health risk factors for foreign students associated with studying at Russian universities, analyzing adaptation strategies aimed at improving physical and mental well-being of foreign citizens and increasing effectiveness of their education.

The study was designed as a descriptive review, which implied qualitative description of scientific literature selected per established keywords. Overall, we analyzed 325 articles; of them, 62 were covered by the present review.

The article outlines certain challenges that foreign students may face during their studies at a higher educational institution. Modern higher education is characterized by academic mobility. The number of foreign students in higher education institutions ranges between 10 and 40 %. Foreign students who come from countries with different geoclimatic and cultural traditions have to go through a long adaptation period.

The intra-university environment was revealed to include a complex of multifaceted factors, namely, educational space, social sphere, physical, technological, financial and psychological aspects. Each factor contributed not only to academic success of foreign students, but also to their personal development and integration into the society.

Success of the educational process for foreign citizens was established to depend on education conditions, time and quality of their adaptation period as well as professional competence of teaching staff.

In addition, health of international students was proven to be an important aspect that included physical, psychological and social well-being. Being influenced by the intra-university environment, students may be susceptible to diseases of the nervous and musculoskeletal systems, respiratory organs, eyes and their adnexa, gastrointestinal tract, as well as parasitic diseases.

Based on the analyzed Russian and foreign works, we can conclude that the intra-university environment is a health risk factor for foreign students

Keywords: *foreign students, risk factors, health, adaptation, intra-university environment, higher education, student mobility.*

At present, the ongoing globalization that involves *"…flows of technologies, economy, knowledge, people, values and ideas…"* promotes, among other things, academic mobility of scientists, teachers, and students[1] [1]. This phenomenon when higher education becomes truly international attracts more and more researchers' attention

Larisa V. Kirichenko – Doctor of Medical Sciences, Head of the Department of Common Hygiene and Specialized Hygiene Disciplines (e-mail: lkv-7@yandex.ru; tel.: +7 (342) 212-10-07; ORCID: https://orcid.org/0000-0001-6306-1757).

Mariya V. Kilanova – lecturer at the Department of Common Hygiene and Specialized Hygiene Disciplines (e-mail: marya.kilanova@yandex.ru; tel.: +7 (342) 212-09-13; ORCID: https://orcid.org/0009-0001-3182-0867).

Maxim V. Glukhikh – Candidate of Medical Sciences, Senior Researcher at the Department for Systemic Procedures of Sanitary-Hygienic Analysis and Monitoring (e-mail: gluhih@fcrisk.ru; tel.: +7 (342) 237-18-04; ORCID: https://orcid.org/0000-0002-4755-8306).

[1] Quality and Internationalisation in Higher Education: Report. In: H. De Wit, J. Knight eds. *OECD*, 1999, 272 p. DOI: 10.1787/9789264173361-en

in its theoretical and practical aspects [2]. Its occurrence has stimulated academic interest involving relevant growth in the body of research [3] using various theories and methods (Push Pull Model, Human Capital Theory, Signaling Theory etc.) on the matter in various contexts (countries) [4]. Over the last decades, several waves of student mobility have been observed; among other things, they are thought to be associated with several global crises [5].

In accordance with the valid Order[2] signed by the President of the Russian Federation (RF), one of the tasks to be solved is to "*increase by not less than twofold the number of foreign citizens who study at higher education institutions* …" together with "*… implementing relevant measures aimed at providing employment for the best of them in the Russian Federation*". To achieve this, the Export of Education Federal Project[3] was developed and implemented; it fixed the target increase in the number of such students up to 425 thousand by 2024. The latest valid document[4], which sets national goals of the development of the Russian Federation, stipulates "*an increase in the number of foreign students by 2030, … up to not less than 500 thousand people*". The first goal as an image-related one is to make Russian education more attractive at the international level. Apart from that, these tasks allow resolving some economic issues as regards growing non-commodity exports as well as integrating the most successful graduates in the Russian economy. In this respect, Russian HEIs are actively involved in education of foreign students, who will play a significant role in creating intellectual and innovative potential of both their own countries and the Russian Federation.

According to the data provided by the Ministry of Science and Higher Education of the Russian Federation, the number of foreign students in Russian HEIs has been growing steadily and amounted to 429.4 thousand people[5] as of January 2025, which is 33.7 % higher against 2018 (321.3 thousand people). This fact may indicate that Russian education is becoming more popular on the international education market. Accordingly, the Russian educational system should be ready for this predicted growth in the number of foreign students considering peculiarities of their adaptation to new conditions.

Apart from the necessity to adapt to health-affecting factors that are common for all HEI students, foreigners have to adapt to new sociocultural and climatic conditions, educational systems and traditions. This requires more reserves and capabilities from their bodies as opposed to 'native' students.

Moving to another country involves the necessity to overcome the language barrier, to adapt to a new unfamiliar education sys-

---

[2] O natsional'nykh tselyakh i strategicheskikh zadachakh razvitiya Rossiiskoi Federatsii na period do 2024 goda: Ukaz Prezidenta RF ot 7 maya 2018 g. № 204 [On national goals and strategic tasks of the Russian Federation development for the period up to 2024: the RF President Order dated May 07, 2018 No. 204]. *Prezident Rossii*. Available at: http://www.kremlin.ru/acts/bank/43027 (April 30, 2025) (in Russian).

[3] Pasport natsional'nogo proekta «Obrazovanie», utv. Prezidiumom Soveta pri Prezidente RF po strategicheskomu razvitiyu i natsional'nym proektam; protokol ot 24.12.2018 № 16 [The profile of the Education National Project, approved by the Presidium of the RF President Council on strategic development and national projects; the meeting report dated December 24, 2018 No. 16] (in Russian).

[4] O natsional'nykh tselyakh razvitiya Rossiiskoi Federatsii na period do 2030 goda i na perspektivu do 2036 goda: Ukaz Prezidenta Rossiiskoi Federatsii ot 7 maya 2024 g. № 309 [On national goals the Russian Federation development for the period up to 2030 and future prospect up to 2036: the RF President Order dated May 07, 2024 No. 309]. *Prezident Rossii*. Available at: http://www.kremlin.ru/acts/bank/50542 (April 30, 2025) (in Russian).

[5] Kolichestvo inostrannykh grazhdan, obuchayushchikhsya v organizatsiyakh, osushchestvlyayushchikh obrazovatel'nuyu deyatel'nost' po obrazovatel'nym programmam vysshego obrazovaniya [The number of foreign students accepted in higher educational institutions]. *EMISS: state statistics*. Available at: https://fedstat.ru/indicator/59950# (March 02, 2025) (in Russian).

tem and changes in customary diets and lifestyles as well as a psychological stress caused by loss of close friends and family.

These factors can have a negative effect on foreign students' physical and mental health deteriorating their academic performance and quality of life [6].

The available publications with their focus on adaptation of foreign students mostly concentrate on social and psychological aspects such as overcoming a cultural shock, integration into a student society, and development of cross-cultural competence [7].

Still, not enough attention is paid to investigating effects produced by learning conditions in Russian higher educational institutions on foreign students' health and to developing effective adaptation strategies aimed at protecting and promoting their physical and mental wellbeing. Failure to adequately estimate effects produced by risk factors related to the intra-university environment on foreign students' health may lead to an increase in incidence, poor academic performance, and, ultimately, expulsion from a HEI and health issues.

Given that, it seems necessary to perform a systemic analysis of risk factors for foreign students' health associated with studying in Russian HEIs as well as to develop complex adaptation programs aimed at mitigating negative effects produced by such factors.

The aim of this review was to summarize and analyze Russian and foreign literary data devoted to investigating health risk factors for foreign students associated with studying in Russian universities, as well as analyzing adaptation methods (strategies) aimed at improving physical and mental wellbeing of foreign students and increasing effectiveness of their education.

The following tasks were set:

– to identify and classify basic health risks for foreign students associated with studying in Russian HEIs (academic, sociocultural, economic, and medical);

– to estimate effects of these factors on foreign students' physical and mental health;

– to analyze the existing approaches and adaptation strategies adopted by foreign students in Russian HEIs.

Materials and methods. This review is a narrative (descriptive) analysis of research literature with its focus on investigating effects produced by education conditions in Russian universities on foreign students' health.

Information sources were represented by research articles published in Russian and English in leading databases such as PubMed, Scopus, Web of Science, eLIBRARY.ru, as well as proceedings of conferences, monographs and reports available in open access. Search for literature sources relied on using key words that reflected the research subject such as 'foreign students', 'adaptation', 'risk factors', 'health', 'higher education institution', and their synonyms, including those in English. The search was not limited to a certain time period so that we would be able to cover the maximum wide range of research articles on the issue. Overall, we selected 325 articles from literature sources relying on the selected key words. This review covers 64 of them based on their availability and completeness of the reported results.

Since we selected a descriptive type for this review, we did not use strict inclusion / exclusion criteria for the analyzed sources. Materials were selected per their relevance to the research subject and quality of the data they provided. The priority was given to studies, which describe experience gained by Russian universities, as well as articles reporting the results of empirical research and systemic reviews. We did not conduct quantitative analysis of the research results reported in the analyzed sources; primarily, attention was paid to qualitative analysis and systematization of information about health risk factors for foreign students and their adaptation strategies.

Our analysis involved systematizing information about risk factors classified per several categories (academic, sociocultural, economic, and medical) as well as estimating effectiveness of various adaptation strategies for foreign students described in the analyzed literature sources.

Results and discussion. ***Health risk factors for foreign students in Russian higher education institutions.*** Intra-university environment factors have multi-aspect influence on foreign students, among other things, causing risks for their physical and mental health during the whole study period. These factors can be tentatively divided into the following categories: academic (related to the educational process), sociocultural, economic, and medical-ecological ones.

Academic factors. The Russian higher education is often described as highly intensive involving a substantial volume of academic hours (more than 1200 lecture hours per year) and self-studies (33 % of the total academic hours). This may result in fatigue, stress, poorer work ability, chronic lack of sleep, and, consequently, weakened immunity and greater susceptibility to communicable diseases. Thus, studies accomplished in the N.F. Katanov's Khakassia State University reported signs of transitory immune deficiency in 43 % of the examined students. First- and second-year students in northern HEIs were found to have immunoglobulin A deficiency and more active reactions of lymph proliferation; strain of cellular-mediated protection mechanisms grew in senior students [8, 9].

Mastery of Russian language is a major obstacle for foreign students when they attend Russian HEIs. It creates difficulties in comprehending lectures, accomplishing study tasks, and passing exams; foreign students start to feel self-doubt and anxiety, their self-esteem goes down and academic performance becomes poor [10–13].

When studying at university, foreign students can face an educational system that is unfamiliar to them and rather different from one in their home country, including the structure of the educational process, control forms, and assessment methods. This requires adaptation of higher nervous activity to these new demands thereby increasing stress-inducing effects on the body [14, 15].

Absent or insufficient support provided by teachers can aggravate adaptation difficulties and this affects academic performance and motivation to continue studies [16–18].

Differences in educational programs adopted in primary and secondary school in different countries can result in inconsistency between the initial knowledge of foreign students and requirements made to this knowledge in Russian universities. This requires some additional efforts from them to eliminate gaps in their knowledge and can also induce some stress states [19].

Sociocultural factors. Studies to get a specialist, bachelor, or a master degree take quite a long time (from two to six years) and are directly connected with a place where students live as well as social and cultural environment around them, which can be health-protecting factors in some situations and health risk factors in others.

Moving to another country, getting acquainted with a new culture, traditions, habits, behavioral patterns, and values can lead to a 'cultural shock' when a person feels disoriented, anxious, disappointed and irritated [20]. Difficulties of cross-cultural communication associated with the language barrier and differences in communication styles can result in failure to understand each other, conflicts, and feeling alienated [21–23].

Being cut off from one's family, friends, and a customary social environment, difficulties in making social contacts in a new country can make foreign students feel lonely, socially isolated, and depressed [24–26].

In addition, foreign students can face discrimination, biased attitudes, racism and xenophobia, which affects their self-esteem, mental state and social integration [27–29].

Absence of a developed social support system for foreign students (for example, some mentor programs or mutual aid groups) can aggravate adaptation challenges and decrease feeling of safety and comfort [30, 31].

Economic factors. High costs of education and accommodation, low scholarships, and difficulties in finding a job can result in financial problems, limited access to healthcare services, low quality diets and poor living conditions [32, 33].

Living in dormitories with very small comfort, long distances between housing and a university, high rental costs (between 15 and 40 thousand rubles) can have negative effects on foreign students' physical and mental health [34].

Poor knowledge about local cuisine / limited choice of foods, high food prices (in 2025, the minimum expenditure basket costs 7326 rubles) can lead to imbalanced diets, deficiency of macro- and microelements and, as a result, health deterioration [35, 36].

Medical and ecological factors. Most part of Russia has very pronounced climatic seasonality with clear division into cold and warm periods with respective changes in insolation, temperature and humidity. Depending on a Russian region, air temperatures and humidity can vary between -50 °C in cold season to 35 °C and higher in warm one. These circumstances are associated with multiple other factors: insufficient illumination and insolation that promote vitamin D deficiency, especially in foreign students who come from countries with warm climate (prevalence of vitamin D deficiency is 60–90 %); poor availability of fresh vegetables and fruits (grown on open ground) in some seasons; endemic diseases (tick-borne virus encephalitis, hemorrhagic fevers, etc. with frequency equal to 5–10 cases per 1000 foreign students, especially in spring and summer). Each such factor can be a potential health risk factor. Therefore, moving to another climatic zone involves forced acclimatization, adaptation to a new day regime, diets, and other living conditions, which can influence physical and mental health of foreign students [37].

For foreign students, moving to another country involves not only changes in weather and climate but also other customary environmental factors (quality of ambient air, drinking water, and soils, noise levels) especially in urbanized areas where universities are located the most often and where sanitary-epidemiological problems are much more likely to occur[6].

Living in dormitories with high population density (2–6 people per room; depending on a dormitory type, 80–90 % of the places in them are filled during an academic year), contacts with many people, failure to stick to sanitary and personal hygiene rules or absence of any knowledge about them can create elevated risks of communicable diseases, especially during early adaptation periods [38, 39].

Difficulties in getting medical insurance, the language barrier in communication with doctors, and absence of knowledge about the healthcare system in Russia can make it difficult for foreign students to get medical assistance on time creating a delay in diagnosing and treating a disease[7].

---

[6] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2023 godu: Gosudarstvennyi doklad [On sanitary-epidemiological well-being of the population in the Russian Federation in 2023: the State Report]. Moscow, Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2024, 364 p. (in Russian).

[7] Miller D.F., Harwell D.J. International students at an American university: health problems and status. *J. Sch. Health*, 1983, vol. 53, no. 1, pp. 45–49. DOI: 10.1111/j.1746-1561.1983.tb04053.x

Ultimately, stress, anxiety, and depression caused by adaptation to new conditions are health risk factors in themselves and can stimulate development of psychosomatic disorders (headaches, stomachaches, sleep disorders, etc.) [40–42].

Effects produced by factors of the intra-university environment on foreign students' health. Exposure to all the foregoing health risk factors can lead to elevated morbidity among foreign students if relevant adaptation measures have not been implemented. This morbidity can be manifested through various diseases.

Diseases of the nervous system. During the first study year, students tend to experience chronic stress upon exposure to some intra-university environment conditions identified as risk factors. This stress induces changes in some morphofucntional indicators of the body such as body mass index, vital capacity, blood pressure, and heart rate. It can be caused by such education-related factors as high educational loads, poor academic performance, inability to systematize newly acquired knowledge, sleeplessness, low interest in a chosen specialty, poor living conditions, and conflicts with peers and teachers. V.V. Ruzhenkova mentions in her article that frequency of neurotic states and stress-related somatoform disorders reaches 15 % among students. In addition, some researchers report that students with low resistance to stress are susceptible to anxiety and depression (10–15 %), are more irritable and emotionally unstable [43–46].

Diseases of the musculoskeletal system and connective tissue. Modern lifestyles adopted by students including foreign ones determine high morbidity of diseases of the musculoskeletal system (bad posture, scoliosis, etc.) caused by high educational loads combined with low physical activity. Many studies report that frequency of diseases of joints and musculoskeletal system can reach approximately 10–20 % among foreign students. High stress levels related to studying and cultural adaptation stimulate muscle tension; gradual change in foreign students' eating habits is accompanied with lower blood calcium and vitamin D, which accordingly affects bone and joint health [47].

Diseases of the respiratory system. Studies at university involve frequent contacts with other people in the educational environment; consequently, this involves higher risks of airborne and droplet infections. Frequency of such diseases is also determined by the fact that foreign students' immunity often does not correspond to an epidemiological situation in a new place of living as regards presence of specific antibodies and is not ready for new communicable diseases and species diversity of microorganisms. Given that, frequency of such diseases reaches 30–40 % among foreign students [48].

Diseases of the eye and adnexa. Diseases of the eye and adnexa (myopia, 15–25 %; allergic conjunctivitis, 10–15 %; dry eye syndrome, 20–30 %) hold the leading place per morbidity levels among students including foreign ones. Students fail to keep a good posture during classes or study in lecture rooms with insufficient illumination, which, in its turn, affects their eyes. More frequent use of digital technologies and mobile devices can promote development of such pathologies as accommodation disorders, weaker eyesight, myopia included, computer vision syndrome, and dry eye syndrome [49].

Diseases of the digestive system, endocrine, nutritional and metabolic diseases. Issues related to having a healthy and balanced diet are a significant aspect of foreign students' health. N.A. Agadzhanyan points out that *"according to the recommendations by the UN Committee on Nutrition and Calorie Needs, each 10 °C decline in mean monthly temperature requires a 5 % rise in caloric contents if +10 °C is taken as the initial level"* [35]. Based on that, discrepancy between initial diets of foreign students and diets they usually have in Russia can be a risk factor that causes or aggravates already existing diseases of the digestive system, frequency of which reaches 20–40 % among foreign students [50, 51].

Some infectious and parasitic diseases. Infectious and parasitic diseases are another significant disease category among foreign students. Some studies use people who often travel for a long time as an example to show that the gut microbiota composition can change due to changing a place of living and this can lead to dysbiotic and immune-deficient states [52] creating additional adaptation costs for foreign students. Major risk factors of infectious and parasitic diseases include absence or poor awareness about infectious agents and pathways of infection transfer as well as failure to follow personal hygiene rules, partially or completely. Combined with unfavorable sociocultural and economic factors, these conditions can decrease the overall sanitation level thereby increasing likelihood of getting infected and spreading communicable diseases and parasitic invasions among this student group (5–15 % of foreign students from warm countries have infections or parasites) [53]. On the other hand, living in Russia can decrease likelihood of some vaccine-managed infections (measles, rubella, poliomyelitis, etc.) and sanitation-caused parasitic diseases (helminthiasis) for foreign students relative to countries they come from.

Approaches to foreign students' adaptation. Health risk factors and states / diseases they are able to cause are numerous and diverse. Bearing this in mind, we should remember that effectiveness of adaptation activities provided for foreign students depends on a complex approach including language training and support, sociocultural adaptation, psychological support, medical support, financial support, and providing better housing.

Language training and support. Organizing and developing intensive Russian language courses is an important stage in training provided for foreign students prior to the study period as well as during it. This approach creates a solid language base and self-confidence for successful learning. In addition, providing learning materials in several languages promotes better perception and understanding of the Russian language. Any complications that arise within the educational process can be easily overcome by having access to resources in different languages. Special attention is paid to language support, providing students with interpreters' and tutors' services, which creates comfortable learning conditions and helps master new knowledge more effectively. Access to such specialists allows explaining difficult subjects and minimizes language barriers. Language clubs and talk groups create additional opportunities for practicing the language among foreign students. In such informal situation, students can discuss various topics, share their experience and master their language skills, which, in its turn, promote their language development and confidence in communication using the Russian language [54].

Sociocultural adaptation. Organization of on-boarding courses in Russian history, culture and traditions plays a key role in foreign students' adaptation to new conditions. Such courses not only help them to get acquainted with Russia's rich heritage but provide a better insight into the Russian society. No doubt, this enriches the educational process, facilitates adaptation and potential integration of future experts into the society. Various cultural events, excursions and festivals are an important component in adaptation since they help foreign students to not only know Russian culture and the society better but also actively interact with locals. Creation of mentor programs aimed at supporting foreign students can facilitate adaptation considerably and help first-year students to enter the educational process and student life more successfully. Senior students can act as such mentors / tutors by sharing their experience and knowledge. Meetings with representatives of various national diasporas are also an important aspect of students' adaptation, which creates opportunities for sharing cultural experience and helps students to feel themselves a part of a multi-national society. This undoubtedly promotes their personal development and social integration [55–57].

Psychological support. An opportunity to get psychological consultations and group therapy in a native language is another adaptation aspect aimed at providing necessary support for foreign students. An insight into cross-cultural differences and language barriers can have substantial influence on adaptation quality; therefore, it is necessary to create a safe space where students can openly discuss their feelings, fears and anxiety in their native language. This facilitates deeper and more sincere communication, which, in its turn, promotes positive adaptation. It is also very important to organize trainings in stress-management, adaptation to a new environment and cross-cultural communication. Such trainings aim to develop skills necessary for foreign students since they help tackle challenges associated with staying in a foreign country. Creation of psychological support services in universities logically continues adaptation. Access to qualified mental therapy should be open and available to all students [58, 59].

Medical support. Providing foreign students with available healthcare services includes many aspects facilitating their comfortable and safe staying in a new country. Special attention is paid to possibility to communicate with healthcare workers using a native language; this allows overcoming language barriers and facilitates better understanding between doctors and patients. In addition, periodical medical check-ups, vaccination and information campaigns about healthy lifestyles are a significant component in health promotion and successful adaptation of foreign students [60].

Financial support. Scholarships and grants for foreign students reduce financial burdens and allow them concentrate their efforts on learning. Such programs can be aimed at attracting talented students and creating a diverse educational environment. Help in finding an additional income source can also be a considerable adaptation element. Another significant factor is consulting foreign students on financial planning and managing their budgets. This is especially important for people who have to face unclear / unfamiliar peculiarities of the financial system existing in a host country [5, 61].

Providing better housing. Foreign students' adaptation involves providing them with comfortable living conditions, which play a key role in their integration into the educational process. It can resolve several related issues. Providing students with places in dormitories includes creation of comfortable and safe conditions, which facilitate their socialization. Dormitories should consider cultural specificity of foreign students and be equipped with all necessities including communal areas for rest and studies so that students can easily communicate with each other and share their experience [62, 63].

Analysis of available research literature has revealed that studying at Russian universities can create elevated health risks for foreign students. A complex approach is advisable for effective adaptation and diseases prevention. It should consider academic, sociocultural, economic and medical and ecological health risks for foreign students. Effectiveness of adaptation programs directly depends on creating favorable learning and living conditions as well as proving available and qualitative healthcare services.

Our findings expand the overview of possible risk factors and adaptation issues for foreign students and this allows developing more effective strategies for resolving them. They can be used for developing new adaptation programs for foreign students in Russian HEIs or updating the existing ones as well as for developing methodical guidelines for teachers, experts of international departments and other specialists working with foreign students.

Study limitations and prospects. Despite our effort to conduct a comprehensive analysis, this literature review has certain limitations caused by the selected study design:

1. Data selection and interpretation are subjective. In contrast to systemic reviews and meta-analyses, a narrative (descriptive) review involves greater subjectivity in data selection and interpretation;

2. Absence of quantitative assessments. All conclusions in this review are based on qualitative analysis and generalization of available data and not on their statistical analysis;

3. Limited number of analyzed literature sources. Despite rather wide search, the review does not claim full coverage of all existing literature in the subject matter. Some research works might have been missed and not included in the analysis due to limited access to databases or the authors' language barrier.

These limitations taken into account, the conclusions made in this review should be considered structured generalization of existing knowledge on health and adaptation issues of foreign students in Russian HEIs and as a basis for further research. More wide-scale investigations are necessary including systemic reviews and meta-analyses for obtaining more precise and well-grounded quantitative data.

Conclusion. The results of this literature review have made it possible to analyze and systematize key health risk factors for foreign students attending Russian higher education institutions. The intra-university environment has been found to have complex influence on physical and mental well-being of this student cohort caused by academic, sociocultural, economic and medical and environmental factors. High educational loads, language barriers, cultural shock, financial difficulties, and limited access to healthcare services are only some of health risk factors foreign students have to face when they adapt to new conditions. Our analysis of research literature has revealed that exposure to these factors can lead to elevated morbidity, poor academic performance and social deadaptation, which, in its turn, has a negative effects on foreign students' quality of life and can prevent them from achieving their educational goals. We have systematized the most common and effective adaptation measures for foreign students, which can mitigate adverse effects of risk factors.

This review has practical significance for developing and updating adaptation programs for foreign students in Russian universities as well as for developing methodical guidelines for teachers, experts of international departments and other specialists working with foreign students. Our findings can be used for developing targeted activities aimed at mitigating negative effects produced by risk factors on foreign students' health and at making their learning more effective. It is necessary to develop and implement complex and effective adaptation programs aimed at creating favorable conditions for learning, living, and social integration of foreign students attending Russian higher education institutions.

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. International Encyclopedia of Education, 4th ed. In: R.J. Tierney, F. Rizvi, K. Ercikan eds. Elsevier Science, 2023, pp. 239–249.

2. Smaliakou D.A. The Genesis of Higher Education Internationalisation. *Obrazovanie i nauka*, 2019, vol. 21, no. 8, pp. 9–28. DOI: 10.17853/1994-5639-2019-8-9-28 (in Russian).

3. Gümüş S., Gök E., Esen M. A Review of Research on International Student Mobility: Science Mapping the Existing Knowledge Base. *Journal of Studies in International Education*, 2020, vol. 24, no. 5, pp. 495–517. DOI: 10.1177/1028315319893651

4. Khanam S., Joshi R.M. International Student Mobility: Advancing the Internationalisation of Higher Education through a Systematic Review. *Journal of Informatics Education and Research*, 2025, vol. 5, no. 1, pp. 1957–1973. DOI: 10.52783/jier.v5i1.2185

5. Choudaha R. Three waves of international student mobility (1999–2020). *Studies in Higher Education*, 2017, vol. 42, no. 5, pp. 825–832. DOI: 10.1080/03075079.2017.1293872

6. Chevzhik Yu.V., Milushkina O.Yu. Adaptation of medical students to learning. *Metodologiya i tekhnologiya nepreryvnogo professional'nogo obrazovaniya*, 2023, no. 4, pp. 5–18. DOI: 10.24075/MTCPE.2023.016 (in Russian).

7. Velikanova L.P. Psychological aspects of adaptation of foreign students to study at the university. *Aktual'nye problemy obucheniya inostrannykh studentov v meditsinskom vuze: Materialy nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem, posvyashchennoi 100-letiyu Astrakhanskogo GMU*, 2018. In: A.Kh. Satretdinova ed. pp. 16–19 (in Russian).

8. Lyalyuk A.V., Tuchina O.R. Investigation of risk factors of the educational environment of modern higher education institution: students' positions. *Vestnik Adygeiskogo gosudarstvennogo universiteta. Seriya 3: Pedagogika i psikhologiya*, 2018, no. 3 (223), pp. 57–65 (in Russian).

9. Ivanova I.V., Shcherbakova A.O., Sorokina A.S. Problems of adaptation of students to a new educational and socio-cultural environment. *Dostizheniya vuzovskoi nauki 2018: sbornik statei Mezhdunarodnogo nauchno-prakticheskogo konkursa: in 3 parts*, 2018, pt 3, pp. 23–25 (in Russian).

10. Belikova R.M., Pyatunina O.I. Problems of students' adaptation to studying in a higher educational institution. *Uchenye zapiski Zabaikal'skogo gosudarstvennogo universiteta. Seriya: Biologicheskie nauki*, 2009, no. 1 (24), pp. 129–130 (in Russian).

11. Nasonova N.A., Il'icheva V.N., Sokolov D.A. Slozhnosti adaptatsii inostrannykh studentov pervogo kursa obucheniya v meditsinskom vuze [Difficulties in Adaptation of First-Year Foreign Students at a Medical University]. *Sovremennye tendentsii razvitiya sistemy obrazovaniya: sbornik statei*, 2019, pp. 315–317 (in Russian).

12. Alekseeva N.T., Klochkova S.V., Nikityuk D.B., Kvaratskheliya A.G. Rol' latinskogo yazyka v meditsinskoi terminologii [The Role of Latin in Medical Terminology]. *Innovatsionnye obuchayushchie tekhnologii v meditsine: sbornik materialov Respublikanskoi nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem*, 2017, pp. 553–556 (in Russian).

13. Belozerov V.S., Shchitova N.A., Chikhichin V.V., Soloviev I.A. Foreign students in the North Caucasus: regional differences and problems of adaptation (on the example of the North Caucasus Federal University). *Nauka. Innovatsii. Tekhnologii*, 2017, no. 4, pp. 79–92 (in Russian).

14. Othman I.W., Abd Rahim S., Bakar A.L. The Factors Contributing to The Different Self-Sufficiency Adaptation and Academic Performance the Case of International Students at Malaysian Public Universities. *International Journal of Education Psychology and Counseling*, 2022, vol. 7, no. 47, pp. 750–772. DOI: 10.35631/IJEPC.747056

15. Starikova I.V., Radyshevskaya T.N., Chaplieva E.M., Zhuravleva M.V., Alexandrina E.S. State of alert and motivation for educational activity of foreign and Russian students in the medical university. *Nauchnoe obozrenie. Pedagogicheskie nauki*, 2018, no. 3, pp. 58–61 (in Russian).

16. Susilawati S., Zuhro N. Cultural Adaptation and Social Experience of International Students at UIN Maulana Malik Ibrahim Malang. *Education Quarterly Reviews*, 2022, vol. 5, no. 3, pp. 134–141. DOI: 10.31014/aior.1993.05.03.531

17. Peng R.-Z., Wu W.-P. Measuring communication patterns and intercultural transformation of international students in cross-cultural adaptation. *International Journal of Intercultural Relations*, 2019, vol. 70, pp. 78–88. DOI: 10.1016/j.ijintrel.2019.03.004

18. Gadzhiev A.A., Bobrova O.E. Adaptatsiya i sotsializatsiya inostrannykh studentov v rossiiskikh universitetakh [Adaptation and socialization of foreign students in Russian universities]. *Sinergiya Nauk*, 2019, no. 33, pp. 639–651 (in Russian).

19. Vasil'eva S.V. Adaptatsiya studentov k vuzam s razlichnymi usloviyami obucheniya [Adaptation of students to universities with different educational conditions]. *Psikhologo-pedagogicheskie problemy razvitiya lichnosti v sovremennykh usloviyakh: psikhologiya i pedagogika v obshchestvennoi praktike: Sbornik nauchnykh trudov*. Saint Petersburg, RGPU im. A.I. Gertsena Publ., 2000, pp. 84–93 (in Russian).

20. Bahri S.S., Hum M., Wardani Sitorus W. An Analysis cultural shock of education system by Indonesian students in Singapore: Analisis gegar budaya sistem pendidikan pelajar Indonesia di Singapura. *JURNAL RISET RUMPUN ILMU BAHASA*, 2022, vol. 1, no. 2, pp. 01–13. DOI: 10.55606/jurribah.v1i2.121

21. Hrehova D., Žiaran P., Seňová A. Learning and growing in a foreign context: Review of literature on cross-cultural adaptation of international students. *SHS Web of Conferences*, 2021, vol. 129, no. 27, pp. 10005. DOI: 10.1051/shsconf/202112910005

22. Abunavas Kh.A., Berestneva O.G. Issledovanie problem adaptatsii inostrannykh studentov na primere Tomskogo politekhnicheskogo universiteta [Research on problems of adaptation of foreign students on the example of Tomsk Polytechnic University]. *Metodologiya obucheniya i povyshenie effektivnosti akademicheskoi, sotsial'no-kul'turnoi i psikhologicheskoi adaptatsii inostrannykh studentov v rossiiskom vuze: teoreticheskie i prikladnye aspekty: materialy vserossiiskogo seminara*, 2008, vol. 2, pp. 7–12 (in Russian).

23. Shcheka N.Yu., Kal'nitskaya Ya.V. Social adaptation of foreign students in universities in the second half of the XX – the end of the XX century: practice-oriented analysis. *Vestnik AmGU*, 2019, no. 84, pp. 45–49 (in Russian).

24. Rathakrishnan B., Singh S.S.B., Kamaluddin M.R., Ghazali M.F., Yahaya A., Mohamed N.H., Krishnan A.R. Homesickness and Socio-Cultural Adaptation towards Perceived Stress among International Students of a Public University in Sabah: An Exploration Study for Social Sustainability. *Sustainability*, 2021, vol. 13, no. 9, pp. 4924. DOI: 10.3390/su13094924

25. Ruzhenkova V.V. Educational stress as a factor of the risk of formation of additive behavior, alert and depressive disorders in foreign medical students. *Nauchnyi rezul'tat. Meditsina i farmatsiya*, 2018, vol. 4, no. 2, pp. 55–68. DOI: 10.18413/2313-8955-2018-4-2-0-6 (in Russian).

26. Ristic D.I., Hinić D., Ristić I., Milanović P., Milenković N., Selaković D., Rosić G. Adverse mental health outcomes in medical students in Serbia during the COVID-19 pandemic. *Acta Clin. Croat.*, 2024, vol. 63, no. 1, pp. 73–79. DOI: 10.20471/acc.2024.63.01.9

27. Erturk S., Nguyen Luu L.A. Adaptation of Turkish international students in Hungary and the United States: A comparative case study. *International Journal of Intercultural Relations*, 2022, vol. 86, no. 2, pp. 1–13. DOI: 10.1016/j.ijintrel.2021.10.006

28. Aisner L.Yu., Trashkova S.M. Features of adaptation of foreign students (on the example of studying FGBOU in the Krasnoyarsk State Agrarian University). *Innovatsionnaya ekonomika: perspektivy razvitiya i sovershenstvovaniya*, 2017, no. 7 (25), pp. 12–17 (in Russian).

29. Schmitt M.T., Spears R., Branscombe N.R. Constructing a minority group identity out of shared rejection: The case of international students. *European Journal of Social Psychology*, 2003, vol. 33, no. 1, pp. 1–12. DOI: 10.1002/ejsp.131

30. Sahakyan L.S., Mkrthchyan N.S., Patel A.N., Rather Mohd R.R., Barapatre D.S., Saharyan A.V., Shaljyan A.L., Antonyan A.G. Psychological and sociocultural adaptation of medical university international students in Armenia. *GSC Advanced Research and Reviews*, 2022, vol. 13, no. 02, pp. 269–276. DOI: 10.30574/gscarr.2022.13.2.0304

31. Morozova Yu.V. On the issue of foreign student adaptation in multinational higher school: the regional aspects. *Azimut nauchnykh issledovanii: pedagogika i psikhologiya*, 2018, vol. 7, no. 1 (22), pp. 129–132 (in Russian).

32. Anfalova L.V. Problemy mezhkul'turnoi adaptatsii inostrannykh studentov v obrazovatel'noi organizatsii [Problems of intercultural adaptation of foreign students in an educational organization]. *Teoriya i metodika obucheniya i vospitaniya v sovremennom obrazovatel'nom prostranstve: Sbornik materialov IV Mezhdunarodnoi nauchno-prakticheskoi konferentsii*, 2018, pp. 145–152 (in Russian).

33. Vinokurova E.I. Management of socio-economic adaptation and integration of external migrants in the Russian society. *Voprosy upravleniya*, 2016, no. 1 (38), pp. 28–34 (in Russian).

34. Güneş M., Aydar Ö. Being Student in a Foreign Country: Adaptation Problems and Solution Strategies. *Sakarya University Journal of Education*, 2019, vol. 9, no. 2, pp. 363–382. DOI: 10.19126/suje.544761

35. Panchenko L.S. To the problem of nutrition of foreign students during the period of adaptation to the sharply continental climate of Volgograd. *Mezhdunarodnyi zhurnal prikladnykh i fundamental'nykh issledovanii*, 2024, no. 4, pp. 5–9. DOI: 10.17513/mjpfi.13619 (in Russian).

36. Sycheva E.A., Tolmachev D.A. Kharakteristika pitaniya studentov meditsinskogo vuza [Characteristics of nutrition of medical university students]. *Modern science*, 2019, no. 12–4, pp. 174–177 (in Russian).

37. Holubnycha L., Matsapura L., Miroshnik L., Hetmanets I. Psychophysiological Adaptation of International Students to Learning Abroad. *International Journal of Innovative Research and Scientific Studies*, 2022, vol. 5, no. 1, pp. 37–46. DOI: 10.53894/ijirss.v5i1.359

38. Udah H., Francis A. Vulnerability and Well-Being: International Students' Experience in North Queensland, Australia. *Journal of Comparative & International Higher Education*, 2022, vol. 14, no. 5, pp. 171–196. DOI: 10.32674/jcihe.v14i5.3942

39. Ndamukunda O.N.U., Mutuyimana M.T., Umubano F., Tuyishime E. Socioeconomic and academic consequences of COVID-19 pandemic on medical students from the University of Rwanda. *PLoS One*, 2025, vol. 20, no. 2, pp. e0318066. DOI: 10.1371/journal.pone.0318066

40. Irawan A.W., Dwisona D., Lestari M. Psychological Impacts of Students on Online Learning During the Pandemic COVID-19. *KONSELI: Jurnal Bimbingan dan Konseling (E-Journal)*, 2020, vol. 7, no. 1, pp. 53–60. DOI: 10.24042/kons.v7i1.6389

41. Ruslyakova E.E., Golub' A.A., Kiseleva U.P. Features of neuropsychic stress in modern students in conditions of forced self-isolation. *Mir nauki. Pedagogika i psikhologiya*, 2020, vol. 8, no. 3, pp. 69 (in Russian).

42. Prasath P.R., Xiong Y., Zhang Q., Jeon L. Psychological Capital, Well-being, and Distress of International Students. *Int. J. Adv. Couns.*, 2022, vol. 44, no. 3, pp. 529–549. DOI: 10.1007/s10447-022-09473-1

43. Ruzhenkova V.V., Khamskaya I.S., Gomelak Yu.N. Depression in medical students: prevalence, dynamics and comorbidity. *Zhurnal nevrologii i psikhiatrii im. S.S. Korsakova*, 2022, vol. 122, no. 6–2, pp. 7–11. DOI: 10.17116/jnevro20221220627 (in Russian).

44. Cherbieva S.V. Features of the influence of educational stress on the student life of students of different courses. *Uchenye zapiski universiteta im. P.F. Lesgafta*, 2022, no. 4 (206), pp. 607–615. DOI: 10.34835/issn.2308-1961.2022.4.p607-615 (in Russian).

45. Kandaurova A.V., Surtaeva N.N., Afanasiev V.V., Ivanova O.A., Rezakov R.G. A research of the psycho-emotional state of a teacher under the influence of social changes. *ESPACIOS*, 2018, vol. 39, no. 46, pp. 19.

46. Shurkevich N.P., Vetoshkin A.S., Gapon L.I., Gubin D.G. The role of natural light in circadian blood pressure rhythm and desynchronosis in the Arctic. *European Heart Journal*, 2018, vol. 39, suppl. 1, pp. 1407. DOI: 10.1093/eurheartj/ehy566.P6578

47. Stratakis K., Todorovic J., Mirkovic M., Nešić D., Tesanovic T., Terzić-Šupić Z. Examination of factors associated with physical activity among medical students pre and post-COVID-19 in Serbia. *Sci. Rep.*, 2025, vol. 15, no. 1, pp. 5791. DOI: 10.1038/s41598-025-90544-9

48. Antwi C.O., Belle M.A., Ntim S.Y., Wu Y., Affum-Osei E., Aboagye M.O., Ren J. COVID-19 Pandemic and International Students' Mental Health in China: Age, Gender, Chronic Health Condition and Having Infected Relative as Risk Factors. *Int. J. Environ. Res. Public Health*, 2022, vol. 19, no. 13, pp. 7916. DOI: 10.3390/ijerph19137916

49. Plotnikov D.Yu., Agliullina S.T., Ashryatova L.Sh., Pankratova S.A., Lushanina K.A., Zakirov I.K., Shulaev A.V. Analysis of the Prevalence of Myopia in Medical Students. *Meditsina*, 2023, vol. 11, no. 1, pp. 25–34. DOI: 10.29234/2308-9113-2023-11-1-25-34 (in Russian).

50. Arde B.O.A. Jr, Claudio E.A. Health behaviors and protective factors of international students in the university of northern Philippines. *Surabaya Internasional Health Conference 2019*, 2019, vol. 1, no. 1, pp. 56–74.

51. Ribeiro José M.E., Costa Ramos I.E., de Sousa T.M., Canella D.S. Food consumption associated with depression, anxiety and stress in students entering a public university. *J. Nutr. Sci.*, 2025, vol. 14, pp. e3. DOI: 10.1017/jns.2024.90

52. Henares D., Monsalvez V., Brotons P., Machado M.L., Capilla S., Gomila-Grange A., Bierge P., Cubero M. [et al.]. Human gut microbiota composition associated with international travels. *Travel Med. Infect. Dis.*, 2024, vol. 61, pp. 102747. DOI: 10.1016/j.tmaid.2024.102747

53. Gasanov R., Kobzar V., Zhuravleva A. Student information level and users internet about parasitic diseases. *Byulleten' nauki i praktiki*, 2020, vol. 6, no. 7, pp. 218–225. DOI: 10.33619/2414-2948/56/22 (in Russian).

54. Gahwal J.K., Urkunova A., Kuzembayeva G., Kupenova A., Nurgaliyeva G. Adaptation of International Students to University Reality in Kazakhstan (on the Example of WKMU). *Journal of Humanities and Social Sciences Studies*, 2021, vol. 3, no. 5, pp. 31–35. DOI: 10.32996/jhsss.2021.3.5.4

55. Luo M., Zhang X. Research Status about Influence Factors of International Students' Cross-Cultural Adaptation with Different Models. *Open Journal of Social Sciences*, 2021, vol. 09, no. 06, pp. 51–63. DOI: 10.4236/jss.2021.96006

56. Chernikova T.V., Sokal'skiy E.A., Boluchevskaya V.V., Shutova O.I. Ethnocultural features of academic adaptation of students of the near and far abroad. *Nauchno-pedagogicheskoe obozrenie. Pedagogical Review*, 2021, vol. 6, no. 40, pp. 178–189. DOI: 10.23951/2307-6127-2021-6-178-189 (in Russian).

57. Yu L., Cao Y., Wang Y., Liu T., MacDonald A., Bian F., Li X., Wang X. [et al.]. Mental health conditions of Chinese international students and associated predictors amidst the pandemic. *J. Migr. Health*, 2023, vol. 7, pp. 100185. DOI: 10.1016/j.jmh.2023.100185

58. Maharaj R., Ndwiga D., Chutiyami M. Mental health and wellbeing of international students in Australia: a systematic review. *J. Ment. Health*, 2024, pp. 1–19. DOI: 10.1080/09638237.2024.2390393

59. Koval E.D. Theoretical aspects of investigation of process of foreign students' adaptation in socio-cultural end educational environment of Russian higher school. *Uchenye zapiski universiteta imeni P.F. Lesgafta*, 2017, no. 10 (152), pp. 110–115 (in Russian).

60. Barsegyan V., Maas I. First-generation students' educational outcomes: the role of parental educational, cultural, and economic capital – a 9-years panel study. *Research in Social Stratification and Mobility*, 2024, vol. 91, pp. 100939. DOI: 10.1016/j.rssm.2024.100939

61. Maksimchuk E.D. The application of social and psychological support of intercultural adaptation of foreign students. *Vestnik Kemerovskogo gosudarstvennogo universiteta*, 2016, no. 4, pp. 171–175. DOI: 10.21603/2078-8975-2016-4-171-175 (in Russian).

62. Abdelkader A., Joseph B., Anish L., Kaur M., Bourke S., Hayes C., Thong M., Plummer V., Jacob S. International students' perspectives on factors impacting psychological well-being and early help-seeking behaviours in undergraduate nursing programs. *Nurs. Open*, 2024, vol. 11, no. 9, pp. e70030. DOI: 10.1002/nop2.70030

Kirichenko L.V., Kilanova M.V., Glukhikh M.V. Factors and strategies for reducing health risks for foreign students attending Russian universities. Health Risk Analysis, 2025, no. 2, pp. 73–86. DOI: 10.21668/health.risk/2025.2.06.eng

Received: 06.05.2025
Approved: 17.06.2025
Accepted for publication: 26.06.2025

UDC 628.1.033:578.8(470+571)
DOI: 10.21668/health.risk/2025.2.07.eng

Read online

Research article

VIRUS CONTAMINATION OF CENTRALIZED WATER SUPPLY SYSTEMS AS A HEALTH RISK FACTOR: FEATURES OF LONG-TERM DYNAMICS IN THE RUSSIAN FEDERATION

G.M. Trukhina[1], G.G. Badamshina[2,3], E.A. Poptsova[4], M.A. Yaroslavtseva[5], E.S. Volostnova[2], E.R. Guzairova[6]

[1]Federal Scientific Center of Hygiene named after F.F. Erisman, 2 Semashko St., Mytishchi, Moscow Region, 141014, Russian Federation
[2]Center of Hygiene and Epidemiology in the Republic of Tatarstan, 13a Sechenova St., Kazan, 420061, Russian Federation
[3]Kazan State Medical University, 49 Butlerova St., Kazan, 420012, Russian Federation
[4]Center of Hygiene and Epidemiology in Mari El, 121 Mashinostroitelei St., Yoshkar-Ola, 424007, Russian Federation
[5]Federal Center for Hygiene and Epidemiology, 19A Varshavskoe Shosse, Moscow, 117105, Russian Federation
[6]Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, Republic of Bashkortostan Regional Office, 58 Rikharda Zorge St., Ufa, 450054, Russian Federation

Despite the fact that high-quality water treatment is currently being carried out, there is a possibility that pathogens of infectious diseases may enter a water supply network. Sanitary and virologic control of water sources and drinking water is currently performed directly by direct detection of viruses, as well as by using indirect indicators of viral contamination, namely, coliphages.

The aim was to study the spectrum of viruses contained in water from a centralized water supply system using laboratory diagnostic methods regulated to determine rated indicators.

Over 2014–2023, water samples were taken from a centralized water supply system (n = 2,847,568). The dynamics and structure of water contamination with hepatitis A (HAV), Rotavirus, Enterovirus, Norovirus, Astrovirus, COVID-19 (SARs-CoV-2) viruses, and Adenovirus in a centralized water supply system were described using classical cultural methods of bacteriology and virology, molecular-genetic and serological methods.

The conducted research found that over 2014–2023, the average proportion of centralized water supply samples that did not meet sanitary and hygienic standards in the Russian Federation was 0.57 % for the presence of viruses and 0.21 % for the presence of coliphages for the period from 2014 to 2023. The proportion of centralized water supply samples per non-conforming Rotavirus contents was 1.41 % (95 % CI: 1.33–1.49) (according to serological studies, 0.08 % of samples (95 % CI: 0.06–0.10); Enterovirus, 0.71 % (95 % CI: 0.57–0.86) (according to PCR studies, 0.37 % (95 % CI: 0.33–0.42); Adenovirus, 0.52 % (95 % CI: 0.38–0.70); Norovirus, 0.20 % (95 % CI: 0.16–0.24); Astrovirus, 0.14 % (95 % CI: 0.11–0.18);

Galina M. Trukhina – Doctor of Medical Sciences, Professor, Head of the Department of Microbiological Methods for Studying Environmental Factors (e-mail: trukhina@list.ru; tel.: +7 (905) 568-47-50; ORCID: https://orcid.org/0000-0001-9955-7447).

Gulnara G. Badamshina – Doctor of Medical Sciences, Head of the Department of Microbiological Research; Associate Professor of Hygiene and Labour Medicine Department (e-mail: ggbadamshina@yandex.ru; tel.: +7 (919) 692-04-90; ORCID: https://orcid.org/0000-0003-0088-6422).

Elena A. Poptsova – chief physician (e-mail: e-poptsova@mail.ru; tel.: +7 (905) 182-86-15; ORCID: https://orcid.org/0009-0007-3542-008X).

Marina A. Yaroslavtseva – Head of Laboratory-bacteriologist (e-mail: m-yaroslavceva@mail.ru; tel.: +7 (926) 536-95-01; ORCID: https://orcid.org/0000-0001-6719-4848).

Elena S. Volostnova – Acting Head of the Department of Particularly Dangerous Infections Diagnostics, bacteriologist (e-mail: e.s.volostnova@mail.ru; tel.: +7 (903) 344-81-45; ORCID: https://orcid.org/0009-0009-6718-504X).

El'giza R. Guzairova – specialist-expert of the Department of Epidemiological Surveillance and Sanitary Protection of the Territory (e-mail: g_elgiza@mail.ru; tel.: +7 (937) 300-52-64; ORCID: https://orcid.org/0009-0004-3086-0623).

SARs-CoV-2, 0.09 % (95 % CI: 0.03–0.20); HAV, 0.2 % (95 % CI: 0.01–0.04) (according to serological studies, it was slightly higher (0.09 % (95 % CI: 0.07–0.11). A direct strong correlation was established between the proportion of centralized water supply samples that did not meet sanitary and epidemiological requirements and the presence of coliphages and pathogens of viral infections (r > 0.8); however, determination of nucleic acids in drinking water by molecular genetic methods indicates higher prevalence of viruses as compared to classical coliphage testing.

The structure of virus contamination in water from a centralized water supply system was represented by Rotavirus (52.6 ± 0.3 %), Adenovirus (18.8 ± 0.5 %), Enterovirus (13.9 ± 0.2 %), Norovirus (7.5 ± 0.1 %), Astrovirus (3.8 ± 0.1 %), and SARs-CoV-2 (3.4 ± 0.2 %) over 2020–2023.

Keywords: *water microflora, centralized water supply, water of centralized water supply, viruses, contamination, biological safety, adenovirus, rotavirus, enterovirus, risk factors.*

Providing people with safe water is among the most significant challenges the global community has been facing over the last decades [1, 2]. In the Russian Federation, the state of centralized drinking water supply to the population requires some specific activities aimed at improving the water treatment system [3]. Despite the fact that high-quality water treatment is currently being carried out, there is a possibility that pathogens of infectious diseases may enter a water supply network, for example, due to wear and tear of water distribution systems and / or accidents at water supply networks and wastewater infiltrating them from damaged sewage pipelines [1, 4, 5].

In the Russian Federation, complex investigations of virus species prevalence in drinking water are rather scarce. Some foreign researchers give evidence of three enteric viruses holding the leading place in virus contamination of drinking water, namely *Adenovirus*, *Rotavirus*, and *Enterovirus* [6]. Prevalence of adenoviruses, enteroviruses, and rotaviruses has been shown in samples of treated drinking water taken at water treatment facilities [6–8]. Enterovirus was found in 21 % of treated drinking water samples in China (Wuhan) (5/24) [6]; adenovirus was found in 20 %, enterovirus in 43 % and rotavirus in 23 % of tap water samples in Pakistan [8]; in Brazil, enterovirus was identified in 27.4 % of water samples, adenovirus in 23.3 % and rotavirus in 16.4 % [7]. A review made by C. Mejías-Molina et al. (2024), covered works published over the last five years and reported Norovirus (0.5–6.7 %) and Hepatitis E virus (0.8 %) in water in addition to adenovirus (17–48 %), rotavirus (1,5–44 %) and enterovirus (0.9 %) [9].

Long-term studies confirm the role that belongs to viruses in occurrence of waterborne infections[1] [10]. According to the World Health Organization data (2022), drinking water contamination is among the most significant risk factors of acute enteric infections caused by *Adenovirus*, *Rotavirus*, *Enterovirus*, as well as by other enteric viruses[1]. In some RF regions, additional disease cases are associated with poor drinking water quality including non-conformity with safe standards per microbiological indicators. In 2023, incidence associated with poor drinking water quality was established in 84 regions of the Russian Federation within the range between 18.6 and 4167.7 additional disease cases per 100 thousand people[2].

At present, Rospotrebnadzor makes schedules of planned monitoring examinations aimed at establishing levels of drinking water contamination as a risk factor of enteric viral infections. The main aim is to prevent waterborne infections. The present sanitary and virologic control of water sources and drinking water relies on using indirect indicators of virus contamination such as coliphages and on direct virus identification [5, 11]. Molecular-genetic methods are widely used by sanitary-epidemiological laboratories for direct identification of viruses per their genome (RNA and

---

[1] WHO. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. *WHO*, 2022, 614 p. Available at: https://www.who.int/publications/i/item/9789240045064 (October 03, 2024).

[2] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2023 godu: Gosudarstvennyi doklad [On sanitary-epidemiological wellbeing of the population in the Russian Federation in 2023: the State Report]. Moscow, Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2024, 364 p. (in Russian).

DNA). In particular, polymerase chain reaction (PCR) is a common method due to its high sensitivity, availability and a possibility to identify contamination over one day [5, 11]. Parallel PCR use and coliphage identification in water ensure high reliability as regards establishing virus contamination in water since they make it possible to establish epidemiological significance of viruses identified per their RNA and DNA [5]. At present, it is quite relevant to investigate tap water contamination as a health risk factor using up-to-date laboratory diagnostic techniques.

Given all foregoing, **the aim of this study** was to examine the spectrum of viruses contained in water from a centralized water supply system (tap water) using laboratory diagnostic methods regulated to determine rated indicators.

Materials and methods. Over 2014–2023, tap water samples (*n* = 2,847,568) were analyzed in accordance with the valid methodical documents[3] annually within scheduled, unscheduled and industrial control, sanitary-hygienic monitoring conducted in RF regions by Rospotrebnadzor offices. Water samples were taken in sterile containers prior to supply and from distribution networks for centralized water supply in 89 regions of the Russian Federation. The samples were then delivered to laboratories of regional Centers for Hygiene and Epidemiology in conformity with all requirements to sample delivery with preliminary concentration of viruses predominantly using filter membranes[4].

Water samples were analyzed in conformity with the valid documents[4,5] using conventional bacteriological and virologic methods to identify coliphages and enteroviruses *(Enterovirus)*, respectively; molecular-genetic methods to identify RNA of hepatitis A virus *(HAV)*, *Rotavirus, Enterovirus, Norovirus* (since 2018), *Astrovirus* and *SARs-CoV-2* (since 2018 and 2020, respectively); *Adenovirus* DNA (since 2018); serological methods to identify *HAV* and *Rotavirus* antigens. The tests were conducted using tests-systems predominantly manufactured by Rospotrebnadzor's Central Scientific Research Institute for Epidemiology (AmpliSens OKI viro-screen-FL», AmpliSens Cov-Bat), Rospotrebnadzor's Saint Petersburg Scientific Research Institute of

---

[3] MUK 4.2.3963-23. Bakteriologicheskie metody issledovaniya vody: metodicheskie ukazaniya, utv. rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii A.Yu. Popovoi 1 sentyabrya 2023 g. [MUK 4.2.3963-23. Bacteriological methods for water analysis: methodical guidelines, approved by A.Yu. Popova, Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on September 01, 2023]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1304575302 (March 27, 2025) (in Russian); MUK 4.2.1018-01. Sanitarno-mikrobiologicheskii analiz pit'evoi vody: metodicheskie ukazaniya, utv. Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii – Pervym zamestitelem ministra zdravookhraneniya Rossiiskoi Federatsii 9 fevralya 2001 g. [MUK 4.2.1018-01. Sanitary-microbiological analysis of drinking water: methodical guidelines, approved by the RF Chief Sanitary Inspector – the First Deputy to the RF Minister of Health on February 09, 2001]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200029648 (March 27, 2025) (in Russian).

[4] MUK 4.2.2029-05. Sanitarno-virusologicheskii kontrol' vodnykh ob"ektov: metodicheskie ukazaniya, utv. i vved. v deistvie Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 18 noyabrya 2005 goda [MUK 4.2.2029-05. Sanitary-virologic control of water objects: methodical guidelines, approved and enacted by G.G. Onishchenko, Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on November 18, 2005]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200042984 (April 16, 2025) (in Russian).

[5] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2023 godu: Gosudarstvennyi doklad [On sanitary-epidemiological wellbeing of the population in the Russian Federation in 2023: the State Report]. Moscow, Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2024, 364 p. (in Russian); MUK 4.2.2746-10. Poryadok primeneniya molekulyarno-geneticheskikh metodov pri obsledovanii ochagov ostrykh kishechnykh infektsii s gruppovoi zabolevaemost'yu: metodicheskie ukazaniya, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 30 sentyabrya 2010 g. [MUK 4.2.2746-10. The Procedure for using molecular-genetic methods when examining foci of acute enteric infections with group incidence: methodical guidelines, approved by G.G. Onishchenko, Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on September 30, 2010]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200084387 (April 16, 2025) (in Russian).

Epidemiology and Microbiology named after Paster (COVID-2019 Amp), Vector-Best JSC (VGA-antigen-IFA-BEST), BioloT LLC (Igla MEM nutrient medium). The test results were included in the Federal Statistical Report Form No. 18 Data on Sanitary Situation in a region of the Russian Federation and No. 2.14–2.23 Data on Activities Accomplished by Sanitary-Hygienic, Microbiological and Parasitological Laboratories of Centers for Hygiene and Epidemiology. Primary data were collected and obtained from the Rospotrebnadzor's Federal Center for Hygiene and Epidemiology.

Water samples that did not conform to safe standards due to containing coliphages and viruses were denoted as 'non-standard samples' (n/s)[6]. Test results were analyzed using Microsoft Office v.2016. A 95 % confidence interval (CI) for the proportion of detected non-standard samples was calculated with BinomCI function using the Wilson method and DescTools libraries of R software. Trend significance to declining (growing) proportions of tap water samples was estimated per the Mann – Kendall method using Mann Kendall function of R software. A trend was considered significant at $p < 0.05$.

Results and discussion. The accomplished investigation found that the mean specific weight of tap water samples not conforming to safe standards valid in the Russian Federation equaled 0.57 % per viruses and 0.21 % per coliphages over the period between 2014 and 2023. In 2023, a descending trend was established for the specific weight of non-standard samples ($p < 0.05$) (Figure 1). A strong direct correlation was found for the proportion of tap water samples not conforming to sanitary-epidemiological requirements per coliphage and virus occurrence ($r = 0.82$, $p = 0.04$); however, the proportion of non-standard samples per presence of virus nucleic acids was on average 2.7 times higher. This probably indicates that molecular-genetic examinations are more informative for assessing safety of drinking water supply per microbiological indicators.

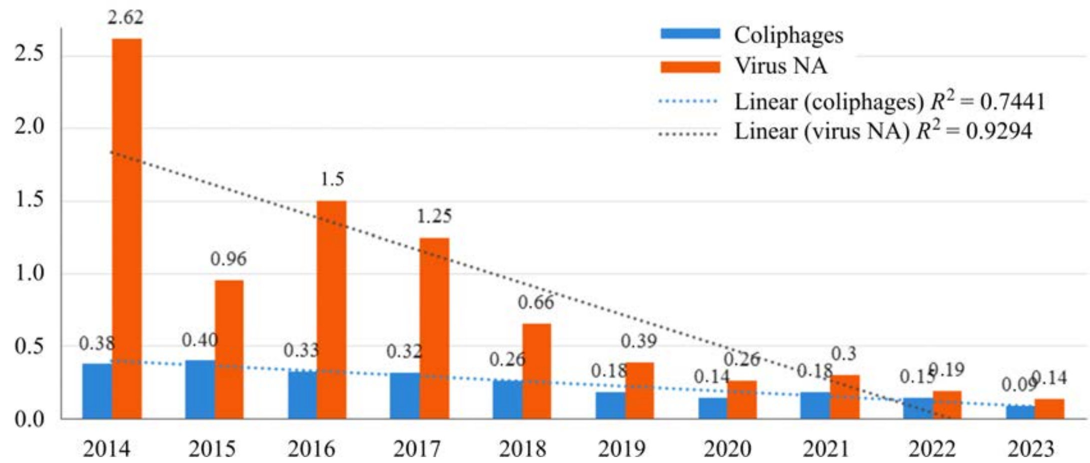

Figure 1. The proportion (%) of tap water samples not conforming to safe standards per occurrence of viruses and coliphages in the RF over 2014–2023

---

[6] SanPiN 1.2.3685-21. Gigienicheskie normativy i trebovaniya k obespecheniyu bezopasnosti i (ili) bezvrednosti dlya cheloveka faktorov sredy obitaniya: sanitarnye pravila i normy, utv. postanovleniem Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 28 yanvarya 2021 goda № 2 (s izmeneniyami na 30 dekabrya 2022 goda) [SanPiN 1.2.3685-21. Hygienic standards and requirements to providing safety and (or) harmlessness of environmental factors for people: sanitary rules and norms, approved by the Order of the RF Chief Sanitary Inspector on January 28, 2021 No. 2 (last edited as of December 30, 2022)]. *KODEKS: electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/573500115 (April 16, 2025) (in Russian).

Tests aimed at identifying antigens of enterovirus (*Enterovirus*), rotavirus (*Rotavirus*), and Hepatitis A (*HAV*) infectious agents in drinking water were accomplished using various laboratory diagnostic methods (cultural, serological, and molecular-genetic), Their results are provided in Tables 1–3.

These data over the period 2014–2023 show that *Rotavirus* contamination was more frequently identified by the molecular-genetic method within assessing safety of drinking water supply; *HAV* contamination, by the serological method; *Enterovirus* contamination, by the cultural method. This requires further investigation considering territorial peculiarities of infectious incidence and use of different laboratory testing methods for monitoring in different RF regions.

These data also indicate that the proportion of tap water samples contaminated with *Rotavirus* declined considerably by 2023 ($p = 0.003$). We did not establish any significant decline in the proportion of tap water samples contaminated with *HAV* or *Enterovirus*.

Infectious agents of other virus infections have been monitored in tap water using molecular-biological methods since 2018 (to identify nucleic acids of *Adenovirus*, *Norovirus*, and *Astrovirus*) and 2020 (to identify *SARs-CoV-2* RNA); this allows annual identification of tap water contamination with infectious agents of enteric and respiratory infections (Table 4).

Table 1

Rotavirus contamination in tap water over 2014–2023

Years	PCR				Serological method			
	Total	Of them, positive			Total	Of them, positive		
	n	*n*	% n/s samples	95 % CI	*n*	*n*	% n/s samples	95 % CI
2014	5485	113	2.06	1.70–2.47	9112	11	0.12	0.16–0.22
2015	6962	144	2.07	1.75–2.43	8639	9	0.10	0.05–0.20
2016	7650	260	3.40	3.01–3.83	9463	11	0.12	0.06–0.21
2017	9454	262	2.77	2.45–3.12	8193	11	0.13	0.07–0.24
2018	10,375	188	1.81	1.56–2.09	9406	11	0.12	0.06–0.21
2019	10,816	131	1.21	1.01–1.44	9893	6	0.06	0.02–0.13
2020	6919	55	0.79	0.60–1.03	9169	3	0.03	0.01–0.10
2021	10,684	65	0.61	0.47–0.77	7823	2	0.03	0.00–0.09
2022	10,974	47	0.43	0.31–0.57	7783	2	0.03	0.00–0.09
2023	11,751	19	0.16	0.10–0.25	7241	1	0.01	0.00–0.08
Total (2014–2023)	91,070	1284	1.41	1.33–1.49	86,722	67	0.08	0.06–0.10

Table 2

HAV contamination in tap water over 2014–2023

Years	PCR				Serological method			
	Total	Of them, positive			Total	Of them, positive		
	n	*n*	% n/s samples	95 % CI	*n*	*n*	% n/s samples	95 % CI
2014	1579	0	0.00	0.00–0.23	8844	13	0.15	0.08–0.25
2015	2947	0	0.00	0.00–0.12	9037	19	0.21	0.13–0.33
2016	3160	2	0.06	0.01–0.23	9237	3	0.03	0.01–0.09
2017	3416	1	0.03	0.03–0.16	9490	10	0.11	0.05–0.19
2018	2964	2	0.07	0.01–0.24	9898	3	0.03	0.01–0.09
2019	2819	0	0.00	0.00–0.13	10,512	11	0.10	0.05–0.19
2020	2051	0	0.00	0.00–0.18	8022	12	0.15	0.08–0.26
2021	2770	0	0.00	0.00–0.13	6897	4	0.06	0.02–0.15
2022	3090	0	0.00	0.00–0.12	8446	1	0.01	0.00–0.07
2023	3931	0	0.00	0.00–0.09	8515	4	0.05	0.01–0.12
Total (2014–2023)	28,727	5	0.02	0.01–0.04	88,898	80	0.09	0.07–0.11

Table 3

Enterovirus contamination in tap water over 2014–2023

Years	PCR				Cultural method			
	Total	Of them, positive			Total	Of them, positive		
	n	*n*	% n/s samples	95 % CI	*n*	*n*	% n/s samples	95 % CI
2014	5341	212	3.97	3.46–4.53	1431	6	0.42	0.15–0.91
2015	6629	14	0.21	0.12–0.35	1440	1	0.07	0.00–0.39
2016	7783	17	0.22	0.13–0.35	1867	73	3.91	3.08–4.89
2017	8813	9	0.10	0.05–0.19	2297	1	0.04	0.00–0.24
2018	8632	15	0.17	0.10–0.29	1398	0	0.00	0.00–0.26
2019	10,216	8	0.08	0.03–0.15	1115	1	0.09	0.00–0.50
2020	4586	0	0.00	0.00–0.08	852	0	0.00	0.00–0.43
2021	6768	3	0.04	0.01–0.13	1119	0	0.00	0.00–0.33
2022	8589	8	0.09	0.04–0.18	825	0	0.00	0.00–0.45
2023	10,427	3	0.03	0.01–0.08	1385	15	1.08	0.61–1.78
Total (2014–2023)	77,784	289	0.37	0.33–0.42	13,729	97	0.71	0.57–0.86

Table 4

Viral contamination in tap water in 2018–2023

Name	Indicator	Years						
		2018	2019	2020	2021	2022	2023	Total
Adenovirus	*n*	1014	1639	451	1495	1344	2697	8640
Of them, positive	*n*	17	8	2	2	6	10	45
	% n/s samples	1.68	0.49	0.44	0.13	0.45	0.37	0.52
	95 % CI	0.98–2.67	0.21–0.96	0.05–1.59	0.02–0.48	0.16–0.97	0.18–0.68	0.38–0.70
Norovirus	*n*	8286	8966	6235	9927	10,385	11,033	54,832
Of them, positive	*n*	23	11	11	25	13	27	110
	% n/s samples	0.28	0.12	0.18	0.25	0.13	0.25	0.20
	95 % CI	0.18–0.42	0.06–0.22	0.09–0.32	0.16–0.37	0.07–0.21	0.16–0.36	0.16–0.24
Astrovirus	*n*	6382	8142	5765	8743	9112	9913	48,057
Of them, positive	*n*	4	7	5	28	12	13	69
	% n/s samples	0.06	0.09	0.09	0.32	0.13	0.13	0.14
	95 % CI	0.02–0.16	0.03–0.18	0.03–0.20	0.21–0.46	0.07–0.23	0.07–0.22	0.11–0.18
SARs-CoV-2	*n*	–	–	2019	2084	1375	296	5774
Of them, positive	*n*	–	–	1	4	0	0	5
	% n/s samples	–	–	0.05	0.19	0.00	0.00	0.09
	95 % CI			0.00–0.28	0.05–0.49	0.00–0.27	0.00–1.24	0.03–0.20

Analysis of the test data showed practically no changes in the proportion of tap water samples not conforming to safe standards per *Adenovirus*, *Astrovirus*, *Norovirus*, and *SARs-CoV-2* contents over the analyzed period.

Monitoring data collected by using molecular-biological methods in 2020–2023 were analyzed to show the structure of infection viruses identified in tap water (Figure 2).

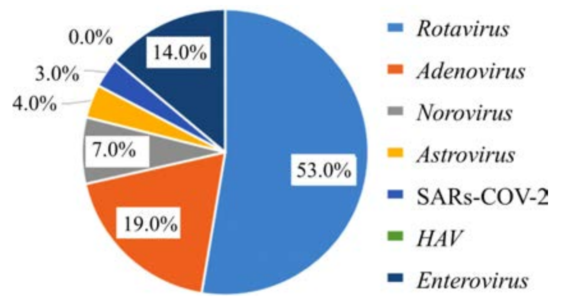

Figure 2. Infectious agents (viruses) identified in tap water in 2020–2023

It is noteworthy that *Rotavirus* prevailed among infection viruses while *Adenovirus* and *Enterovirus* were identified more than twofold less frequently. *Norovirus* and *Astrovirus* had the lowest specific weights. *HAV* was not identified in tap water in 2020–2023; however, it was occasionally detected in water samples between 2014 and 2019. The virus antigen was also annually identified by serological methods (Table 2).

Our findings show that despite the established descending trend, tap water samples did not conform to safe standards in 0.57 % cases per occurring viruses and per a basic indicator (coliphages) in 0.21 % cases on average in Russia over the last decade. This indicates the necessity to continuously monitor safety of drinking water and water sources per microbiological indicators [5, 12, 13] and existing likelihood of virus infections among the population in Russia due to contaminated water intake[8].

Our analysis showed that identification of a basic indicator (coliphages) reflected water contamination with enteric viruses to a certain extent (r = 0.82). However, within this study, the proportion of non-standards samples was on average 2.7 times higher per detected virus nucleic acids against coliphages. Bearing this in mind, we can state that coliphage contents as an indicator does not fully reflect virus contamination of water, enteric viruses included. This is consistent with opinions expressed by the authors who do not believe coliphages to be reliable indicators of enteric viruses in water safety assessments[8] [14] and is confirmed by enteric viruses isolated from treated and disinfected drinking water, which was negative in conventional coliphage-detecting tests[8]. Studies did not find any correlation between water contamination with Norovirus, adenoviruses, rotaviruses and coliphages in water objects and tap water [6, 14]. Occurrence of enteric viruses in water samples indicates contamination with wastewaters [15] and a risk of getting infected with enteric viruses for people due to contaminated drinking water intake[8].

Absence of contaminated water samples per coliphage contents as a basic indicator together with present contamination with enteric viruses can also be evidence of molecular-genetic tests being more informative within assessing safety of drinking water supply per microbiological indicators [15, 16].

At the same time, PCR-based methods are usually used for detecting and identifying virus infections in water although PCR alone does not allow the discrimination between infectious and non-infectious viral particles [15]. In addition, water can contain certain inhibitors, which prevent nucleic acids from amplifying by polymerase chain reaction [17]. PCR-based tests aimed at detecting *Enterovirus* identified the virus NA only in 0.37 % of samples, which was almost twofold lower relative to the cultural method, 0.71 % of samples. This can result from PCR inhibitors in water and be explained by virus interference [2, 18, 19]. The review by O.N. Savostikova and others (2021) established that various chemicals could occur in water sources [20], including those potentially able to inhibit PCR. *HAV* RNA and antigen identified in 0.02 % and 0.09 % of water samples respectively can also give evidence of water contamination with infectious and non-infectious virus particles along with various informative values of the molecular-genetic and serologic methods and assumed occurrence of PCR inhibitors in water as well as conditions suitable for virus interference. This requires further investigation considering territorial peculiarities of infection incidence and use of different laboratory testing methods in different regions of Russia.

Given all foregoing, it should be noted that examining water safety with complex laboratory diagnostic methods is becoming more significant for assessing risk factors of enteric virus infections. Foreign researchers emphasize the significance of combining the virologic method relying on using cell cultures

[1] WHO. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. *WHO*, 2022, 614 p. Available at: https://www.who.int/publications/i/item/9789240045064 (October 03, 2024).

with PCR identifying infection viruses, which grow slow or do not produce any cytotoxic effects in a cell culture [15]. Several studies have revealed an association between virus persistence (determined by molecular methods) and infectivity (determined by analyzing cytopathic effects) indicating that degradation of virus nucleic acids has a strong correlation with loss of virus infectivity [16].

Prevalence of virus contamination was considerably lower in Russia in comparison with the data reported by foreign researchers (in this study, *Rotavirus* was detected in 1.41 % of samples; *Enterovirus,* 0.70 %; *Adenovirus,* 0.52 %), which means disinfection activities accomplished within water treatment in the country are quite effective for managing risks of enteric infection incidence. Differences established in discussing the obtained results including those identified in contamination of subterranean and surface water sources require special attention and give grounds for setting new research tasks. Foreign researchers have established the total prevalence of hepatitis E virus to be 4.7 % in drinking water [21], which also requires special attention from Russian researchers.

Overall, over 2014–2023, the proportion of tap water samples that did not conform to basic safe sanitary-microbiological indicators equaled to 0.20 % per *Norovirus*, 0.14 % per *Astrovirus*, 0.09 % per *SARs-CoV-2*, and 0.02 % per *HAV*. Studies with their focus on drinking water contamination with *Astrovirus*, *Norovirus*, *SARs-CoV-2*, and *HAV* have been given very little attention in literature available to us. However, there are some available data on virus prevalence in Thailand in a water reservoir, river water, etc., where various *Astrovirus* genotypes were detected in 4.8 % of the samples with different prevalence (in RF, according to this study, *Astrovirus* was detected in 0.14 % of drinking water samples) [16]. Researchers assert that *Astrovirus* is a prevailing species in the water environment [16], and cases of inter-species transmission have been described for some genotypes [22]. Risk of enteric infections accused by *Astrovirus* due to drinking water intake should be given special attention by researchers.

Virus contamination of drinking water gives evidence of viruses being resistant to disinfectants used in water treatment. The highest virus elimination in water treatment was reported for *Enterovirus* (97 % of viruses are eliminated by disinfection) and *Rotavirus* (82 % removed by disinfection); lower levels are reported for *Adenovirus* (73 %) [6]. Considering the structure of infectious agents identified in tap water in 2020–2023 even after mandatory disinfection, as shown in Figure 2, we should assume *Rotavirus* to be the most resistant to disinfectants used in water treatment in the RF; *Adenovirus*, *Norovirus* and *Enterovirus* were a bit less resistant. Studies reported that some of these viruses were resistant to water disinfection and ecological stressors, especially to inactivation by ultraviolet light [15, 23]. Given that, researchers emphasize the necessity to use the combined water disinfection method [5] to prevent risks of infections.

Bearing in mind that viruses causing Hepatitis A, COVID-19 and Astrovirus infection were either not detected or had very small proportions, we can assume that disinfection activities accomplished within water treatment in 2020–2023 in the RF were effective as regards elimination of these viruses.

Conclusion. Therefore, we analyzed the results obtained by testing tap water samples taken both prior to supply and directly from distribution networks. The analysis revealed that despite the descending trend identified over the period 2014–2023 in the proportion of samples not meeting sanitary-epidemiological requirements, such samples were still detected (on average, 0.57 % contained viruses and 0.21 % coliphages). This means risks of infectious diseases caused by intake of drinking water contaminated with enteric viruses.

Over 2014–2023, the proportion of tap water samples that did not meet safe standards per sanitary-microbiological indicators equaled the following: for *Rotavirus,* 1.41 % of samples (95 % CI: 1.33–1.49) (according to serological tests, 0.08 % of samples (0.06–0.10)); *Enterovirus,* 0.71 % of samples (95 % CI: 0.57–0.86) (according to PCR tests, 0.37 % of samples (95 % CI: 0.33–0.42)); *Adenovirus,* 0.52 % of samples (95 % CI: 0.38–0.70); *Norovirus,* 0.20 % samples (95 % CI: 0.16–0.24); *Astrovirus,* 0.14 % of samples (95 % CI: 0.11–0.18); *SARs-CoV-2,* 0.09 % (95 % CI: 0.03–0.20); *HAV,* 0.02 % of samples (95 % CI: 0.01–0.04) (according to serological tests, a bit higher, 0.09 % of samples (0.07–0.11)). The structure of virus contamination in tap water in 2020–2023 contained *Rotavirus* (52.6 ± 0.3 %), *Adenovirus* (18.8 ± 0.5 %), *Enterovirus* (13.9 ± 0.2 %), *Norovirus* (7.5 ± 0.1 %), *Astrovirus* (3.8 ± 0.1 %), and *SARs-CoV-2* (3.4 ± 0.2 %). This indicates the necessity to further investigate risk factors of some specific enteric virus infections associated with drinking water intake to predict incidence rates and take relevant preventive measures.

We found a strong direct correlation between the proportions of tap water samples deviating from safe standards per contents of coliphages and virus infectious agents ($r > 0.8$); however, use of molecular-genetic methods to identify nucleic acids in water established higher virus prevalence in comparison with conventional coliphage testing. This probably requires special attention when water safety is assessed using ‘coliphages contents’ as the basic regulated indicator. Absence of virus contamination in treated water confirmed by cultural methods and high levels of contaminations with antigens when PCR-tests are negative indicate it is necessary to develop additional reliable tests, which can confirm virus infectivity and presence of amplification reaction inhibitors.

Identification of viruses from various genera in drinking water indicates that drinking water intake can be a risk factor of enteric infections in Russia and that it is necessary to monitor virus resistance to disinfectants, to use combined disinfection techniques in water treatment, and to investigate a role that belongs to drinking water in occurrence of virus infections. The obtained data can be used for predicting incidence of some specific enteric infections and economic losses as well as for planning relevant preventive activities.

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. Popova A.Yu., Kuzmin S.V., Mehantyev I.I. Assessment of Implementation Efficiency of the System Approach to Ensuring Safety of Public Drinking and Recreational Water Use on the Example of the Voronezh Region. *ZNiSO*, 2021, vol. 29, no. 8, pp. 7–14. DOI: 10.35627/2219-5238/2021-29-8-7-14 (in Russian).

2. Lavrik E.P., Trukhina G.M., Kravchenko A.G., Vysotin S.A., Vysotina A.T., Dmitrieva N.A. Sanitary and epidemiological features of recreational water use of the Tuapse district of the Krasnodar region. *Gigiena i sanitariya*, 2021, vol. 100, no. 9, pp. 910–916. DOI: 10.47470/0016-9900-2021-100-9-910-916 (in Russian).

3. Baydakova E.V., Unguryanu T.N., Mikhaylova R.I. On quantitative assessment of microbe risk caused by exposure to enteric viruses in drinking water. *Health Risk Analysis*, 2019, no. 2, pp. 108–114. DOI: 10.21668/health.risk/2019.2.12.eng

4. Erkhov A.A. SARS-CoV-2: Sewerage as a transmission route. *Stroitel'stvo i tekhnogennaya bezopasnost'*, 2023, no. 30 (82), pp. 105–112 (in Russian).

5. Rakhmanin Yu.A., Onishchenko G.G. Hygienic assessment of drinking water supply of the population of the Russian Federation: problems and the way their rational decision. *Gigiena i sanitariya*, 2022, vol. 101, no. 10, pp. 1158–1166. DOI: 10.47470/0016-9900-2022-101-10-1158-1166 (in Russian).

6. Ye X.Y., Ming X., Zhang Y.L., Xiao W.Q., Huang X.N., Cao Y.G., Gu K.D. Real-time PCR detection of enteric viruses in source water and treated drinking water in Wuhan, China. *Curr. Microbiol.*, 2012, vol. 65, no. 3, pp. 244–253. DOI: 10.1007/s00284-012-0152-1

7. Kluge M., Fleck J.D., Soliman M.C., Luz R.B., Fabres R.B., Comerlato J., Silva J.V.S., Staggemeier R. [et al.]. Human adenovirus (HAdV), human enterovirus (hEV), and genogroup A

rotavirus (GARV) in tap water in southern Brazil. *J. Water Health*, 2014, vol. 12, no. 3, pp. 526–532. DOI: 10.2166/wh.2014.202

8. Rashid M., Khan M.N., Jalbani N. Detection of Human Adenovirus, Rotavirus, and Enterovirus in Tap Water and Their Association with the Overall Quality of Water in Karachi, Pakistan. *Food Environ. Virol.*, 2021, vol. 13, no. 1, pp. 44–52. DOI: 10.1007/s12560-020-09448-8

9. Mejías-Molina C., Estarlich-Landajo I., Martínez-Puchol S., Bofill-Mas S., Rusiñol M. Exploring waterborne viruses in groundwater: Quantification and Virome characterization via passive sampling and targeted enrichment sequencing. *Water Res.*, 2024, vol. 266, pp. 122305. DOI: 10.1016/j.watres.2024.122305

10. Jacqueline C., Del Valle Arrojo M., Bellver Moreira P., Rodríguez Feijóo M.A., Cabrerizo M., Fernandez-Garcia M.D. Norovirus GII.3[P12] Outbreak Associated with the Drinking Water Supply in a Rural Area in Galicia, Spain, 2021. *Microbiol. Spectr.*, 2022, vol. 10, no. 4, pp. e0104822. DOI: 10.1128/spectrum.01048-22

11. Nedachin A.E., Dmitrieva R.A., Doskina T.V., Dolgin V.A. Comparative stability of poliovirus, hepatitis a virus and their RNA to the impact of ultraviolet radiation. *Gigiena i sanitariya*, 2019, vol. 98, no. 11, pp. 1240–1244. DOI: 10.47470/0016-9900-2019-98-11-1240-1244 (in Russian).

12. Bobun I.I., Buzinov R.V., Shishko L.A., Boltenkov V.P., Gudkov A.B. Features of Viral Contamination of Drinking Water in Arkhangelsk Region. *Ekologiya cheloveka*, 2016, no. 2, pp. 3–7 (in Russian).

13. Paklonskaya N.V., Amvrosieva T.V., Kaltunova Yu.B., Shilova Yu.A., Belskaya I.V. Wastewater based epidemiology as an effective tool for infection surveillance and biological hazards prevention. *Novosti mediko-biologicheskikh nauk*, 2022, vol. 22, no. 2, pp. 104–111 (in Russian).

14. Liang L., Goh S.G., Vergara G.G.R.V., Fang H.M., Rezaeinejad S., Chang S.Y., Bayen S., Lee W.A. [et al.]. Alternative fecal indicators and their empirical relationships with enteric viruses, *Salmonella enterica*, and *Pseudomonas aeruginosa* in surface waters of a tropical urban catchment. *Appl. Environ. Microbiol.*, 2015, vol. 81, no. 3, pp. 850–860. DOI: 10.1128/AEM.02670-14

15. Fongaro G., Nascimento M.A., Rigotto C., Ritterbusch G., da Silva A.D., Esteves P.A., Barardi C.R. Evaluation and molecular characterization of human adenovirus in drinking water supplies: viral integrity and viability assays. *Virol. J.*, 2013, vol. 10, pp. 166. DOI: 10.1186/1743-422X-10-166

16. Kumthip K., Khamrin P., Ushijima H., Maneekarn N. Detection of Six Different Human Enteric Viruses Contaminating Environmental Water in Chiang Mai, Thailand. *Microbiol. Spectr.*, 2023, vol. 11, no. 1, pp. e0351222. DOI: 10.1128/spectrum.03512-22

17. Chemeris A.V., Chemeris D.A., Magdanov E.G., Garafutdinov R.R., Nagaev N.R., Vakhitov V.A. Causes of false-negative PCR and how to avoid some of them. *Biomics*, 2012, vol. 4, no. 1, pp. 31–47 (in Russian).

18. Larin V.F., Zhukova L.I., Lebedev V.V., Rafeyenko G.K. Interference interaction of viruses in the regulation of an epidemic process. *Epidemiologiya i infektsionnye bolezni*, 2012, no. 1, pp. 25–29 (in Russian).

19. Sano D., Watanabe R., Oishi W., Amarasiri M., Kitajima M., Okabe S. Viral Interference as a Factor of False-Negative in the Infectious Adenovirus Detection Using Integrated Cell Culture-PCR with a BGM Cell Line. *Food Environ. Virol.*, 2021, vol. 13, no. 1, pp. 84–92. DOI: 10.1007/s12560-020-09453-x

20. Savostikova O.N., Mamonov R.A., Turina I.A., Alekseeva A.V., Nikolaeva N.I. Xenobiotics and products of their transformation in wastewater (literature review). *Gigiena i sanitariya*, 2021, vol. 100, no. 11, pp. 1218–1223. DOI: 10.47470/0016-9900-2021-100-11-1218-1223 (in Russian).

21. Takuissu G.R., Kenmoe S., Ndip L., Ebogo-Belobo J.T., Kengne-Ndé C., Mbaga D.S., Bowo-Ngandji A., Oyono M.G. [et al.]. Hepatitis E Virus in Water Environments: A Systematic Review and Meta-analysis. *Food Environ. Virol.*, 2022, vol. 14, no. 3, pp. 223–235. DOI: 10.1007/s12560-022-09530-3

22. Mendenhall I.H., Smith G.J.D., Vijaykrishna D. Ecological Drivers of Virus Evolution: Astrovirus as a Case Study. *J. Virol.*, 2015, vol. 89, no. 14, pp. 6978–6981. DOI: 10.1128/JVI.02971-14

23. Zaitseva N.V., Kleyn S.V., May I.V., Savochkina A.A., Kiryanov D.A., Kamaltdinov M.R., Vekovshinina S.A. Methodical grounds and experience gained in implementing complex assessment of activities aimed at risk to public health and effectiveness of measures to improve the quality of drinking water in centralized water supply systems. *Gigiena i sanitariya*, 2022, vol. 101, no. 11, pp. 1403–1411. DOI: 10.47470/0016-9900-2022-101-11-1403-1411 (in Russian).

Trukhina G.M., Badamshina G.G., Poptsova E.A., Yaroslavtseva M.A., Volostnova E.S., Guzairova E.R. Virus contamination of centralized water supply systems as a health risk factor: features of long-term dynamics in the Russian Federation. Health Risk Analysis, 2025, no. 2, pp. 87–97. DOI: 10.21668/health.risk/2025.2.07.eng

Received: 23.04.2025
Approved: 22.05.2025
Accepted for publication: 22.06.2025

UDC 614.446
DOI: 10.21668/health.risk/2025.2.08.eng

Read online

Research article

ISOLATION AND CHARACTERIZATION OF *BACILLUS CEREUS* STRAINS ISOLATED FROM A BEEF PIZZA FOOD POISONING INCIDENT IN HANOI

Pham Ngoc Ha[1], Ninh Thi Hanh[1], Vu Khanh Van[1], Tran Le Minh[2], Nguyen Thanh Trung[1]

[1]National Institute for Food Control, 65 Pham Than Duat St., Hanoi, Vietnam
[2]Hanoi Medical University, 1 Ton That Tung St., Hanoi, Vietnam

Bacillus cereus is one of the global causes of food poisoning. In this study, we isolated 10 strains of B. cereus from beef pizza samples identified as the cause of food poisoning among students at two kindergartens A and B in Vietnam in 2024.

Species identification was carried out using biochemical tests and MALDI-TOF technology; antibiotic resistance profile was constructed according to the M45 CLSI guidelines, and examined the presence of cytK, bceT, hbl (hblA, hblC, and hblD) and nhe (nheA, nheB, and nheC) toxin genes among these isolated strains. The antibiotic resistance testing results showed that isolated B. cereus strains were significantly resistant to several strong antibiotics, including penicillin (100 %), vancomycin (100 %), streptomycin (90 %), tetracycline (80 %), ampicillin (70 %), and erythomycin (70 %). In addition, 100 % of B. cereus strains (10/10) in the beef pizza sample were positive for the bceT toxin gene, 80 % of strains (8/10) were positive for the cytK toxin gene, and 60 % of strains (6/10) were positive for the nheA and nheC toxin genes, and negative for the NRPS emetic toxin gene. Our study contributes to the antibiotic resistance database for B. cereus associated with food poisoning in Vietnam and provides a valuable resource for developing reference materials aimed at the rapid diagnosis of food poisoning caused by diarrhea B. cereus type.

Keywords: *Bacillus cereus, food poisoning, antibiotic resistance, M45 CLSI, bceT, cytK, hbl, nhe.*

B. cereus is estimated to be responsible for nearly 12 % of total food poisoning incidents worldwide. The Centers for Disease Control and Prevention (CDC) in the United States reported that from 1998 to 2015, there were 619 food poisoning outbreaks caused by *Bacillus* spp., with 7,385 illnesses, 75 serious illnesses, and three deaths [1]. In China, a total of 419 food poisoning incidents caused by *B. cereus* were recorded from 2010 to 2020, including 7,892 people poisoned, 2,786 hospitalized and 5 fatalities [2]. Vietnam has also recorded numerous outbreaks involving *B. cereus*; for example, in 2020, 230 people were poisoned after eating food from a vegetarian restaurant in Da Nang contaminated with *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),* and *B. cereus,* while in the same year dozens of preschool children in Can Tho were hospitalized after eating pho and yogurt contaminated with *B. cereus*. In 2022, more than 600 students of a school in Nha Trang were hospitalized after consuming fried chicken wings contaminated with *Salmonella*, *B. cereus* and *E. coli*, with one fatality reported.

B. cereus is a gram-positive, rod-shaped bacterium belonging to the Bacillaceae family and is one of the most common food poisoning agents. *B. cereus* is commonly found in soil, water, and food, especially rice products, processed foods and dairy[1]. Notably, *B. cereus* is heat-resistant and produces highly drug-

Nguyen Thanh Trung – PhD, Head of Laboratory of Food Microbiology (e-mail: trungnt@nifc.gov.vn; tel.: + 84349363269; ORCID: https://orcid.org/0000-0002-8732-9911).

Pham Ngoc Ha – MSc, Researcher at Laboratory of Food Microbiology (e-mail: hapn0411@gmail.com; tel.: + 84963991575; ORCID: https://orcid.org/0009-0001-1248-3404).

Hanh Ninh Thi – Researcher at Laboratory of Food Microbiology (e-mail: ninhhanh891997@gmail.com; tel.: + 84338273077; ORCID: https://orcid.org/0000-0001-9693-3507).

Vu Khanh Van – Researcher at Laboratory of Food Microbiology (e-mail: khanhvan2180@gmail.com; tel.: + 84345096509).

Tran Le Minh – student (e-mail: minhtranle.work@gmail.com; tel.: + 84985364352).

[1] Ash C., Farrow J.A., Dorsch M., Stackebrandt E., Collins M.D. Comparative analysis of Bacillus anthracis, Bacillus cereus, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int. J. Syst. Bacteriol.*, 1991, vol. 41, no. 3, pp. 343–346. DOI: 10.1099/00207713-41-3-343

resistant spores, allowing this strain to survive in harsh environmental conditions. *B. cereus* is capable of producing toxins and causes two main types of poisoning: emetic toxin, which usually occurs when contaminated food is consumed, and diarrheal toxin, which is produced by the bacteria after the contaminated food has entered the gastrointestinal tract [3].

Widespread use of antibiotics has resulted in drug resistance in many bacterial strains, including *B. cereus*, which often produces β-lactamase enzymes that confer strong resistance to β-lactam antibiotics [4]. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, B. cereus strains are generally susceptible to aminoglycosides, clindamycin, chloramphenicol, erythromycin, and vancomycin. However, some studies indicate that B. cereus is resistant to tetracycline, streptomycin, ciprofloxacin, cloxacillin, erythromycin, and rifampicin [5].

Regarding diarrheal toxin-induced poisoning, *B. cereus* toxins associated with the diarrheal syndrome include non-hemolytic enterotoxin (*nhe*), hemolysin BL (*hbl*), cytotoxin K (*cytK*), and enterotoxin T (*bceT*)[2] [6–8]. However, the enterotoxic potential of *bceT* remains controversial in some studies [9, 10]. For emetic toxin-induced poisoning, the emetic toxin gene NRPS (Non-Ribosomal Peptide Synthetase) of *B. cereus* is responsible for producing toxic peptides such as Cereulide and isocereulides A-G. This gene functions independently of conventional protein synthesis and allows the bacteria to efficiently synthesize harmful compounds that increase the virulence of *B. cereus* in food poisoning cases [11].

In Hanoi, where traditional food preparation methods prevail, monitoring and characterizing B. cereus strains linked to outbreaks is essential to identify their origins, understand their epidemiological traits, and develop effective preventive measures.

The aim of this study is to construct antibiotic resistance profiles and detect virulence genes of *B. cereus* strains isolated from beef pizza food and related to the poisoning outbreak at two kindergartens A and B in Hanoi in 2024.

Materials and methods. Ten strains of *B. cereus* isolated from beef pizza samples were identified as the cause of food poisoning in students at two kindergartens A and B. Both preschool facilities are located in Hanoi and share a management system, with incidents occurring in 2024. Kindergarten A reported 135 students monitored for gastrointestinal disorders, while kindergarten B had 77 students in a similar condition. All relevant food samples were collected, preserved in a freezer, and sent to the National Institute for Food Control for testing and analysis.

Isolation and identification of B. cereus in beef pizza sample. The detection method for *B. cereus* was performed according to ISO 7932:2004[3] (TCVN 4992:2005). Specifically, 10 g of each food sample was homogenized in 90 mL of peptone water (Merck). A dilution series up to 10^{-5} was prepared for each sample, and 100 µL was inoculated onto Mannitol Egg Yolk Polymyxin Agar (MYP; Merck) at each dilution. All plates were incubated overnight at 37°C. Colonies exhibiting typical morphology (flat, 2–3 mm diameter, serrated edges, pinkish color, surrounded by a clear zone) were selected for hemolysis testing on blood agar and biochemical tests using the API 50 Carbohydrate kit (bioMerieux, France). The typical *B. cereus* colonies that tested positive in biochemical tests were stored at -80 °C. For identification, these colonies were streaked onto Tryptone Soya Agar (TSA; Merck), incubated for 24 hours at 37 °C, and identified using MALDI-TOF Matrix-Assisted Laser Desorption Ionization - Time of Flight on the VITEK® MS system

[2] Agata N., Ohta M., Arakawa Y., Mori M. The bceT gene of Bacillus cereus encodes an enterotoxic protein. *Microbiology (Reading)*, 1995, vol. 141, pt 4, pp. 983–988. DOI: 10.1099/13500872-141-4-983

[3] ISO 7932:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive Bacillus cereus – Colony-count technique at 30 degrees C, 3rd ed. *ISO*, 2004. Available at: https://www.iso.org/standard/38219.html (November 15, 2024).

(BioMérieux SA, Marcy l'Etoile, France). *E. coli* ATCC 8739 was used as a control strain during the run.

Evaluation of antibiotic resistance characteristics of B. cereus in beef pizza sample. The antibiotic resistance of *B. cereus* strains was tested using the Kirby-Bauer disk diffusion method[4]. *B. cereus* was grown in 10 mL of sterile BHI medium until a cell density equivalent to a 0.5 McFarland standard was reached, approximately 1.5×10^8 CFU/mL. A sterile swab was used to spread the bacterial suspension on Muller-Hinton agar. A total of 11 types of antibiotics were tested according to M45 CLSI, including ampicillin (AMP, 10 µg/disk), chloramphenicol (C, 30 µg/disk), ciprofloxacin (CIP, 5 µg/disk), erythromycin (ERY, 15 µg/disk), imipenem (IPM, 10 µg/disk), meropenem (MRP, 10 µg/disk), ofloxacin (OFX, 5 µg/disk), penicillin (PRL, 10 IU/disk), streptomycin (S, 10 µg/disk), tetracycline (TE, 30 µg/disk), and vancomycin (VAN, 30 µg/disk). The disks were placed evenly on the agar surface using sterile forceps and incubated at 37 °C for 18 hours. The antibiotic susceptibility results were determined by measuring the diameter of the inhibition zone, which is the transparent area surrounding the antibiotic disk.

Detection of toxin genes of B. cereus in beef pizza sample. DNA extraction method. Ten strains *of B. cereus* isolated from beef pizza samples were stored at -80 °C. They were streaked on blood agar to capture pure strains, cultured in BHI (Brain Heart Infusion broth) broth, and incubated for 18–24 hours at 37 °C. Total DNA of *B. cereus* was isolated according to the protocol for gram-positive bacteria of the GeneJET Genomic DNA Purification kit (Thermofisher; C5042). The total DNA concentration of *B. cereus* strains was quantified using a nanodrop spectrophotometer at 260 nm. The DNA solution was stored at -20 °C until use.

PCR and multiplex PCR reactions. Primer pairs used in this study for the detection of diarrheal and emetic toxin genes of *B. cereus* strains in beef pizza samples are listed in Table 1. PCR technique was applied for primer pairs bceT-F/R, EM1F/R, and *cytK*F/R, while the remaining primer pairs were subjected to multiplex PCR.

PCR mixture (25 µL) contained 12.5 µL of 2X PCR Master Mix (Thermo Scientific), 1 µL of forward primer (10 pmol), 1 µL of reverse primer (10 pmol), 3 µL of template DNA, and 7.5 µL of deionized water. The thermal cycling conditions for the primer pair EM1F/EM1R were set at 95 °C for 15 minutes; (95 °C for 30 seconds; 60 °C for 30 seconds; 72 °C for 60 seconds) for 30 cycles, followed by 72 °C for 5 minutes and holding at 4 °C. The thermal cycling conditions for the primer pair bceT-F/bceT-R were set at 94 °C for 5 minutes; (94 °C for 45 seconds; 55 °C for 45 seconds; 72 °C for 2 minutes) for 30 cycles, followed by 72 °C for 10 minutes and holding at 4 °C. The thermal cycling conditions for the primer pair cytKF/cytKR were set at 94 °C for 1 minute; (95 °C for 45 seconds; 54 °C for 1 minute; 72 °C for 2 minutes) for 35 cycles, followed by 72 °C for 5 minutes, and holding at 4 °C.

PCR mixture (25 µL) contained 12.5 µL of 2X PCR Master Mix (Thermo Scientific), 0.5 µL of each forward primer (20 pmol), 0.5 µL of each reverse primer (20 pmol), 3 µL of template DNA, and 3.5 µL of deionized water. The thermal cycling parameters for multiplex PCR amplification of the hblA, hblC, hblD, nheA, nheB, and nheC genes were as follows: 94 °C/2 min; (95 °C/15 s; 55 °C/45 s; 72 °C/2 min) x 35 cycles and 72 °C/5 min, and holding at 4 °C. PCR products were separated using a 1.5 % agarose gel made with 1X TAE buffer and Redsafe dye, subjected to electrophoresis at 110V for 50 minutes, followed by UV visualization, and were stored at -80 °C for future studies.

[4] Bauer A.W., Kirby W.M., Sherris J.C., Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 1966, vol. 45, no. 4, pp. 493–496.

Table 1

Primer pairs used for detection of toxin genes of *B. cereus*

Name	Sequence (5'-3')	Gene	Size (bp)	Reference
bceT-F	CGT ATC GGT CGT TCA CTC GG	Enterotoxin *bceT*	662	[12]
bceT-R	GTT GAT TTT CCG TAG CCT GGG			
EM1F	GAC AAG AGA AAT TTC TAC GAG CAA GTA CAA T	NRPS	635	[13]
EM1R	GCA GCC TTC CAA TTA CTC CTT CTG CCA CAG T			
nheAF	TAC GCT AAG GAG GGG C	*nheA*	499	[14]
nheAR	GTT TTT ATT GCT TCA TCG GCT			
nheBF	CTA TCA GCA CTT ATG GCA G	*nheB*	769	
nheBR	ACT CCT AGC GGT GTT CC			
nhCF	CGG TAG TGA TTG CTG GG	*nheC*	581	
nhCR	CAG CAT TCG TAC TTG CCA A			
hblAF	GTG CAG ATG TTG ATG CCG AT	*hbl*A	1154	
hblAR	ATG CCA CTG CCT GGA CAT A			
HbICF	GAT ACT AAT GTG GCA ACT GC	*hbl*C	740	
HbICR	TTG AGA CTG CTC GTT AGT TG			
HbIDF	AAT CAA GAG CTG TCA CGA AT	*hbl*D	829	
HbIDR	CAC CAA TTG ACC ATG CTA AT			
*CytK*F	CGA CGT CAC AAG TTG TAA CA	Cytotoxin-K	565	
*cytK*R	CGT GTG TAA ATA CCC CAG TT			

Results and discussion. Isolation, identification, and assessment of B. cereus contamination in beef pizza samples collected from kindergartens A and B. B. cereus was found in various dishes, with the highest concentration in beef pizza at $6.0 \cdot 10^5$ CFU/g in Kindergarten A and $6.8 \cdot 10^5$ CFU/g in Kindergarten B (Table 2).

It should be noted that both kindergartens sourced beef pizza from the same supplier. Currently, Vietnam does not have standards of microbial limits in pizza samples. When referring to microbial limits from other countries in the world, such as Law No. 329 of the Republic of Estonia in 2000 or the microbiological criteria established by the National Advisory Committee on Microbiological Standards for Foods under the U.S. Department of Agriculture, the maximum limit of *B. cereus* in pizza[5] or ready-to-eat food [15] is 10^3 CFU/g. Consequently, the beef pizza consumed by students in kindergartens A and B exceeded the permissible limit by 600 times and 680 times, respectively. In cases where microbiological limits for ready-to-eat foods of the Australia-New Zealand Food Standards Code 2022[6] or the Microbiological Guide for Food in 2014 of the Hong Kong Centre for Food Safety[7] are applied, the acceptable limit for ready-to-eat foods is 10^5 CFU/g. Consequently, the beef pizza from both kindergartens exceeded the limit by 6 and 6.8 times, respectively. In addition, the fried pork tenderloin, boiled mixed vegetables and seafood egg noodles of

---

[5] Governmental Regulation No. 166 of 2000 regarding validation of microbiological requirements for food groups. *FAOLEX Database*, 2000. Available at: https://www.fao.org/faolex/results/details/en/c/LEX-FAOC037807/ (September 19, 2024).

[6] Standard 1.6.1. Microbiological limits for food. *Food Standards Australia New Zealand*. Available at: https://www.foodstandards.gov.au/business/microbiological-limits (September 19, 2024).

[7] Food Legislation / Guidelines. *Centre for Food Safety*. Available at: https://www.cfs.gov.hk/english/food_leg/food_leg.html (September 19, 2024).

Table 2

Contamination levels of *B. cereus* (CFU/g) in food samples collected from kindergartens A and B

	Kindergarten A	Kindergarten B
Fried pork tenderloin	ND	$5.0 \cdot 10^1$
Boiled mixed vegetables	ND	$6.0 \cdot 10^1$
Seafood egg noodles	ND	$8.0 \cdot 10^1$
Beef pizza	$6.0 \cdot 10^5$	$6.8 \cdot 10^5$

Note: ND, not detected.

kindergarten B (Table 2) also showed *B. cereus* concentrations of $5.0 \cdot 10^1$ CFU/g, $6.0 \cdot 10^1$ CFU/g and $8.0 \cdot 10^1$ CFU/g indicating potential transmission of *B. cereus* from beef pizza to other dishes. To better understand the epidemiological characteristics and develop effective preventive measures, *B. cereus* strains isolated from beef pizza samples in both Kindergartens A and B were evaluated for antibiotic resistance.

The results of the hemolysis tests showed that all isolated strains produced beta-hemolytic zones, a characteristic feature of *B. cereus* (Figure 1). The MALDI-TOF identification results of 10 colonies isolated from beef pizza samples of kindergartens A and B showed that all ten tested colonies achieved MS MALDI–TOF scores greater than 2.0, allowing identification at the species level as *B. cereus* (10/10; 100 %). Other biochemical characteristics of the strains were evaluated using the API 50 CHE kit, in which 100 % (10/10) of the *B. cereus* strains were positive for D-Ribose, D-Glucose, D-Fructose, N-Acetylglucosamine, Arbutin, Esculin/Ferric citrate, Salicin, D-Maltose, D-Trehalose, Starch (amidon) and Glycogen, 80 % (8/10) of the strains were positive for and D-Cellobiose; 70 % (7/10) of the strains were positive for D-Saccharose (sucrose); and 50 % (5/10) of the strains were positive for Gentiobiose (Table 3).

These results are consistent with the data published in Bergey's Manual of Systematic Bacteriology. Glycerol, glycogen and starch fermentation, and Esculin/Ferric citrate-reducing properties confirmed in this biochemical test are also the properties used to differentiate *B. cereus* from closely related *Bacillus* sp. [8].

Antibiotic resistance profile of B. cereus strains isolated from beef pizza samples. All strains were susceptible (n = 10; 100 %) to meropenem and exhibited high sensitivity to ciprofloxacin (n = 9; 90 %). The results also indicated an average resistance rate of *B. cereus* to ofloxacin (n = 6; 60 %) and chloramphenicol (n = 7; 70 %). *B. cereus* strains isolated from pizza samples had absolute resistance rates to penicillin (n = 10; 100 %) and vancomycin (n = 10; 100 %); they also showed high resistance rates to streptomycin (n = 9; 90 %), tetracycline (n = 8; 80 %), ampicillin (n = 7; 70 %) and erythromycin (n = 7; 70 %); and moderate resistance rates to imipenem (n = 6; 60 %) (Figure 2).

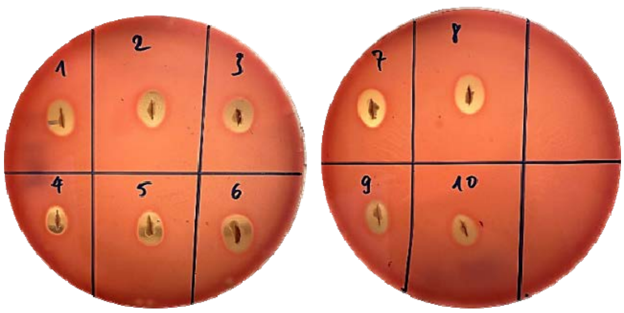

Figure 1. Hemolytic test results of 10 *B. cereus* strains isolated from beef pizza samples. 1–10: *B. cereus* strains isolated

[8] Bergey's Manual of Systematic Bacteriology. Volume One: The Archaea and the Deeply Branching and Phototrophic Bacteria, 2nd ed. In: D.R. Boone, R.W. Castenholz, G.M. Garrity eds. NY, Springer Publ., 2001, 722 p.

Table 3

Percentage of *B. cereus* strains (n = 10) positive for tests in the API 50 CH kit

No.	Test	Conc. (mg)	%	No.	Test	Conc. (mg)	%
0	Negative control		0	25	Esculin / Ferric citrate	1.16 / 0.152	100
1	Glycerol	1.64	100	26	Salicin	1.04	100
2	Erythritol	1.44	0	27	D-Cellobiose	1.32	80
3	D-Arabinose	1.4	0	28	D-Maltose	1.4	100
4	L-Arabinose	1.4	0	29	D-Lactose	1.4	0
5	D-Ribose	1.4	100	30	D-Melibiose	1.32	0
6	D-Xylose	1.4	0	31	D-Saccharose	1.32	70
7	L-Xylose	1.4	0	32	D-Trehalose	1.32	100
8	D-Adonitol	1.36	0	33	Inulin	1.28	0
9	Methyl-ß-D-xylopyranoside	1.28	0	34	D-Melezitose	1.32	0
10	D-Galactose	1.4	0	35	D-Raffinose	1.56	0
11	D-Glucose	1.56	100	36	Starch	1.28	100
12	D-Fructose	1.4	100	37	Glycogen	1.28	100
13	D-Mannose	1.4	0	38	Xylitol	1.4	0
14	L-Sorbose	1.4	0	39	Gentiobiose	0.5	50
15	L-Rhamnose	1.36	0	40	D-Turanose	1.32	0
16	Dulcitol	1.36	0	41	D-Lyxose	1.4	0
17	Inositol	1.4	0	42	D-Tagatose	1.4	0
18	D-Mannitol	1.36	0	43	D-Fucose	1.28	0
19	D-Sorbitol	1.36	0	44	L-Fucose	1.28	0
20	Methyl-α-D-mannopyranoside	1.28	0	45	D-Arabitol	1.4	0
21	Methyl-α-D-glucopyranoside	1.28	0	46	L-Arabitol	1.4	0
22	N-Acetylglucosamine	1.28	100	47	Potassium gluconate	1.84	0
23	Amygdalin	1.08	0	48	Potassium 2-ketogluconate	2.12	0
24	Arbutin	1.08	100	49	Potassium 5-ketogluconate	1.8	0

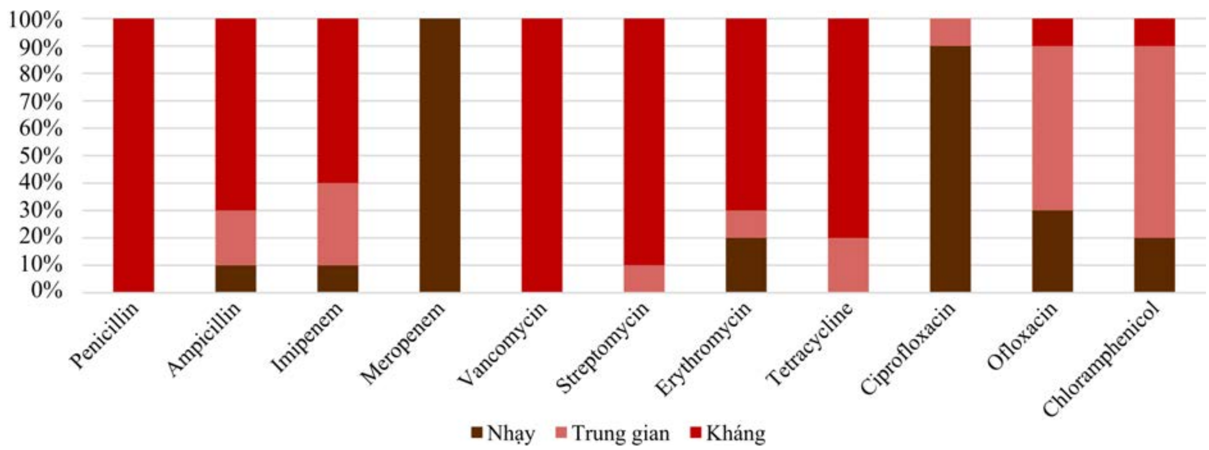

Figure 2. Antibiotic profile of *B. cereus* based on inhibition zone diameter (mm); (brown) resistant, (orange) intermediate, (red) susceptible

From the above results, meropenem and ciprofloxacin demonstrated promising antibacterial activity against the *B. cereus* strains in the study. In contrast to M45 CLSI guidelines, which indicate *B. cereus* is typically resistant to penicillin but often susceptible to vancomycin and macrolides, our study found that *B. cereus* strains from beef pizza samples exhibited 100 % resistance to vancomycin and 70 % resistance to erythromycin. Abdelaziz

ISSN (Print) 2308-1155 ISSN (Online) 2308-1163 ISSN (Eng-online) 2542-2308

et al. (2024) examined the antibiotic resistance patterns of *B. cereus* strains in food in Japan and reported high levels of resistance to vancomycin [16], which is similar to our study. Nakayama (2021) showed that *B. cereus* strains isolated from chicken in Ho Chi Minh City were resistant to ampicillin, ciprofloxacin, and tetracycline [17], while *B. cereus* strains analyzed in this study were sensitive to ciprofloxacin and had resistance rates to ampicillin and tetracycline. When comparing the antibiotic resistance profiles of 10 *B. cereus* strains isolated from beef pizza samples in the kindergarten poisoning case with *B. cereus* strains circulating globally, our results were similar to those of Algammal et al. (2022). *B. cereus* strains circulating in Egypt were susceptible to meropemen and exhibited resistance to several antibacterial agents such as erythromycin, streptomycin and tetracycline [18], similar to *B. cereus* strains in this study. Further investigations at the DNA level regarding virulence and antibiotic resistance genes are necessary to thoroughly explore the epidemiological characteristics and implement preventive measures against *B. cereus* in beef pizza samples.

Presence of toxin genes of B. cereus in beef pizza sample. Ten strains *of B. cereus* isolated from beef pizza samples involved in the poisoning incidents at kindergartens A and B in Hanoi were analyzed for emetic (NRPS) and diarrheal (*hblA, hblC, hblD, nheA, nheB, nheC, bceT* and *cytK*) toxin gene using PCR (NRPS, *bceT* and *cytK)* and multiplex PCR *(hblA, hblC, hblD, nheA, nheB,* and *nheC).* The electrophoresis results (Figures 3–5) indicate that 100 % of the *B. cereus* strains (10/10) from the beef pizza sample were positive for the *bceT* gene, 80 % of the strains (8/10) were positive for the *cytK* gene and 60 % of the strains (6/10) were positive for the *nheA* and *nheC* genes. Additionally, 100 % of the strains in this study (10/10) were negative for NRPS gene (electrophoresis image not included in this paper).

Our findings are consistent with other studies in the world. In Iraq, B.M.S. Saeed et al. (2021) reported a very low detection rate of the emetic toxin gene in all food samples, approximately 7.69 %, indicating a low prevalence of emetic *B. cereus* strains in foods [14]. N. Jessberger et al. (2021) [19] and M. Bağcioğlu et al. (2019) [20] also reported that emetic *B. cereus* type was less commonly found in food compared to diarrheal type. Our study contributes to the database of *B. cereus* strains circulating in Vietnam; moreover, the *B. cereus* strains carrying the diarrheal toxin gene identified in this research provide material for developing a reference material to support rapid and accurate food poisoning diagnostics.

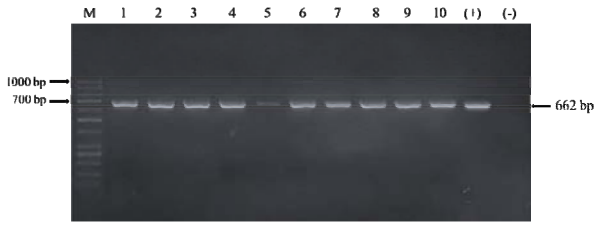

Figure 3. PCR product electrophoresis of *bceT* toxin gene. 1–10: *bceT* positive (662bp). M: DNA ladder 50bp; (+): Positive control; (-): Negative control

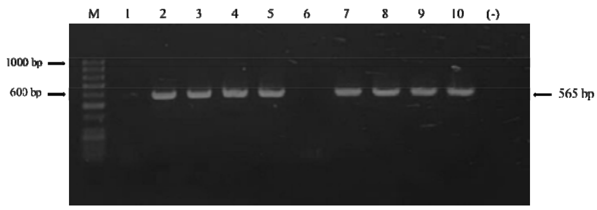

Figure 4. PCR product electrophoresis image of *cytK* toxin. 1, 6: Negative; 2–5 and 7–10: *cytK* positive (565bp). M: DNA ladder 50bp; (-): Negative control

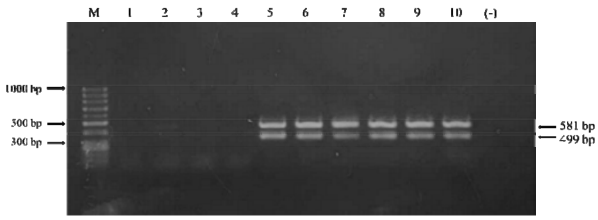

Figure 5. Electrophoresis images of multiplex PCR products of the *hbl* (*hblA*, *hblC*, and *hblD*) and *nhe* (*nheA*, *nheB*, and *nheC*) toxin genes: 1–4: Negative; 5–10: *nhA* and *nhC* positive (581bp, 499bp). M: DNA ladder 50bp; (-): Negative control

Conclusion. The study isolated 10 strains of *B. cereus* from beef pizza samples identified as the cause of poisoning among students at two kindergartens A and B, revealing *B. cereus* concentrations exceeding permissible limits set by current global regulations, specifically $6.0 \cdot 10^5$ CFU/g at Kindergarten A and $6.8 \cdot 10^5$ CFU/g at Kindergarten B. Among the 11 antibiotics used to evaluate the antibiotic resistance of isolated strains, *B. cereus* exhibited absolute resistance (100 %) to penicillin and vancomycin, high resistance rates to streptomycin (90 %), tetracycline (80 %), ampicillin (70 %) and erythromycin (70 %); moderate resistance to imipenem (60 %) and were susceptible to meropenem (100 %) and ciprofloxacin (90 %). Furthermore, 100 % of *B. cereus* strains (10/10) in the beef pizza sample were positive for *bceT* gene, 80 % of the strains (8/10) were positive for *cytK* gene and 60 % of the strains (6/10) were positive for *nheA* and *nheC* genes indicating that these *B. cereus* strains belong to the diarrhea-causing type. *B. cereus* remains a major pathogenic threat due to its rapid evolution, virulence genes, and antibiotic resistance, making continuous monitoring of the genetic profile and antibiotic resistance of circulating strains in Vietnam crucial for disease prevention and response.

Funding. The authors express their deepest gratitude for financial support provided by the National Institute for Food Control, Vietnam.

Competing interests. The authors declare no competing interests.

References

1. McDowell R.H., Sands E.M., Friedman H. Bacillus Cereus. *StatPearls*. Treasure Island (FL), StatPearls Publ., 2024. Available at: http://www.ncbi.nlm.nih.gov/books/NBK459121/ (September 19, 2024).

2. Duan S., Yu Y., Guo Y., Lu D., Li N., Liu Z., Liang J., Jiang Y. [et al.]. Epidemiological Evaluation of *Bacillus cereus*-Induced Foodborne Outbreaks – China, 2010–2020. *China CDC Wkly*, 2023, vol. 5, no. 33, pp. 737–741. DOI: 10.46234/ccdcw2023.140

3. Bottone E.J. Bacillus cereus, a Volatile Human Pathogen. *Clin. Microbiol. Rev.*, 2010, vol. 23, no. 2, pp. 382–398. DOI: 10.1128/CMR.00073-09

4. Etikala A., Thamburaj S., Johnson A.M., Sarma C., Mummaleti G., Kalakandan S.K. Incidence, toxin gene profile, antibiotic resistance and antibacterial activity of *Allium parvum* and *Allium cepa* extracts on *Bacillus cereus* isolated from fermented millet-based food. *LWT*, 2022, vol. 160, no. 11, pp. 113314. DOI: 10.1016/j.lwt.2022.113314

5. Jensen L.B., Baloda S., Boye M., Aarestrup F.M. Antimicrobial resistance among Pseudomonas spp. and the Bacillus cereus group isolated from Danish agricultural soil. *Environ. Int.*, 2001, vol. 26, no. 7–8, pp. 581–587. DOI: 10.1016/s0160-4120(01)00045-9

6. Dietrich R., Jessberger N., Ehling-Schulz M., Märtlbauer E., Granum P.E. The Food Poisoning Toxins of Bacillus cereus. *Toxins (Basel)*, 2021, vol. 13, no. 2, pp. 98. DOI: 10.3390/toxins13020098

7. Ramm F., Stech M., Zemella A., Frentzel H., Kubick S. The Pore-Forming Hemolysin BL Enterotoxin from Bacillus cereus: Subunit Interactions in Cell-Free Systems. *Toxins (Basel)*, 2021, vol. 13, no. 11, pp. 807. DOI: 10.3390/toxins13110807

8. Zhao Y., Sun L. Bacillus cereus cytotoxin K triggers gasdermin D-dependent pyroptosis. *Cell Death Discov.*, 2022, vol. 8, no. 1, pp. 305. DOI: 10.1038/s41420-022-01091-5

9. Choma C., Granum P.E. The enterotoxin T (BcET) from Bacillus cereus can probably not contribute to food poisoning. *FEMS Microbiol. Lett.*, 2002, vol. 217, no. 1, pp. 115–119. DOI: 10.1111/j.1574-6968.2002.tb11464.x

10. Hansen B.M., Høiby P.E., Jensen G.B., Hendriksen N.B. The Bacillus cereus bceT enterotoxin sequence reappraised. *FEMS Microbiol. Lett.*, 2003, vol. 223, no. 1, pp. 21–24. DOI: 10.1016/S0378-1097(03)00249-0

11. Marxen S., Stark T.D., Rütschle A., Lücking G., Frenzel E., Scherer S., Ehling-Schulz M., Hofmann T. Depsipeptide Intermediates Interrogate Proposed Biosynthesis of Cereulide, the Emetic Toxin of Bacillus cereus. *Sci. Rep.*, 2015, vol. 5, pp. 10637. DOI: 10.1038/srep10637

12. Ehling-Schulz M., Fricker M., Scherer S. Identification of emetic toxin producing Bacillus cereus strains by a novel molecular assay. *FEMS Microbiol. Lett.*, 2004, vol. 232, no. 2, pp. 189–195. DOI: 10.1016/S0378-1097(04)00066-7

13. Cở sở dữ liệu nhiệm vụ KHCN – Đánh giá khả năng phát hiện trực tiếp gen độc tố của Bacillus Cereus trong một số thực phẩm có nguồn gốc từ gạo bằng kỹ thuật Multiplex PCR. *BỘ KHOA HỌC VÀ*

CÔNG NGHỆ HỆ THỐNG THÔNG TIN KHOA HỌC VÀ CÔNG NGHỆ. Available at: https://sti.vista.gov.vn/tw/Pages/tai-lieu-khcn.aspx?ItemID=199450&Type_CSDL=TAILIEUKHCN&Keyword=&searchInFields... (October 08, 2024) (in Vietnamese).

14. Saeed B.M.S., Abbas B.A., Al-Jadaan S.A.N. Detection of Bacillus cereus genes responsible for diarrheal and emetic toxins. *J. Phys. Conf. Ser.*, 2021, vol. 1879, no. 2, pp. 022034. DOI: 10.1088/1742-6596/1879/2/022034

15. National Advisory Committee On Microbiological Criteria For Foods. Response to Questions Posed by the Department of Defense Regarding Microbiological Criteria as Indicators of Process Control or Insanitary Conditions. *J. Food Prot.*, 2018, vol. 81, no. 1, pp. 115–141. DOI: 10.4315/0362-028X.JFP-17-294

16. Abdelaziz M.N.S., Zayda M.G., Maung A.T., El-Telbany M., Mohammadi T.N., Lwin S.Z.C., Linn K.Z., Wang C. [et al.]. Genetic Characterization, Antibiotic Resistance, and Virulence Genes Profiling of *Bacillus cereus* Strains from Various Foods in Japan. *Antibiotics (Basel)*, 2024, vol. 13, no. 8, pp. 774. DOI: 10.3390/antibiotics13080774

17. Nakayama T., Yamaguchi T., Jinnai M., Yamamoto S., Li H.T., Ngo P.T., Tran D.N.M., Nguyen O.T.H. [et al.]. Untargeted Phylogenetic Group III of Multi-drug-Resistant Bacillus cereus Isolated Using Fraser Medium from Retail Chickens in Ho Chi Minh City. *Curr. Microbiol.*, 2021, vol. 78, no. 8, pp. 3115–3123. DOI: 10.1007/s00284-021-02562-1

18. Algammal A.M., Alfifi K.J., Mabrok M., Alatawy M., Abdel-Moneam D.A., Alghamdi S., Azab M.M., Ibrahim R.A. [et al.]. Newly Emerging MDR *B. cereus* in *Mugil seheli* as the First Report Commonly Harbor *nhe, hbl, cytK*, and *pc-plc* Virulence Genes and *bla*1, *bla*2, *tetA*, and *erm*A Resistance Genes. *Infect. Drug Resist.*, 2022, vol. 15, pp. 2167–2185. DOI: 10.2147/IDR.S365254

19. Jessberger N., Dietrich R., Granum P.E., Märtlbauer E. The *Bacillus cereus* Food Infection as Multifactorial Process. *Toxins*, 2020, vol. 12, no. 11, pp. 701. DOI: 10.3390/toxins12110701

20. Bağcıoğlu M., Fricker M., Johler S., Ehling-Schulz M. Detection and Identification of *Bacillus cereus, Bacillus cytotoxicus, Bacillus thuringiensis, Bacillus mycoides* and *Bacillus weihenstephanensis* via Machine Learning Based FTIR Spectroscopy. *Front. Microbiol.*, 2019, vol. 10. DOI: 10.3389/fmicb.2019.00902

Pham Ngoc Ha, Ninh Thi Hanh, Vu Khanh Van, Tran Le Minh, Nguyen Thanh Trung. Isolation and characterization of Bacillus cereus strains isolated from a beef pizza food poisoning incident in Hanoi. Health Risk Analysis, 2025, no. 2, pp. 98–106. DOI: 10.21668/health.risk/2025.2.08.eng

Received: 03.12.2024
Approved: 25.04.2025
Accepted for publication: 14.06.2025

UDC 613.6.01: 614.3
DOI: 10.21668/health.risk/2025.2.09.eng

Read online

Research article

SANITARY-EDUCATIONAL ACTIVITIES AS AN EFFECTIVE METHOD OF NONSPECIFIC PLAGUE PREVENTION IN THE NATURAL PLAGUE FOCUS IN THE KOSH-AGACH DISTRICT OF THE REPUBLIC OF ALTAI

S.V. Balakhonov[1], E.S. Kulikova[1], A.I. Mishchenko[2], V.M. Korzun[1], A.V. Rogaleva[1], S.M. Lyashenko[1], A.A. Itashev[2], S.L. Sarikova[3], N.B. Kalybayeva[4], N.M. Madinova[4]

[1] Irkutsk Anti-Plague Research Institute, 78 Trilissera St., Irkutsk, 664047, Russian Federation
[2]Altai Anti-Plague Station, 2 Zavodskaya, Gorno-Altaisk, 649002, Russian Federation
[3] Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, Altai Republic Regional Office, 173 Kommunisticheskii Av., Gorno-Altaisk, 649002, Russian Federation
[4]Kosh-Agach district hospital, 3 Meditsinskaya, Kosh-Agach, 649780, Republic of Altai, Russian Federation

The Altai Mountains are an area of ecological tourism with high potential for further development. At the same time, intensive epizootics of plague are typical for the region including those registered in close proximity to settlements and shepherds' camps raising likelihood of potential contacts between people and plague vectors and carriers. Potential hazard of plague cases among the population persists in this area and this can result in serious epidemiological complications. From 2010 to 2024, 397 plague microbe strains, 182 Yersinia pestis ssp. pestis and 215 Y. pestis ssp. central asiatica bv. Altaica, were isolated and investigated in the Gorno-Altaisk high-mountain natural plague focus. After cases of the infection were identified in humans (2014–2016), the coverage of the sanitary-educational work increased considerably. Overall, 282 lectures were delivered to the population, more than 15 thousand leaflets and booklets were handed out, and more than 6630 talks were organized over 2016–2022. Annually, sanitary-educational activities are organized for an audience between 20 and 63 thousand people.

The aim of this study was to assess effectiveness of sanitary-educational activities aimed at informing people about risk factors associated with living in a natural plague focus.

The study method was a survey conducted among the regional population using a specifically designed questionnaire. Totally, 1650 residents of the Kosh-Agach districts, including 135 shepherds and their family members, took part in the survey; the district was selected as a plague-endemic area.

According to the survey results, people from the Kosh-Agach district of the Altai Republic (more than 90 % of the respondents) were established to be well aware about risk factors associated with living in a natural plague focus as well as the infection sources, namely, plague vectors and carriers. Most people who lived in this endemic area had means of communication and transportation necessary for applying for medical aid and were ready to inform healthcare workers in case plague was suspected or the first plague signs were manifested in them or their friends or acquaintances

Keywords: *Gorno-Altaisk high-mountain natural plague focus, survey, sanitary-educational work, risk factors.*

Sergey V. Balakhonov – Doctor of Medical Sciences, Professor, director (e-mail: adm@chumin.irkutsk.ru; tel.: +7 (395) 222-01-35; ORCID: https://orcid.org/0000-0003-4201-5828).

Elena S. Kulikalova – Candidate of Medical Sciences, Head of the Department of Epidemiology (e-mail: adm@chumin.irkutsk.ru; tel.: +7 (395) 222-01-35; ORCID: https://orcid.org/0000-0001-7034-5125).

Aleksandr I. Mishchenko – doctor of Epidemiology Department (e-mail: chumagorny@mail.ru; tel.: +7 (388) 226-43-19).

Vladimir M. Korzun – Doctor of Biological Sciences, Head of Zoological and Parasitological Department (e-mail: adm@chumin.irkutsk.ru; tel.: +7 (395) 222-01-35; ORCID: https://orcid.org/0000-0003-1947-5252).

Anastasiya V. Rogaleva – Junior Researcher of the Department of Epidemiology (e-mail: nastyarogaleva77@gmail.com; tel.: +7 (914) 923-80-90; ORCID: https://orcid.org/0009-0009-3951-714X).

Semyon M. Lyashchenko – doctor of the Department of Epidemiology (e-mail: adm@chumin.irkutsk.ru; tel.: +7 (395) 222-01-35; ORCID: https://orcid.org/0009-0003-9778-3485).

Arzhan A. Itashev – Deputy Director for Epidemiological Work (e-mail: chumagorny@mail.ru; tel.: +7 (388) 226-43-19; ORCID: https://orcid.org/0009-0002-1183-9728).

Svetlana L. Sarikova – Deputy Head (e-mail: Tagizova_SL@04.rospotrebnadzor.ru; tel.: +7 (923) 664-62-72).

Nataliya B. Kalybaeva – chief physician (e-mail: kosh-agach@med04.ru; tel.: +7 (388) 422-26-55).

Natal'ya M. Madinova – Head of the Infectious Diseases Department (e-mail: kosh-agach@med04.ru; tel.: +7 (388) 422-26-55).

The trans-border Sailyugemskii natural plague focus is located in the north border area of the vast Central Asia zone with natural foci of the infection and covers territories in two countries, Russia and Mongolia. Its northern Russian part (Gorno-Altaisk high-mountain natural plague focus) is located in the South-Eastern Altai Mountains or, according to administrative division, in the Kosh-Agach district of the Altai Republic [1]. The Altai Mountains are becoming more and more popular as an area for environmental tourism and this area is being visited by multiple Russian and foreign tourists. At the same time, it is endemic per plague and potential zoonotic plague foci are often located in close proximity to settlements and shepherds' camps as well as to major transport routes with high shares of trans-border trips. All this raises likelihood of potential contacts between people and plague vectors and carriers.

A considerable part of the Kosh-Agach district of the Altai Republic has been characterized with high epizootic activity per plague for a long time (since early 1990ties) [2]. All three plague cases among people (2014–2016) were caused by hunting marmots and eating their meat [3–6]. For Altai people, marmot meat is a traditional delicacy and is often given as a present to esteemed guests; this can cause the infection. Thus, when an epidemiological investigation was conducted after the epidemic situation per plague became complicated in 2014, three marmot carcasses were found in a fridge that belonged to the plague patient A. in Mukhor-Tarkhata settlement. One of them was infected with plague. Those marmots had been hunted on purpose to be served as a delicacy during a holiday feast. In 2015, two marmot carcasses (plague microbe strains of the basic sub-species were isolated from both during microbiological testing) were taken from a fridge in the house of the patient M. in Kyzyl-Tash settlement; they had been prepared for a New Year feast [3]. Therefore, there is a risk that the epidemiological situation per plague can get complicated in a season not typical for the disease and that the plague agent can spread far beyond the boundaries of an area enzootic per plague, marmot meat contaminated with the plague microbe acting as an infection source [3–6].

High epizootic activity that persists in the Gorno-Altaisk high-mountain natural plague focus determines its highest epidemic significance in Russia. Here, over the period between 2010 and 2014, 397 plague microbe strains were isolated from field materials; of them, *Yersinia pestis* ssp. *pestis,* 182; *Y. pestis* ssp. *central asiatica* bv. *Altaica,* 215.

Given high epizootic activity in the natural plague focus, sanitary education provided for the population becomes a significant component of preventive activities together with immune prevention of the infection and timely disinsection measures. This sanitary education should be aimed at informing people about health risk factors associated with living and performing economic and other activities in a plague-enzootic area. These activities are accomplished in accordance with the Complex Plan of Scheduled Activities by Rospotrebnadzor Aimed at Improving the Situation in the Gorno-Altaisk High-Mountain Natural Plague Focus located in the Kosh-Agach district of the Altai Republic; the plan is annually approved and prolonged by the Head of Rospotrebnadzor. Basic activities aimed at plague prevention, which were accomplished to localize and eliminate plague cases as well as annually taken measures, were described in works published in 2018 [7, 8]. It seems very important to have a feedback as solid grounds for improving preventive activities, sanitary education included, which involve local population. Use of medical investigative questionnaires for surveys is a powerful tool for getting such a feedback. Surveys allow reaching the maximum number of respondents and obtain large enough samples, which are representative for using proved statistical models [9, 10].

The aim of this study was to assess effectiveness of sanitary-educational activities aimed at informing people about risk factors associated with living in a natural plague focus.

Materials and methods. The study relied on using questionnaires developed by Rospotrebnadzor experts in 2017 and updated in 2024. The questionnaires were made of three blocks: general information (9 questions), social and household conditions (12 questions), and types of contacts between people and the natural plague focus (21 questions). People who were exposed to high risks of plague infection took part in the survey; they lived in a plague-endemic area located in the Kosh-Agach district. Experts of the Altai Anti-plague Station distributed the questionnaires through paramedic and obstetric stations. The respondents were asked to give detailed answers or select the most suitable options. All 132 questionnaires filled by shepherds and their family members who lived in summer camps were put into a database created in Microsoft Excel. The survey results were analyzed using conventional variation statistics with calculating the extensive indicator and 95 % confidence intervals (CI)[1] :

$$CL = \overline{x} \pm Z \cdot \frac{S}{\sqrt{n}},$$

where CL is confidence interval;

$\overline{x}$ is the sample mean;

Z is the confidence level value for calculating 95 % likelihood = 1.96;

S is the sample standard deviation;

N is the sample size.

Conclusions on effectiveness of sanitary-educational activities performed in the natural plague focus were made based on the survey results; in addition, relevant recommendations were developed.

Results and discussion. High epizootic activity of the natural plague focus in the Kosh-Agach district indicates how significant it is to inform people about risk factors able to cause plague. Overall, 282 lectures were delivered to the population in the area over 2016–2022 (on average, approximately 40 lectures a year); 15,263 leaflets and booklets were handed out and more than 6630 talks were held. The total number of people involved in sanitary education every year grew from 20,000 in 2016–2020 to 64,580 in 2022 [1]. Effectiveness of activities performed in 2016–2017 is confirmed by the survey conducted by an epidemiologic group among locals June to September 2017. Overall, 1650 people who lived in the Kosh-Agach district took part in it. The survey established that people knew there was a threat of getting infected with plague and about basic signs of the disease; they had positive attitudes towards vaccination, disinsection, and deratization. Most respondents (91 %) were aware what role marmots had in plague transmission to humans. Fleas as the infection vectors were mentioned by 77 % of the respondents; 89 % knew it was prohibited to hunt marmots but only 61 % of them were ready to cooperate with law enforcement agencies in fighting against poachers who hunted grey (Altai) marmots. These data indicate it is necessary to intensify sanitary-educational work with people as regards responsibility for violating prohibitions and limitations imposed on marmot hunting; preventive activities are also advisable [1].

In 2024, 17 lectures were delivered in the Gorno-Altaisk High-Mountain Natural Plague Focus, 730 healthcare workers and 57 veterinaries were consulted on plague prevention methods; 816 healthcare workers took part in workshops with their focus on clinical mani-

———————————

[1] Rokitskii P.F. Biologicheskaya statistika [Biological statistics], 3rd ed., revised. Minsk, Vysheish. shkola Publ., 1973, 320 p. (in Russian); Glantz S. Mediko-biologicheskaya statistika [Biomedical statistics], translated from English. Moscow, Praktika Publ., 1998, 459 p. (in Russian).

festation and prevention of the disease. Overall, 18,966 people living in the area were involved in all types of sanitary-educational work with its focus on plague.

In 2024, the screening group of the respondents who took part in the survey was made of men and women in approximately equal proportions (men accounted for 54.1 % and women 45.9 %) (Figure 1).

It is noteworthy that not all respondents gave answers to each question in the questionnaire; therefore, the sum of all answers does not equal 100 percent everywhere. The age structure of the population in settlements and shepherd camps in the natural plague focus was as follows: people aged 30–49 years accounted for 37.1 %; 50–64 years, 27.2 %; older than 65 years, 3.0 %; the smallest group was people aged 18–29 years, 1.5 %. Altai people were the prevailing nationality accounting for 58.3 %; they were followed by Kazakhs, 30.3 %, and Telengits, 1.5 %. The remaining 9.9 % were mixed families consisting of people with these nationalities. Two-person families lived in a summer camp in almost half of the cases (43.2 %), but sometimes families who lived there were made of more people. Eighty-one point one percent of the respondents did not have children or their children did not live in these camps.

Most respondents (97.7 %) estimated sanitary conditions in their housing as having medium quality. Among outhouses, many respondents (88.6 %; CI: 83.2–94.0 %) mentioned sheep yards. Most respondents (82.6 %; CI: 76.1–89.1 %) stated there were no rodents, fleas, ticks or lice in their homes. The respondents predominantly estimated their hygienic knowledge as medium (68.2 %; CI: 60.3–76.1 %). Dogs were kept as pets the most often; cats were a rarer occasion. Camels were kept at two camps (Ak-Kel area, Beltirkii paramedic and obstetric station and Dzudut-Zhan-Aulskii paramedic and obstetric stations). Rest in camps as a leisure activity was chosen by 47.0 % (CI: 38.5–55.5 %) of the respondents.

Most respondents, 65.9 % (CI: 57.8–74.0 %) lived between 11 and 50 km away from the nearest healthcare facility; 68.9 % (CI: 61.0–76.8 %) of the respondents applied for healthcare services once a month or more often. Absence of any communication means was mentioned by 16.7 % of the respondents (CI: 10.3–23.1 %) living in this area; this could make it difficult for them to apply for medical aid in time in case some plague signs were manifested. The emergency phone number was known to 56.1 % (CI: 47.6–64.6 %); in addition, 25.8 % (CI: 18.3–33.3 %) of the respondents said they knew a healthcare worker's personal mobile number. A long distance to healthcare facilities was to a certain extent compensated by more than a half of the respondents having a car and another 15.2 % having a bike (CI: 9.1–21.3 %).

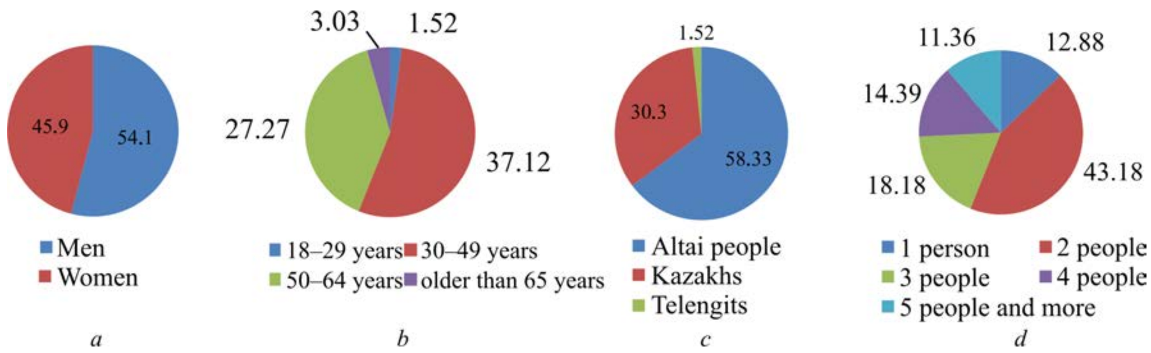

Figure 1. The profile of the screening respondent group (2024). Distribution per:
a) sex; b) age; c) nationality; d) number of family members

Most respondents were shepherds 71.2 % (CI: 63.5–78.9 %); of them, 7.6 % (CI: 3.1–12.1 %) let their sheep graze on wild pastures. Seventy-six point five percent (CI: 69.3–83.7 %) were involved in making hay: 10.6 % (CI: 5.3–15.9 %) by hand and 53.8 % (CI: 45.3–62.3 %) using mowing machines; 12.1 % (CI: 6.5–17.7 %) used both ways. Half of the respondents stocked fuel (pressed dung). Thirty-five point six percent gathered berries, mushrooms and herbs (CI: 27.4–43.8 %); 3.0 % dealt with some construction (CI: 0.1–5.9 %). Doing these works was associated with staying in camps for 60.0 % of the respondents (CI: 51.6–68.4 %); these camps were located in areas with rodents' colonies, which raised likelihood of contacts with the parasitic system of the natural plague focus.

Almost all respondents (97.0 %; CI: 94.1–99.9 %) knew about the prohibition imposed on marmot hunting. Still, 16.7 % (CI: 10.3–23.1 %) pointed out that they knew about some cases of marmot hunting involving their relatives, friends, or acquaintances. The respondents did not mention their pets brining home dead small mammals; still, rodents' carcasses were found by four respondents (3.0 %; CI: 0.1–5.9 %). More than a half of the respondents, 61.4 % (CI: 53.1–69.7 %) believed that children should be kept away from summer camps. Interestingly, 78.0 % of the respondents (CI: 79.4–88.8 %) gave a negative answer to the question about eating marmot meat but 21.2 % (CI: 14.2–28.2 %) did not give any answer at all. This is rather alerting and requires some thinking about the actual situation. Most respondents, 72.0 % (CI: 64.3–79.7 %) lived in close proximity (within 1 km) to rodent colonies.

Anti-plague vaccination was considered useful by 91.0 % (CI: 86.1–95.9 %) of the respondents whereas 9.0 % (CI: 4.1–13.9 %) of them had a negative attitude towards specific prevention; deratization was considered a useful activity by 96.2 % (CI: 92.9–99.5 %). Disease cases with inflammation in the groin or armpit areas in case history were mentioned by 0.8 % (CI: 0–2.3 %) and 80.3 % (CI: 73.5–87.1 %) of the respondents gave the negative answer to this question while the remaining were hesitant to give any.

The study conducted in 2024 established that 95.8 % (CI: 96.4–100.00 %) of the respondents knew about main risk factors associated with living in the natural plague focus, namely, marmots as carriers of the major subspecies of the plague agent; 91.7 % (CI: 87.0–96.4 %) mentioned fleas as plague vectors. Most respondents (75.8 %; CI: 68.5–83.1 %) gave the correct answer that only camels could be sick with plague among all household animals and pets. Almost all respondents (98.5 %; CI: 96.4–100 %) said they were ready to inform healthcare workers in case they suspected plague signs in themselves or people they knew (Figure 2).

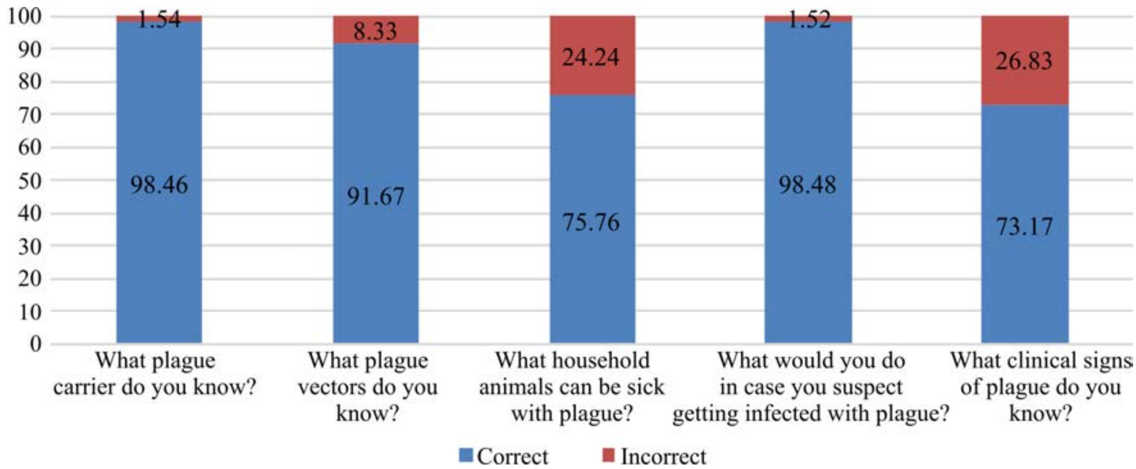

Figure 2. People's awareness about risk factors of plague infection in the Gorno-Altaisk high-mountain natural plague focus and clinical signs of the disease

Therefore, awareness is quite high among people living in the high mountain natural plague focus. The respondents are informed about major infection risk factors such as contacts with basic plague carriers and vectors in the natural plague focus. Most people who live in this plague-endemic area have communication and transport means necessary to reach a healthcare facility in case the first plague signs are manifested.

Conclusion. Endemic plague-related complications were avoided as a result of implemented activities, including sanitary education, after 2016. It is quite relevant to plan and conduct sanitary-education activities by Rospotrebnadzor organizations and healthcare institutions in the Altai Republic. They should inform about health risks and involve permanent and / or temporary residents who live and perform various economic activities in the analyzed natural plague focus.

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. Transgranichnyi Sailyugemskii prirodnyi ochag chumy [Transborder Sailugem natural plague focus]. In: S.V. Balakhonov, V.M. Korzun eds. Novosibirsk, 2022, 248 p. (in Russian).

2. Balakhonov S.V., Korzun V.M., Chipanin E.V., Afanasev M.V., Mikhailov E.P., Denisov A.V., Fomina L.A., Eshelkin I.I. [et al.]. Mountain-Altai natural plague focus: retrospective analysis, epizootological monitoring, current state. In: S.V. Balakhonov, V.M. Korzun eds. Novosibirsk, Nauka-Tsentr Publ., 2014, 272 p. (in Russian).

3. Balakhonov S.V., Popova A.Yu., Mishchenko A.I., Mikhailov E.P., Ezhlova E.B., Demina Yu.V., Denisov A.V., Rozhdestvensky E.N. [et al.]. A Case of Human Infection with Plague in the Kosh-Agach Region of the Republic of Altai in 2015. Communication 1. Clinical-Epidemiological and Epizootiological Aspects. *Problemy osobo opasnykh infektsii*, 2016, no. 1, pp. 55–60. DOI: 10.21055/0370-1069-2016-1-55-60 (in Russian).

4. Balakhonov S.V., Korzun V.M., Kosilko S.A., Mikhailov E.P., Shchuchinov L.V., Mishchenko A.I., Zarubin I.V., Rozhdestvensky E.N., Denisov A.V. Actual Aspects of Epidemiological Well-Being Support Anti-Plague for Population in Altai Republic. *Epidemiologiya i vaktsinoprofilaktika*, 2016, vol. 15, no. 4, pp. 42–48. DOI: 10.31631/2073-3046-2016-15-4-42-48 (in Russian).

5. Korzun V.M., Balakhonov S.V., Kosilko S.A., Mikhailov E.P., Mishchenko A.I., Denisov A.V., Rozhdestvensky E.N., Chipanin E.V. [et al.]. Gorno-Altai Natural Plague Focus Epizootical and Epidemical Activity in 2012–2016. *Epidemiologiya i vaktsinoprofilaktika*, 2017, vol. 16, no. 1, pp. 36–38. DOI: 10.31631/2073-3046-2017-16-1-36-38 (in Russian).

6. Kutyrev V.V., Popova A.Yu., Ezhlova E.B., Demina Yu.V., Pakskina N.D., Shchuchinov L.V., Mikhailov E.P., Mishchenko A.I. [et al.]. Infection of an Individual with Plague in the Gorno-Altaisk High-Mountain Natural Focus in 2014. Communication 1. Epidemiological and Epizootiological Peculiarities of Plague Manifestations in the Gorno-Altaisk High-Mountain (Sailyugemsky) Natural Plague Focus. *Problemy osobo opasnykh infektsii*, 2014, no. 4, pp. 9–16. DOI: 10.21055/0370-1069-2014-4-9-16 (in Russian).

7. Balakhonov S.V., Schuchinov L.V., Mischenko A.I., Matrosov A.N., Denisov A.V., Rozhdestvensky E.N., Korzun V.M., Kosilko S.A. [et al.]. Organization of preventive, anti-epidemic actions to decrease risks of epidemic situation complications for plague in Republic Altai. *Zhurnal mikrobiologii, epidemiologii i immunobiologii*, 2018, no. 6, pp. 85–94 (in Russian).

8. Popova A.Yu., Balakhonov S.V., Shchuchinov L.V., Matrosov A.N., Mikhaylov E.P., Mishchenko A.I., Denisov A.V., Shefer V.V. [et al.]. Organization of plague control and prevention measures on the territory of Kosh-Agach district of the Altai Republic and estimation of their effectiveness. *Infektsionnye bolezni*, 2018, vol. 16, no. 4, pp. 5–15. DOI: 10.20953/1729-9225-2018-4-5-15 (in Russian).

9. Jones T.L., Baxter M.A.J., Khanduja V. A quick guide to survey research. *Ann. R. Coll. Surg. Engl.*, 2013, vol. 95, no. 1, pp. 5–7. DOI: 10.1308/003588413X13511609956372
10. Alderman A.K., Salem B. Survey research. *Plast. Reconstr. Surg.*, 2010, vol. 126, no. 4, pp. 1381–1389. DOI: 10.1097/PRS.0b013e3181ea44f9

Balakhonov S.V., Kulikova E.S., Mishchenko A.I., Korzun V.M., Rogaleva A.V., Lyashenko S.M., Itashev A.A., Sarikova S.L., Kalybayeva N.B., Madinova N.M. Sanitary-educational activities as an effective method of nonspecific plague prevention in the natural plague focus in the Kosh-Agach district of the Republic of Altai. Health Risk Analysis, 2025, no. 2, pp. 107–113. DOI: 10.21668/health.risk/2025.2.09.eng

Received: 27.01.2025
Approved: 17.04.2025
Accepted for publication: 14.06.2025

UDC 613.6.02
DOI: 10.21668/health.risk/2025.2.10.eng

Read online

Research article

ON ASSESSING THE POTENTIAL RISK OF DOSE-DEPENDENT HEPATOTOXIC EFFECTS OF SELENIUM OXIDE NANOPARTICLES

M.P. Sutunkova[1,2], I.A. Minigalieva[1], V.G. Panov[1,3], T.V. Makhorina[1], M.S. Unesikhina[1], I.G. Shelomentsev[1], R.R. Sakhautdinova[1]

[1]Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, 30 Popov St., Yekaterinburg, 620014, Russian Federation
[2]Ural State Medical University, 3 Repin St., Yekaterinburg, 620028, Russian Federation
[3]Institute of Industrial Ecology of the Ural Branch of the Russian Academy of Sciences, 20 Sofia Kovalevskaya St., Yekaterinburg, 620990, Russian Federation

Selenium nanoparticles (Se NPs) have found wide application in many human economic activities. Therefore, it is necessary to predict and assess emerging potential health risks. Nanotoxicants can affect the body causing negative effects that have a non-linear dependence on the dose of a toxic substance. There is no consensus on the LD_{50} of Se NPs. Recent data on the dose-dependent liver response to different exposures of selenium nanoparticles are contradictory.

The aim is to study and characterize potentially adverse dose-dependent effects in the liver under exposure to selenium oxide nanoparticles in a subchronic experiment using mathematical models.

Exposure was modeled on male rats aged 3 to 4 months, 12 animals in each group. We used three levels of selenium nanoxide doses for subchronic exposure: 3.6, 18, and 36 mg/kg. The research was approved by the Local Ethics Committee of the Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers (Protocol No. 2 of April 20, 2021).

We observed an atypical dose-response relationship between selenium nanooxide exposure and hepatic changes. The negative effects included pronounced changes in mitochondria of liver cells as well as an imbalance of blood enzymes and cellular composition of the liver, which may indicate damage to the organ and impaired secretory functions following the exposure to low and moderate concentrations of SeO nanoparticles.

Our findings can be used for determining chemical safety standards for selenium oxide nanoparticles and assessing their health risks.

Keywords: *nanoparticles, in vivo, dose-effect, selenium oxide, hepatotoxicity, Kupffer cells, hepatocytes, health risk.*

Marina P. Sutunkova – Doctor of Medical Sciences, director; Head of the Department of Occupational Hygiene and Medicine (e-mail: sutunkova@ymrc.ru; ORCID: https://orcid.org/0000-0002-1743-7642).

Ilzira A. Minigalieva – Doctor of Biological Sciences, Head of the Department of Toxicology and Bioprophylaxis (e-mail: ilzira@ymrc.ru; ORCID: https://orcid.org/0000-0002-0097-7845).

Vladimir G. Panov – Candidate of Physical and Mathematical Sciences, Senior Researcher at the Department of Toxicology and Bioprophylaxis; Associate Professor at the Department of Mathematical Analysis and Function Theory (e-mail: panov.wlad1mir@yandex.ru; ORCID: https://orcid.org/0000-0001-6718-3217).

Tatyana V. Makhorina – Candidate of Medical Sciences, Senior Researcher at the Department of Toxicology and Bioprophylaxis (e-mail: lisa430@yandex.ru; ORCID: https://orcid.org/0000-0001-6126-9706).

Maria S. Unesikhina – Junior Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: unesihinams@ymrc.ru; ORCID: https://orcid.org/0000-0002-5576-365X).

Ivan G. Shelomentsev – Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: shelomencev@ymrc.ru; ORCID: https://orcid.org/0000-0002-8795-8777).

Renata R. Sakhautdinova – Candidate of Medical Sciences, Head of the Diagnostic Laboratory Department, Research and Production Association of Laboratory Diagnostic Technologies (e-mail: sahautdinova@ymrc.ru; ORCID: https://orcid.org/0000-0002-2726-9259).

Selenium nanoparticles (Se NPs) are generated in the course of human economic activities: in the metallurgical and chemical industries [1], in production of ceramics, glass, and electronics. Use of Se NPs in enrichment of food products and supplements [2], as drug carriers [3], antibacterial and anticancer drugs [4–7], for disease prevention in farm animals [8, 9] and in plant cultivation can pose health risks from excessive accumulation of selenium compounds in the body not only to industrial workers but also to the general population. When analyzing biological effects of nanoselenium, it is necessary to take into account toxicity of NPs determined by physical properties and specific characteristics of the chemical elements that form them. Se NPs exhibit pro- or antioxidant activity depending on the dose and exposure duration [4, 5, 10]. It has been experimentally proven that smaller Se NPs (sized 6.8 nm) have greater penetrating ability and accumulation in organs, higher activity in replacing sulfur in sulfur-containing proteins when participating in the synthesis of selenoproteins [4, 11]. Bypassing the blood–brain barrier with small Se NPs (6.8 nm) leads to a decrease in the number of astrocytes [4], while larger Se NPs exhibit neuroprotective properties by increasing the number of neurons [10]. Adverse toxic effects of Se NPs are known to date. Oral administration of Se NPs to rats at the dose of 0.5 mg/kg body weight (b.w.) per day for 28 days induced local alopecia, a decrease in the body weight gain, and an increase in the relative weight of the liver [12]. Young rats demonstrated more intense accumulation of Se NPs, mainly in the liver, kidneys, and testicles, compared to adult rodents [13]. Se NPs accumulate in the liver [4, 14, 15], kidneys [4, 15], muscles, stomach, and blood [15]. They are potentially toxic to reproduction [16]. The liver is one of the key target organs for Se NPs. It facilitates conversion of Se NPs into selenocysteine and selenomethionine and their incorporation into enzymes [17]. Both pro-oxidant [18] and antioxidant activity of Se NPs [19] cause bioaccumulation of lipid peroxidation products in the liver [15]. The results of studying the dose-dependent effect of Se NPs on the liver seem contradictory: the functional activity and histological picture of the liver change ambiguously. In some cases, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin in the blood remained unchanged, as in the 90-day experiment on fish at the doses of 0.25, 0.5, and 1 mg Se NPs/kg feed [9]. No differences were observed in the activity of AST and alkaline phosphatase (ALP) in rats following intake of Se NPs containing 0.5, 1.5, 3.0, and 5.0 mg Se/kg feed for 28 days; a significant decrease in ALT activity was found in all exposed groups compared to the controls and a decrease in superoxide dismutase activity in the liver in the group receiving Se NPs at 5 mg Se/kg (929 ± 103 U/mL) [14]. Several studies have noted a similar decrease in ALT, alone or in combination with AST [20, 22]. An increase in transaminases was noted in 28-day experiments on mice with oral administration of Se NPs sized 70–90 nm at 1 mg/kg feed and 4 mg/kg b.w. [23] and that by gavage at the doses of 1/10–1/5 LD_{50} (LD_{50} = 88.76 Se mg/kg b.w.) [24]. An increase in transaminases [16, 25] and in alkaline phosphatase activity [15] under effect of Se NPs was reported in earlier studies.

Histopathological studies demonstrated the dose-dependent state of the liver parenchyma varying from the absence [12] or mild dystrophic changes in the groups of rats receiving Se NPs at the doses of 0.5–3.0 mg Se/kg b.w. for 28 days [14] and 2.0 and 4.0 mg/kg b.w. for 14 days [16], to mild multifocal autolytic lesions with signs of congestion in the group receiving Se NPs at the dose of 5.0 mg Se/kg [14], and hepatocyte death [16].

Nanosized selenium compounds have different degrees of toxicity [26], and this is the reason for differences in experimental findings relating to body weight gain, activity of liver and selenium-dependent enzymes, changes in the antioxidant system of the body, and the severity of histological damage.

The question about the median lethal dose of Se NPs remains open, possibly due to the choice of different biological objects for research (fish, turkeys, mice, rats), but even for animals of the same species LD_{50} is uncertain: in mice, it ranges, for instance, from 61.6 mg Se/kg b.w. for SPF ICR mice [27] to 2,000 mg/kg b.w. [4].

The study aimed to establish dose-dependent hepatic effects of subchronic exposure to selenium oxide nanoparticles posing potential health hazard using mathematical models.

Materials and methods. *Description of nanoparticles and the suspension.* Selenium oxide nanoparticles (SeO NPs) in the form of a water-based suspension were generated at the Ural Center for Collective Use "Modern Nanotechnologies" of the Ural Federal University named after the First President of Russia B.N. Yeltsin. Scanning electron microscopy was used to confirm a nearly spherical shape and the size range of 37 to 65 nm (Figure 1). The nanoparticle concentration in the suspension (0.25 mg SeO/mL) was validated at a high zeta potential of up to 42 mV using a Zetasizer Nano ZS analyzer (Malvern Panalytical Ltd., UK).

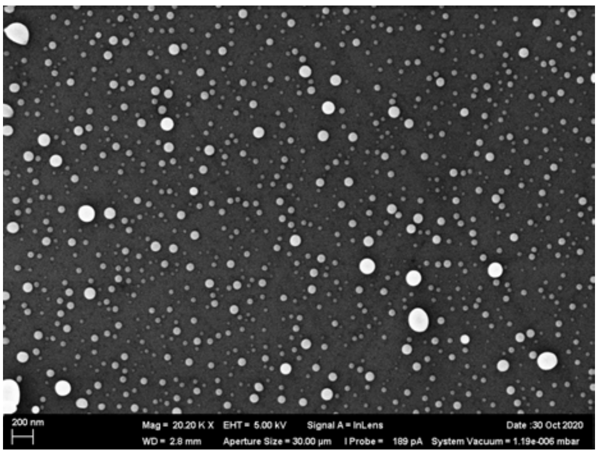

Figure 1. Suspended SeO nanoparticles (SEM image at 20,200 × magnification)

Laboratory animals and experimental exposure. The study was conducted using outbred 3-4-month-old male rats with a body weight of 200 to 270 g. We divided the animals into a control group and three experimental groups of 12 rats each, the latter being exposed to different doses of SeO NPs. Subchronic exposure was modeled over 6 weeks by successive intraperitoneal injections of a stable suspension of SeO NPs made thrice a week. The "SeO NPs 3.6" group was administered the total dose of SeO NPs equal to 3.6 mg/kg b.w., the "SeO NPs 18" group – 18 mg/kg b.w., the "SeO NPs 36" group – 36 mg/kg b.w., and the control group received injections of deionized water at the same intervals. Since there is no consensus on the LD_{50} of selenium NPs, the selection of doses and route of administration was carried out in a pilot experiment taking into account the use of chemically pure SeO NPs to obtain a suspension similar to that used in our previous works [28]. Our findings are comparable with those of other research teams [21, 25].

The limitation of our study was the use of animals of the same species and sex.

The study was approved by the Local Ethics Committee of the Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers (protocol No. 2 of April 20, 2021).

After exposure cessation, we performed biochemical testing of blood serum on a Cobas Integra 400 plus Roche analyzer (Switzerland) using ready-made diagnostic kits for determining transaminases and alkaline phosphatase.

Morphological studies of liver cells and tissue. Cytological preparations were tested to identify post-exposure morphologic hepatic changes in all groups of animals. After Leishman staining, liver smears were examined by light microscopy at 100× and 1000× magnifications using a Primo Star microscope (Carl Zeiss, Germany) with a USCMOS visualization video camera for 300 cells and the percentage composition of cells and the number of damaged cells were calculated.

We scrutinized the histological picture of the liver of the control rats and those in the SeO NPs 36 exposure group receiving the maximum dose. Morphometric studies of enucleated hepatocytes (cytoplasts) and Kupffer cells were performed using the Avtandilov grid.

Electron microscopy. Ultrastructural characteristics of cell damage were determined according to the classification by Sun [13] using a high-resolution scanning electron microscope Hitachi REGULUS SU8220 (Hitachi High-Technologies Corp., Japan) in the STEM mode. Based on the topological characteristics of the inner mitochondrial membrane (number of cristae, homogeneity and density of the matrix), we distinguished normal mitochondria (type A) and a variant of normal vesicular mitochondria (type B), as well as pathological forms, including vesicular (type C), vesicular swollen (type D), and swollen (type E) ones.

Mathematical modeling and statistical analysis. Based on the obtained values of generally accepted indicators reflecting changes in liver functioning, we constructed the relationship between the total dose of SeO NPs and its toxic effects on the liver using the following functions:

- The modified Hill function (1) introduced by Panov et al. [29]:

$$y = \left(b_0 + \frac{b_1 + b_2 x^{b_3}}{1 + (b_4 x)^{b_5}} \right)\left(1 + b_6 x^{b_7}\right), \quad (1)$$

where b_0, …, b_7 are the parameters determined by the least squares method from experimental data;

- The hyperbolic function (2) related to the Michaelis-Menten equation, which is used, for instance, to describe the rate of enzyme reactions [15]:

$$y = \frac{b_0 + b_1 x}{b_2 + b_3 x} \quad (2)$$

- The linear combination of Chebyshev polynomials (3). Chebyshev polynomials of the first kind are defined by the following equality:

$$T_n(x) = \sum_{k=0}^{[n/2]} C_n^{2k} (x^2 - 1) x^{n-2k}, \quad (3)$$

Where $[n/2]$ is the integer part of the number $n/2$ and C_n^{2k} is the number of combinations of n by $2k$; and

- The modified Johnson – Lovett dose-response model [29, 30]:

$$y = b_0 + \frac{b_1}{1 + b_2 e^{b_3 x}} + \frac{b_4}{1 + b_5 e^{b_6 x}}. \quad (4)$$

The statistical significance of intergroup differences in the mean values of all quantities was assessed using the Student's t-test ($p < 0.05$) and the Mann – Whitney U test.

Results and discussion. Damaging effects of SeO NPs on the liver were analyzed at the subcellular, cellular, tissue, and organ levels.

Ultrastructural examination of liver cells using electron microscopy revealed a decrease in the number of type A and B mitochondria, attributed by Sun to the normal morphotype [13], ranging from 94.82 ± 0.95 % in the control to 87.44 ± 1.14 % in the SeO NPs 36 group ($p < 0.05$).

We detected changes at the cellular and tissue levels in rats after SeO NP exposure. Microscopy of histological liver preparations showed an increase in the proportions of cytoplasts and Kupffer cells (KC) in the SeO NPs 36 group ($p < 0.01$).

One of the manifestations of the damaging effect of SeO NPs was an increase in the number of degenerated hepatocytes (Figure 2). The increase in their percentage in line with an increase in the exposure dose is described by a graph using a linear combination of Chebyshev polynomials (Formula 3).

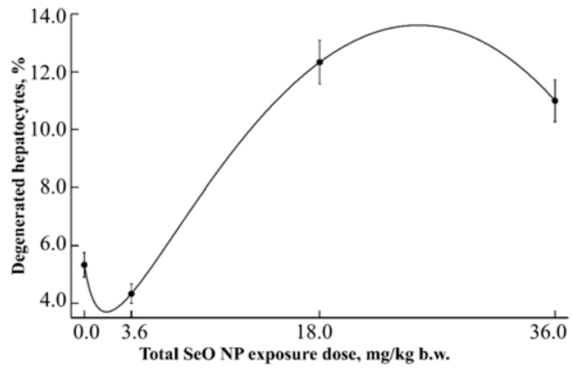

Figure 2. Changes in the proportion of degenerated hepatocytes in liver smears following SeO NP exposure (mean ± error of the mean)

At the lowest SeO NP exposure dose of 3.6 mg/kg b.w., we observed a slight decrease in the percentage of degenerated hepatocytes, but at 18 mg/kg b.w., we detected the maximum number of damaged hepatocytes ($p < 0.05$), which decreased insignificantly at the highest exposure dose of 36 mg/kg b.w. Similar non-monotonicity of the response of biological objects was also noted by other authors [31–34].

The non-linearity of the graphs of the relationship between SeO NP concentrations and the amount of degenerated hepatocytes is explained by toxic damaging mechanisms of SeO NPs in the liver. At low doses (like 3.6 mg/kg b.w. chosen for the experiment), SeO NPs can be consumed for the synthesis of selenium-containing enzymes, including thioredoxin reductase, phospholipid hydroperoxide glutathione peroxidase, and glutathione peroxidase [17], and pose no serious threat. Yet, a multiple (here, 5-fold) increase in the SeO NP exposure causes damage to mitochondria, disruption of the genetic apparatus of cells, damage to cells by oxidative stress products, and early apoptosis [15, 35].

At the same time, a decrease in the reparative potential of the liver is observed. Liver regeneration involves hepatocytes, which make up more than 60 % of the liver cell population [36], sinusoidal cells, 50 % of which are represented by Kupffer cells and leukocytes, connective tissue cells and the extracellular matrix [37].

The ability of the liver to regenerate was assessed by the proportions of Kupffer cells, leukocytes and binuclear hepatocytes (BH) in smears (Figures 3–6). The obtained function (Formula 4) for BH based on the modified Johnson–Lovett model [30] demonstrates a reduced reparative potential against the background of SeO NP accumulation. According to modern concepts, the appearance of binuclear cells occurs as a result of hepatocyte mitosis without cytotomy or amitotic division of hepatocytes and accompanies reparative regeneration of the damaged liver [38].

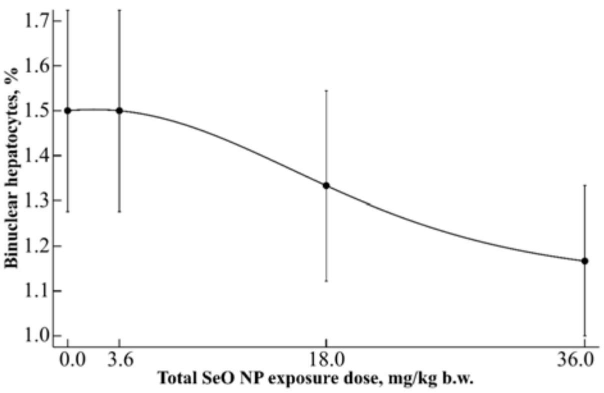

Figure 3. Changes in the proportion of binuclear hepatocytes in liver smears following SeO NP exposure (mean ± error of the mean)

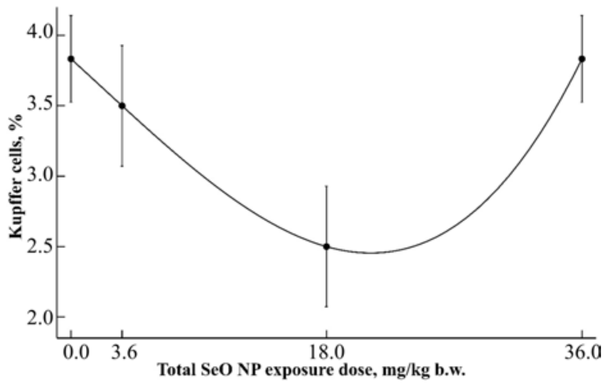

Figure 4. Changes in the proportion of Kupffer cells in liver smears following SeO NP exposure (mean ± error of the mean)

In the absence of a response to the low dose of SeO NPs, we revealed a stable tendency towards a decrease in the proportion of binuclear hepatocytes with a multiple increase in the total dose of NPs to 18 and 36 mg/kg b.w.

At the lowest tested dose of 3.6 mg/kg b.w., the percentage of BH remained similar to that in the control group. With an increase in the dose to 18 and 36 mg/kg b.w., an increase in the percentage of DH (Figure 2) was accompanied by a decrease in the percentage of BH (Figure 3), indicating a decrease in the reparative ability of the liver ($p < 0.01$).

In response to liver cell damage, macrophages migrate to the inflammation zone. Kupffer cells, as well as monocytes and neutrophils coming with the bloodstream, are the representatives of the macrophage community. To describe the dose-dependent effect of SeO NPs on the proportion of Kupffer cells in liver smears, we applied the variant with a linear combination of Chebyshev polynomials (Formula 3).

The percentage of Kupffer cells in liver imprint smears decreased significantly following SeO NP exposure at 3.6 mg/kg b.w. and was more pronounced at 18 mg/kg b.w. ($p < 0.05$); however, upon reaching the concentration of 36 mg/kg b.w., the amount of those cells returned to its initial values, comparable with the control ones (Figure 4). Quantitative restoration of Kupffer cells is explained by *in situ* proliferation [39], the influx and differentiation of blood monocytes into tissue macrophages [40, 41]. When cells are damaged, Kupffer cells and blood leukocytes (monocytes, neutrophils, and eosinophilic leukocytes) migrate to the site of inflammation and play an important role in the macrophage destruction of damaged cells and the replacement of degenerative cells with new cells and even other tissues [38]. Minor damage for a prolonged period can induce chronic inflammation and tissue replacement with connective tissue with fibrosis zones. That is why predicting damaging effects is so important.

When comparing the graphs for degenerated hepatocytes (Figure 2) and Kupffer cells (Figure 4), it becomes obvious that fluctuations in the proportion of the latter can be associated with the destruction of hepatocytes by SeO NPs and reparative processes in the liver, which are supported by the proliferation of Kupffer cells and the influx from outside. During liver regeneration, polymorphonuclear leukocytes, Kupffer cells, and endothelial cells secrete metalloproteinases (collagenases, gelatinases, elastases, and other proteinases), changing the density of the extracellular matrix to deliver regulatory signals to all liver cells (cytokines) thereby [42, 43]. The maximum decrease in the proportion of Kupffer cells upon exposure to SeO NPs at 18 mg/kg b.w. can be associated not only with the direct toxic effect of NPs on cells, but also with their migration to the lymph nodes associated with the antigen-presenting function.

In case of macrophage deficiency, blood cells, specifically neutrophils and eosinophilic leukocytes, join the process of removing damaged cells, the change in the percentage of which is associated with the activation of the inflammatory degenerative process in the liver. Neutrophils are attracted to the damaged liver by inflammation mediators [38]; yet, they themselves secrete a sufficient amount of chemoattractants to induce other cells to migrate towards them. The organism maintains the necessary and sufficient population of neutrophils to participate in inflammation.

To describe the relationship between SeO NP exposure and the proportion of neutrophils found in imprint smears (Figure 5), we used a model [29] based on the Hill function (Formula 1), which reflected the tendency towards a decrease in the proportion of neutrophils in the smear under the toxic effect of NPs.

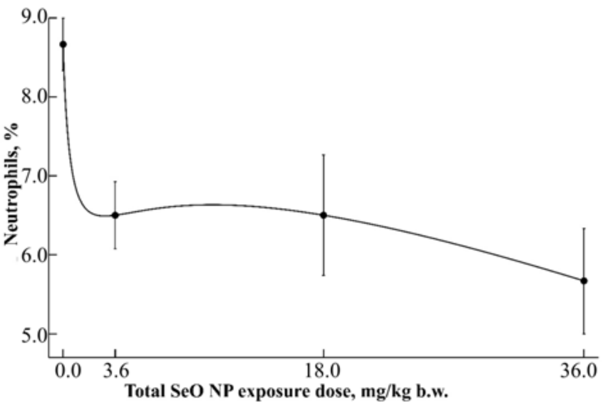

Figure 5. Changes in the proportion of neutrophils in liver smears following SeO NP exposure (mean ± error of the mean)

A comparable plateau-like decrease in the percentage of neutrophils in liver smears occurs at low (3.6 mg/kg b.w.) and moderate (18 mg/kg b.w.) exposure doses of SeO NPs and is aggravated by the high one (36 mg/kg b.w.) ($p < 0.05$).

A decrease in the percentage of neutrophils at low exposure doses leads to mobilization of the body expressed by a slight increase in the proportion of eosinophils

(Figure 6) in liver smears at higher SeO NP exposure doses (18 and 36 mg/kg b.w.). The dose-response relationship [30] for EL is shown in Figure 6.

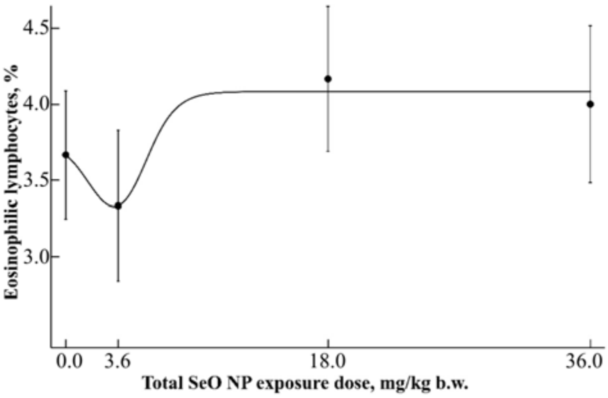

Figure 6. Changes in the proportion of eosinophilic lymphocytes in liver smears following SeO NP exposure (mean ± error of the mean)

The graph of dose-dependent changes in the EL proportion in liver smears is noteworthy for its fluctuations related to inflammatory response development. The destruction of EL at the lowest exposure dose of SeO NPs (3.6 mg/kg b.w.) with the release of cytotoxic EL granules contributes to tissue destruction, but, on the other hand, the release of interleukin-4 (IL-4) stimulates liver regeneration [44, 45]. These findings presented by nonlinear functions are explainable in terms of liver process regulations by migrating cells of the blood and immune system [38]. By producing cytokines (interleukins, chemokines, growth factors) and through direct contact, these cells control the expression of receptors of various liver cells, predetermining its regeneration process [46].

Health and even life threatening disruption of vital organ and system functioning is associated with the ability of NPs to penetrate the bloodstream and cells of various organs [47]. The liver that is well-known for its detoxifying abilities and high blood supply is a target organ for SeO NPs [35]. Functional changes in the liver are observed in animals exposed to SeO NPs, as shown, for instance, by serum enzymes. Maintaining the proportion of degenerated hepatocytes at the level of 11.0 to 12.33 % creates the prerequisites for an increased release of enzymes into the blood. When cells are damaged, enzymes first leak into the intercellular fluid from the cytosol and lysosomes and then, in case of deeper damage, from mitochondria, ribosomes, and nucleus of these cells. The larger the lesion and depth of damage, the greater the enzyme concentration that enters the intercellular space and blood.

To assess the effect of SeO NPs on the activity of ALT and ALP in blood serum, we used the modified Johnson–Lovett model [30] for ALT (Figure 7) and a model close to the Michaelis–Menten equation for ALP when constructing the dose-effect relationship (Figure 8).

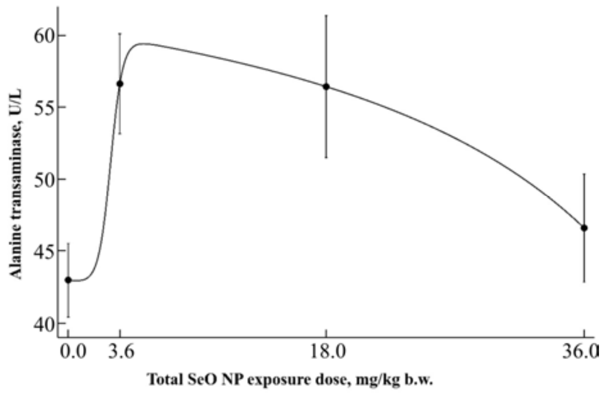

Figure 7. Changes in ALT activity in the blood following SeO NP exposure (mean ± error of the mean)

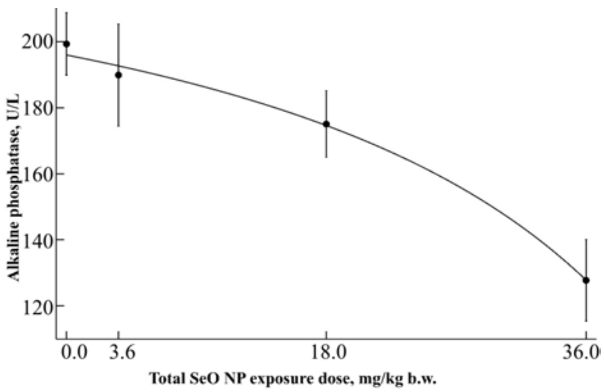

Figure 8. Changes in the activity of alkaline phosphatase in the blood following SeO NP exposure (mean ± error of the mean)

The dose-effect responses of the organism observed during the experiment with SeO NPs were non-monotonic, which was not surprising given the multicomponent nature of the biological system. When examining the charts of the proportion of degenerated hepatocytes and ALT activity, we noted a relationship between the increase in the SeO NP concentration and changes in the concentration of ALT and the above cell count. An insignificant decrease in the proportion of degenerated hepatocytes at low concentrations of the irritant led to a sharp increase in blood ALT concentrations. The observed non-monotonicity of both functions was associated with the damaging effect of SeO NPs on liver cells and the release of ALT, the marker for cytolysis. At the lowest dose of SeO NPs, however, a sharp increase in ALT activity was accompanied by an insignificant increase in the proportion of degenerated hepatocytes, whereas the dependencies of these indicators uniformly decreased at the moderate and high doses ($p < 0.05$). The sharp increase in ALT activity becomes clear when comparing the graphs of changes in the proportion of neutrophils and EL[1], Kupffer cells in liver smears and ALT (Figures 4–7). The contribution of these cells, or rather their destruction [20] due to the toxic effect of SeO NPs, increased the concentration of the cytosolic enzyme ALT at the low NP dose of 3.6 mg/kg b.w.

The toxic effect of SeO NPs on biliary epithelial cells was associated with a dose-dependent decrease in the concentration of alkaline phosphatase in the blood serum in all groups exposed to SeO NPs.

Despite the fact that destroyed epithelial cells of the biliary tract, intestine, brush border of the kidneys, and bone tissue contribute the most to the increase in ALP blood levels, it is important to highlight the role of myeloid cells, including neutrophils and EL, in amending blood ALP concentrations. The entry of ALP into the blood accompanying the destruction of biliary epithelial cells can be enhanced by the destruction of neutrophils and EL, which is manifested upon exposure to SeO NPs at 3.6 mg/kg b.w. (see Figures 5–6). The most pronounced decrease in ALP activity was observed at the highest tested SeO NP dose of 36 mg/kg b.w. ($p < 0.05$) (Figure 8). It is likely that the dose-dependent decrease in ALP activity with an increase in SeO NP exposure is associated with a deficiency in zinc and magnesium, which are part of the enzyme, but data on competitive interactions between magnesium/zinc and selenium are lacking.

Changes in the structure of liver cell mitochondria, an imbalance of blood enzymes and the cellular composition of the liver may indicate liver damage and dysfunction induced by SeO NP exposure. Mathematical modeling can be used to assess the dose-dependent general toxic effect at the level of cells, tissues, and organs and to analyze health risks.

Conclusions:

1. We revealed atypical dose-response relationships between SeO NP exposures and hepatic changes. They were nonlinear and described by non-monotonic functions for such parameters as the proportion of eosinophilic leukocytes, neutrophils, Kupffer cells, degenerated hepatocytes, and the activity of ALT and ALP enzymes.

2. The effectiveness of using mathematical models built on a linear combination of Chebyshev polynomials, the Michaelis–Menten equation, modified Hill functions, and the Johnson–Lovett model for describing dose-dependent adverse effects of SeO NP exposure was proven.

3. The highest health risks were posed by low (3.6 mg/kg b.w.) and moderate (18 mg/kg b.w.) doses of SeO NPs, as shown by changes in ALT and ALP activity and the percentage of degenerated hepatocytes and Kupffer cells.

---

[1] Kishkun A.A., Beganskaya L.A. Klinicheskaya laboratornaya diagnostika [Clinical Laboratory Diagnostics]: A Manual in 2 Volumes, 2nd ed. Moscow, GEOTAR-Media Publ., 2021, 1000 p. (in Russian).

4. The effect of SeO NP exposure dose of 36 mg/kg b.w. on the percentage of degenerated hepatocytes and neutrophils in the liver was most pronounced.

When developing chemical safety standards and assessing health risks, it is therefore necessary to consider that, in case of subchronic exposure, the highest number of pronounced toxic effects and the risk of pathological disorders are observed at low and medium doses of SeO NPs tested.

Funding. This research received no external funding.

Conflict of interest statement. The authors have no conflicts of interest to declare.

References

1. Tsivileva O., Pozdnyakov A., Ivanova A. Polymer Nanocomposites of Selenium Biofabricated Using Fungi. *Molecules*, 2021, vol. 26, no. 12, pp. 3657. DOI: 10.3390/molecules26123657

2. Constantinescu-Aruxandei D., Frîncu R.M., Capră L., Oancea F. Selenium Analysis and Speciation in Dietary Supplements Based on Next-Generation Selenium Ingredients. *Nutrients*, 2018, vol. 10, no. 10, pp. 1466. DOI: 10.3390/nu10101466

3. Khurana A., Tekula S., Saifi M.A., Venkatesh P., Godugu C. Therapeutic applications of selenium nanoparticles. *Biomed. Pharmacother.*, 2019, vol. 111, pp. 802–812. DOI: 10.1016/j.biopha.2018.12.146

4. Lesnichaya M., Shendrik R., Titov E., Sukhov B. Synthesis and comparative assessment of antiradical activity, toxicity, and biodistribution of κ-carrageenan-capped selenium nanoparticles of different size: in vivo and in vitro study. *IET Nanobiotechnol.*, 2020, vol. 14, no. 6, pp. 519–526. DOI: 10.1049/iet-nbt.2020.0023.

5. Fang X., Li C., Zheng L., Yang F., Chen T. Dual-Targeted Selenium Nanoparticles for Synergistic Photothermal Therapy and Chemotherapy of Tumors. *Chem. Asian J.*, 2018, vol. 13, no. 8, pp. 996–1004. DOI: 10.1002/asia.201800048

6. Lesnichaya M., Perfileva A., Nozhkina O., Gazizova A., Graskova I. Synthesis, toxicity evaluation and determination of possible mechanisms of antimicrobial effect of arabinogalactane-capped selenium nanoparticles. *J. Trace Elem. Med. Biol.*, 2022, vol. 69, pp. 126904. DOI: 10.1016/j.jtemb.2021.126904

7. Dukhnovsky E.A. Application of Selenium Nanoparticles in Oncology (Review). *Drug development and registration*, 2023, vol. 12, no. 2, pp. 34–43. DOI: 10.33380/2305-2066-2023-12-2-34-43 (in Russian).

8. Bano I., Skalickova S., Arbab S., Urbankova L., Horky P. Toxicological effects of nanoselenium in animals. *J. Anim. Sci. Biotechnol.*, 2022, vol. 13, no. 1, pp. 72. DOI: 10.1186/s40104-022-00722-2

9. Abd El-Kader M.F., Fath El-Bab A.F., Abd-Elghany M.F., Abdel-Warith A.-W.A., Younis E.M., Dawood M.A.O. Selenium Nanoparticles Act Potentially on the Growth Performance, Hemato-Biochemical Indices, Antioxidative, and Immune-Related Genes of European Seabass (Dicentrarchus labrax). *Biol. Trace Elem. Res.*, 2021, vol. 199, no. 8, pp. 3126–3134. DOI: 10.1007/s12011-020-02431-1

10. Liu X., Mao Y., Huang S., Li W., Zhang W., An J., Jin Y., Guan J. [et al.]. Selenium nanoparticles derived from Proteus mirabilis YC801 alleviate oxidative stress and inflammatory response to promote nerve repair in rats with spinal cord injury. *Regen. Biomater.*, 2022, vol. 9, pp. rbac042. DOI: 10.1093/rb/rbac042

11. Vinceti M., Filippini T., Jablonska E., Saito Y., Wise L.A. Safety of selenium exposure and limitations of selenoprotein maximization: Molecular and epidemiologic perspectives. *Environ. Res.*, 2022, vol. 211, pp. 113092. DOI: 10.1016/j.envres.2022.113092

12. Hadrup N., Loeschner K., Mandrup K., Ravn-Haren G., Frandsen H.L., Larsen E.H., Lam H.R., Mortensen A. Subacute oral toxicity investigation of selenium nanoparticles and selenite in rats. *Drug Chem. Toxicol.*, 2019, vol. 42, no. 1, pp. 76–83. DOI: 10.1080/01480545.2018.1491589

13. Singh H., Kaur J., Datusalia A.K., Naqvi S. Age-Dependent Assessment of Selenium Nanoparticles: Biodistribution and Toxicity Study in Young and Adult Rats. *Nanomedicine (Lond.)*, 2023, vol. 18, no. 27, pp. 2021–2038. DOI: 10.2217/nnm-2023-0204

14. Urbankova L., Skalickova S., Pribilova M., Ridoskova A., Pelcova P., Skladanka J., Horky P. Effects of Sub-Lethal Doses of Selenium Nanoparticles on the Health Status of Rats. *Toxics*, 2021, vol. 9, no. 2, pp. 28. DOI: 10.3390/toxics9020028

15. Loeschner K., Hadrup N., Hansen M., Pereira S.A., Gammelgaard B., Hyrup Møller L., Mortensen A., Lam H.R., Larsen E.H. Absorption, distribution, metabolism and excretion of selenium following oral administration of elemental selenium nanoparticles or selenite in rats. *Metallomics*, 2014, vol. 6, no. 2, pp. 330–337. DOI: 10.1039/c3mt00309d

16. He Y., Chen S., Liu Z., Cheng C., Li H., Wang M. Toxicity of selenium nanoparticles in male Sprague–Dawley rats at supranutritional and nonlethal levels. *Life Sci.*, 2014, vol. 115, no. 1–2, pp. 44–51. DOI: 10.1016/j.lfs.2014.08.023

17. Chaudière J. Biological and Catalytic Properties of Selenoproteins. *Int. J. Mol. Sci.*, 2023, vol. 24, no. 12, pp. 10109. DOI: 10.3390/ijms241210109

18. Lesnichaya M., Karpova E., Sukhov B. Effect of high dose of selenium nanoparticles on antioxidant system and biochemical profile of rats in correction of carbon tetrachloride-induced toxic damage of liver. *Colloids Surf. B Biointerfaces*, 2021, vol. 197, pp. 111381. DOI: 10.1016/j.colsurfb.2020.111381

19. Dumore N.S., Mukhopadhyay M. Antioxidant properties of aqueous selenium nanoparticles (ASeNPs) and its catalysts activity for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reduction. *Journal of Molecular Structure*, 2020, vol. 1205, pp. 127637. DOI: 10.1016/j.molstruc.2019.127637

20. Sohrabi A., Tehrani A.A., Asri-Rezaei S., Zeinali A., Norouzi M. Histopathological assessment of protective effects of selenium nanoparticles on rat hepatocytes exposed to Gamma radiation. *Vet. Res. Forum*, 2020, vol. 11, no. 4, pp. 347–353. DOI: 10.30466/vrf.2018.93499.2260

21. Zhang Z., Du Y., Liu T., Wong K.-H., Chen T. Systematic acute and subchronic toxicity evaluation of polysaccharide–protein complex-functionalized selenium nanoparticles with anticancer potency. *Biomater. Sci.*, 2019, vol. 7, no. 12, pp. 5112–5123. DOI: 10.1039/C9BM01104H

22. Ibrahim S.E., Alzawqari M.H., Eid Y.Z., Zommara M., Hassan A.M., Dawood M.A.O. Comparing the Influences of Selenium Nanospheres, Sodium Selenite, and Biological Selenium on the Growth Performance, Blood Biochemistry, and Antioxidative Capacity of Growing Turkey Pullets. *Biol. Trace Elem. Res.*, 2022, vol. 200, no. 6, pp. 2915–2922. DOI: 10.1007/s12011-021-02894-w

23. Kondaparthi P., Deore M., Naqvi S., Flora S.J.S. Dose-dependent hepatic toxicity and oxidative stress on exposure to nano and bulk selenium in mice. *Environ. Sci. Pollut. Res.*, 2021, vol. 28, no. 38, pp. 53034–53044. DOI: 10.1007/s11356-021-14400-9

24. Zhao H., Liu C., Song J., Fan X. Pilot study of toxicological safety evaluation in acute and 28-day studies of selenium nanoparticles decorated by polysaccharides from Sargassum fusiforme in Kunming mice. *J. Food Sci.*, 2022, vol. 87, no. 9, pp. 4264–4279. DOI: 10.1111/1750-3841.16289

25. Zhang J., Wang X., Xu T. Elemental Selenium at Nano Size (Nano-Se) as a Potential Chemopreventive Agent with Reduced Risk of Selenium Toxicity: Comparison with Se-Methylselenocysteine in Mice. *Toxicol. Sci.*, 2008, vol. 101, no. 1, pp. 22–31. DOI: 10.1093/toxsci/kfm221

26. Sampath S., Sunderam V., Manjusha M., Dlamini Z., Lawrance A.V. Selenium Nanoparticles: A Comprehensive Examination of Synthesis Techniques and Their Diverse Applications in Medical Research and Toxicology Studies. *Molecules*, 2024, vol. 29, no. 4, pp. 801. DOI: 10.3390/molecules29040801

27. Wang H., Zhang J., Yu H. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. *Free Radic. Biol. Med.*, 2007, vol. 42, no. 10, pp. 1524–1533. DOI: 10.1016/j.freeradbiomed.2007.02.013

28. Ryabova Yu.V., Sutunkova M.P., Chemezov A.I., Minigalieva I.A., Bushueva T.V., Shelomentsev I.G., Klinova S.V. Evaluation of Effects of Selenium Nanoparticles as an Occupational and Environmental Chemical Hazard on Cellular Bioenergetic Processes. *ZNiSO*, 2022, no. 9, pp. 29–34. DOI: 10.35627/2219-5238/2022-30-9-29-34 (in Russian).

29. Panov V., Minigalieva I., Bushueva T., Fröhlich E., Meindl C., Absenger-Novak M., Shur V., Shishkina E. [et al.]. Some Peculiarities in the Dose Dependence of Separate and Combined In Vitro Cardiotoxicity Effects Induced by CdS and PbS Nanoparticles With Special Atten-

tion to Hormesis Manifestations. *Dose Response*, 2020, vol. 18, no. 1, pp. 1559325820914180. DOI: 10.1177/1559325820914180

30. Liu Y., Chen X., Duan S., Feng Y., An M. Mathematical Modeling of Plant Allelopathic Hormesis Based on Ecological-Limiting-Factor Models. *Dose Response*, 2010, vol. 9, no. 1, pp. 117–129. DOI: 10.2203/dose-response.09-050.Liu

31. Weiss B. The intersection of neurotoxicology and endocrine disruption. *Neurotoxicology*, 2012, vol. 33, no. 6, pp. 1410–1419. DOI: 10.1016/j.neuro.2012.05.014

32. Ge H.-L., Liu S.-S., Zhu X.-W., Liu H.-L., Wang L.-J. Predicting Hormetic Effects of Ionic Liquid Mixtures on Luciferase Activity Using the Concentration Addition Model. *Environ. Sci. Technol.*, 2011, vol. 45, no. 4, pp. 1623–1629. DOI: 10.1021/es1018948

33. Zhu X.-W., Liu S.-S., Qin L.-T., Chen F., Liu H.-L. Modeling non-monotonic dose–response relationships: Model evaluation and hormetic quantities exploration. *Ecotoxicol. Environ. Saf.*, 2013, vol. 89, pp. 130–136. DOI: 10.1016/j.ecoenv.2012.11.022

34. Nweke C.O., Ogbonna C.J. Statistical models for biphasic dose-response relationships (hormesis) in toxicological studies. *Ecotoxicology and Environmental Contamination*, 2017, vol. 12, no. 1, pp. 39–55. DOI: 10.5132/eec.2017.01.06

35. Wang H., He Y., Liu L., Tao W., Wang G., Sun W., Pei X., Xiao Z. [et al.]. Prooxidation and Cytotoxicity of Selenium Nanoparticles at Nonlethal Level in Sprague-Dawley Rats and Buffalo Rat Liver Cells. *Oxid. Med. Cell. Longev.*, 2020, vol. 2020, pp. 7680276. DOI: 10.1155/2020/7680276

36. Godoy P., Hewitt N.J., Albrecht U., Andersen M.E., Ansari N., Bhattacharya S., Bode J.G., Bolleyn J. [et al.]. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. *Arch. Toxicol.*, 2013, vol. 87, no. 8, pp. 1315–1530. DOI: 10.1007/s00204-013-1078-5

37. Bilzer M., Roggel F., Gerbes A.L. Role of Kupffer cells in host defense and liver disease. *Liver Int.*, 2006, vol. 26, no. 10, pp. 1175–1186. DOI: 10.1111/j.1478-3231.2006.01342.x

38. Hu Y., Wang R., An N., Li C., Wang Q., Cao Y., Li C., Liu J., Wang Y. Unveiling the power of microenvironment in liver regeneration: an in-depth overview. *Front. Genet.*, 2023, vol. 14, pp. 1332190. DOI: 10.3389/fgene.2023.1332190

39. Scott C.L., Zheng F., De Baetselier P., Martens L., Saeys Y., De Prijck S., Lippens S., Abels C. [et al.]. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nat. Commun.*, 2016, vol. 7, pp. 10321. DOI: 10.1038/ncomms10321

40. David B.A., Rezende R.M., Antunes M.M., Morais Santos M., Freitas Lopes M.A., Barros Diniz A., Vaz Sousa Pereira R., Cozzer Marchesi S. [et al.]. Combination of Mass Cytometry and Imaging Analysis Reveals Origin, Location, and Functional Repopulation of Liver Myeloid Cells in Mice. *Gastroenterology*, 2016, vol. 151, no. 6, pp. 1176–1191. DOI: 10.1053/j.gastro.2016.08.024

41. Wang J., Kubes P. Reservoir of Mature Cavity Macrophages that Can Rapidly Invade Visceral Organs to Affect Tissue Repair. *Cell*, 2016, vol. 165, no. 3, pp. 668–678. DOI: 10.1016/j.cell.2016.03.009

42. Kanta J. Collagen matrix as a tool in studying fibroblastic cell behavior. *Cell Adh. Migr.*, 2015, vol. 9, no. 4, pp. 308–316. DOI: 10.1080/19336918.2015.1005469

43. Torok N.J., Dranoff J.A., Schuppan D., Friedman S.L. Strategies and endpoints of antifibrotic drug trials: Summary and recommendations from the AASLD Emerging Trends Conference, Chicago, June 2014. *Hepatology*, 2015, vol. 62, no. 2, pp. 627–634. DOI: 10.1002/hep.27720

44. Goh Y.P.S., Henderson N.C., Heredia J.E., Eagle A.R., Odegaard J.I., Lehwald N., Nguyen K.D., Sheppard D. [et al.]. Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc. Natl Acad. Sci. USA*, 2013, vol. 110, no. 24, pp. 9914–9919. DOI: 10.1073/pnas.1304046110

45. Yang Y., Xu L., Atkins C., Kuhlman L., Zhao J., Jeong J.-M., Wen Y., Moreno N. [et al.]. Novel IL-4/HB-EGF-dependent crosstalk between eosinophils and macrophages controls liver regeneration after ischaemia and reperfusion injury. *Gut*, 2024, vol. 73, no. 9, pp. 1543–1553. DOI: 10.1136/gutjnl-2024-332033

46. Michalopoulos G.K., Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nat. Rev. Gastroenterol. Hepatol.*, 2021, vol. 18, no. 1, pp. 40–55. DOI: 10.1038/s41575-020-0342-4

47. Attarilar S., Yang J., Ebrahimi M., Wang Q., Liu J., Tang Y., Yang J. The Toxicity Phenomenon and the Related Occurrence in Metal and Metal Oxide Nanoparticles: A Brief Review From the Biomedical Perspective. *Front. Bioeng. Biotechnol.*, 2020, vol. 8, pp. 822. DOI: 10.3389/fbioe.2020.00822

Sutunkova M.P., Minigalieva I.A., Panov V.G., Makhorina T.V., Unesikhina M.S., Shelomentsev I.G., Sakhautdinova R.R. On assessing the potential risk of dose-dependent hepatotoxic effects of selenium oxide nanoparticles. Health Risk Analysis, 2025, no. 2, pp. 114–125. DOI: 10.21668/health.risk/2025.2.10.eng

Received: 20.03.2025
Approved: 21.05.2025
Accepted for publication: 22.06.2025

UDC 613.955
DOI: 10.21668/health.risk/2025.2.11.eng

Read online

Research article

COMMON IMMUNOLOGICAL PATHWAYS OF ANTI-INFECTION IMMUNITY AND ALLERGIC REACTIVITY MODIFICATION IN CHILDREN ASSOCIATED WITH PECULIARITIES OF THE MODERN EDUCATIONAL PROCESS AND ENVIRONMENT QUALITY

S.L. Valina, N.V. Zaitseva, O.Yu. Ustinova, I.E. Shtina, O.A. Maklakova

Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation

This study is relevant due to humoral and cellular pathways of the immune response having much in common as well as its high sensitivity to factors of the modern educational process in school when building up anti-infection and anti-allergy protection. Our research objects were secondary schools with profound studies of some subjects (Type 1 schools) and ordinary secondary schools (Type 2 schools); overall, we examined 842 schoolchildren from Type 1 schools and 540 schoolchildren from Type 2 schools.

The aim of this study was to establish conditions and common pathogenetic sections of immunological pathways of anti-infection immunity and allergic reactivity modification associated with environmental exposures and exposure to factors of the modern educational process.

We analyzed how educational activities were organized in the analyzed schools; what food products were consumed by the participating schoolchildren daily and their chemical structure; basic aspects of schoolchildren's lifestyles; quality of indoor air in classrooms; quality of ambient air in areas where the analyzed schools were located; prevalence of allergic diseases (ADs) and anti-infection immunity disorders; results of immunological tests and chemical blood tests; intensity of humoral anti-infection and post-vaccination immunity. The study involved odds ratio calculation, linear regression analysis, and neural network modeling.

As a result, we established that high educational loads, improper duration of breaks and periods of work with electronic teaching aids (ETAs), insufficient sleeping time, too low physical activity and too high digital activity among schoolchildren, manganese, nickel, chromium and formaldehyde in air inside classrooms in levels up to 1.8–8.5 higher than RfCchr, and unhealthy diets created 1.3 times higher risks of ADs, 2.3 times higher risks of insufficient production of IgG to herpesviruses, 3.1–5.4 times higher risks of an increasing proportion of people seronegative to measles and diphtheria antigens (OR = 1.33–5.40). Activation of cellular-mediated reaction of adaptive immunity response (an increase in levels of CD3+-, CD3+CD25+-, CD3+CD8+- lymphocytes) and declining activity of the non-specific resistance system (a decline in absolute phagocytosis, phagocytic number, and levels of CD16+56+- lymphocytes) were common pathogenetic sections of immunological pathways of anti-infection immunity and allergic reactivity modification upon exposure to a set of priority factors. Isolated contributions made by various factors to likelihood of risk-associated ADs amounted to 35.7–74.0 % for peculiarities of the modern educational process; chemical factors, 7.6–33.1 %; lifestyle, 7.6–31.2 %. Contributions to humoral post-infection and post-vaccination immunity disorders amounted to 14.6–44.0 % for diet-related factors; educational process, 13.5–30.8 %; lifestyle, 11.4–29.4 %; chemical factors, 6.5–19.9 %.

Keywords: *schoolchildren, post-infection and post-vaccination immunity, allergic diseases, immunological pathways of modification, neural network modeling, factor contribution.*

Svetlana L. Valina – Candidate of Medical Sciences, Head of the Department for Children and Teenagers Hygiene (email: valina@fcrisk.ru; tel.: +7 (342) 237-27-92; ORCID: https://orcid.org/0000-0003-1719-1598).

Nina V. Zaitseva – Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor, Scientific Director (e-mail: znv@fcrisk.ru; tel.: +7 (342) 237-25-34; ORCID: http://orcid.org/0000-0003-2356-1145).

Olga Yu. Ustinova – Doctor of Medical Sciences, Deputy Director responsible for Clinical Work (e-mail: ustinova@fcrisk.ru; tel.: +7 (342) 236-32-64; ORCID: https://orcid.org/0000-0002-9916-5491).

Irina E. Shtina – Candidate of Medical Sciences, Head of the Laboratory for Complex Issues of Children's Health with a Clinical Group (e-mail: shtina_irina@mail.ru; tel.: +7 (342) 237-27-92; ORCID: http://orcid.org/0000-0002-5017-8232).

Olga A. Maklakova – Doctor of Medical Sciences, Head of the Consulting and Polyclinic Department (e-mail: olga_mcl@fcrisk.ru; tel.: +7 (342) 236-80-98; ORCID: http://orcid.org/0000-0001-9574-9353).

The RF President Order dated June 18, 2024 No. 529 names preventive and personalized healthcare as well as provision of healthy long life a priority trend in scientific and technological development[1].

Schoolchildren's health directly depends on conditions and organization of the educational process in schools, qualitative and quantitative structure of their diet, physical activity, optimal study and rest regime, levels and structure of digital activity, education provided by their families and family environment [1, 2].

At present, the leading role in maintaining homeostasis undoubtedly belongs to the immune system. Its functioning can go beyond physiological parameters inducing immunopathological processes, which implement genetically determined programs upon exposure to various environmental factors [3, 4].

Although chronic stress-induced diseases and anthropogenic diseases gradually displace communicable ones, over the last decade a growth has been observed in incidence of 'eradicated' infections. This has again attracted attention to stability of the specific anti-viral and anti-bacterial immunity created by vaccination [5, 6].

Long-term observations in some European countries indicate that prevalence of allergic diseases (ADs) grows twofold every ten years, which allows describing allergy as a pandemic process [7, 8]. Children with ADs are established to have substantial changes in humoral immunity upon vaccination as compared to healthy peers. Antibody production tends to be less intensive, highly protective specific immunoglobulins do not accumulate well, and post-vaccination immunity is lost very rapidly. At the same time, it is a well-known fact that children with allergy do need protection from infections, which do not only strengthen and prolong the allergic process by determining a cascade of immune reactions but also act as ADs triggers [9–11].

Results obtained by epidemiological studies and medical statistics data give evidence that prevalence of chronic infections as well as their persistence is considerably higher among children with ADs against their peers without any signs of allergic pathology [12, 13]. The range of infectious agents is rather diverse but viruses, as a rule, occupy leading places. Herpesvirus infections remain a priority interdisciplinary challenge for practical healthcare due to high infection rates among the population either with one or more often with several human herpes viruses (Herpes simplex virus (HSV) 1 and 2; Varicella zoster virus; Epstein – Barr virus; Cytomegalovirus; Human herpesvirus 6). All these viruses are able to produce immunotropic effects [14].

At present, the conventional concept highlights the leading role that belongs to imbalance between subpopulations of Th1/Th2-helper T-lymphocytes in pathogenesis of chronic infections, post-vaccination immunity, and ADs. Prevalence of Th2-immune response in patients with allergic pathology involves production of IL-4, IL-5, IL-10, and IL-13 cytokines, induces IgE synthesis and a decline in the level of interferon-γ, which weakens anti-viral protection and promotes long-term virus persistence. Common humoral and cellular pathways of the immune response underlie comorbidity of allergic and infectious diseases as well as disrupted formation of specific immunity as a response to vaccination [15]. Apart from internal factors determined by a patient's individual peculiarities, some external factors can also act as causes for insufficient effectiveness of the global fight with its aim to both eliminate vaccine-managed infections and decrease ADs incidence. Industrial and transport emissions, changes in lifestyle and diets, more intensive educational activities and stress play a significant role in changes of immunological reactivity [16–18].

---

[1] Ob utverzhdenii prioritetnykh napravlenii nauchno-tekhnologicheskogo razvitiya i perechnya vazhneishikh naukoemkikh tekhnologii: Ukaz Prezidenta Rossiiskoi Federatsii ot 18.06.2024 № 529 [On Approval of priority trends in scientific and technological development and the list of the most significant science-intense technologies: the Order by the RF President dated June 18, 2024 No. 529]. *Prezident Rossii*. Available at: http://www.kremlin.ru/acts/bank/50755 (April 10, 2025) (in Russian).

The modern school environment is a multicomponent dynamic system. Given that, it is becoming especially vital to establish factors and biomarkers, which correlate with intensity of vaccine-induced, post-infection and allergic immune responses [19, 20].

Therefore, issues related to conjugate post-vaccination immunity disorders and allergic reactivity in children and adolescents remain in a focus of intensive researchers' attention, first of all, due to the immune system being highly sensitive to external exposures, the necessity to develop methods for predicting intensity of an immune response as well as activities aimed at establishing, eliminating, or mitigating adverse impacts exerted by relevant risk factors.

The aim of this study was to establish conditions and common pathogenetic regularities of immunological pathways of anti-infection immunity and allergic reactivity modification in schoolchildren associated with environmental exposures and exposure to factors of the modern educational process.

Materials and methods. The article relies on using data obtained by studies accomplished over 2019–2024 within scientific research conducted in conformity with Rospotrebnadzor's Brunch Scientific Research Program. All studies were accomplished in compliance with the valid principles of biomedical ethics and approved by the local ethics committee (LEC) of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (the LEC Meeting Protocols No. 3 dated March 01, 2019; No. 1 dated February 06, 2020; No. 1 dated February 04, 2021; No. 3 dated February 17, 2022; No. 3 dated February 14, 2023). Our research objects were secondary schools with profound studies of some subjects (Type 1 schools) and ordinary secondary schools (Type 2 schools); overall, we performed profound clinical and laboratory examinations of 1382 primary, middle and high school students from the analyzed schools. The observation group was made of 842 schoolchildren from Type 1 schools, their median age being 12 (10; 15) years; the reference group consisted of 540 schoolchildren from Type 2 schools, the median age being 12 (9; 15) years.

Educational processes in two different types of the analyzed schools were comparatively assessed as regards their conformity with the valid sanitary legislation[2]. The assessment involved analyzing school schedules, breaks, and time studies of work with electronic teaching aids (ETAs) during classes.

Consumption of food products by schoolchildren was analyzed by comparing averaged data taken from school menus with recommended average daily sets of food products[3]. We determined how well a diet satisfied average daily needs in basic nutrients and energy by calculating the chemical structure and caloric contents of a schoolchild's daily diet with the

---

[2] SP 2.4.3648-20. Sanitarno-epidemiologicheskie trebovaniya k organizatsiyam vospitaniya i obucheniya, otdykha i ozdorovleniya detei i molodezhi; utv. postanovleniem Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 28.09.2020 g. № 28; vved. v deistvie s 01.01.2021 g. [Sanitary Rules SP 2.4.3648-20. Sanitary-epidemiological requirements to organizing education, leisure and health improvement of children and youth; approved by the Order of the RF Chief Sanitary Inspector on September 28, 2020 No. 28; came into force on January 01, 2021]. *GARANT: information and legal portal*. Available at: https://base.garant.ru/75093644/ (April 11, 2025) (in Russian); SanPiN 1.2.3685-21. Gigienicheskie normativy i trebovaniya k obespecheniyu bezopasnosti i (ili) bezvrednosti dlya cheloveka faktorov sredy obitaniya (s izmeneniyami na 30 dekabrya 2022 goda), utv. postanovleniem Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 28 yanvarya 2021 goda № 2; vved. v deistvie s 01.03.2021 g. [SanPiN 1.2.3685-21. Hygienic standards and requirements to providing safety and (or) harmlessness of environmental factors for people (edited as of December 30, 2022), approved by the Order of the RF Chief Sanitary Inspector on January 28, 2021 No. 2; came into force on March 01, 2021]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573500115 (April 11, 2025) (in Russian).

[3] SanPiN 2.3/2.4.3590-20. Sanitarno-epidemiologicheskie trebovaniya k organizatsii obshchestvennogo pitaniya naseleniya (s izmeneniyami na 22 avgusta 2024 goda), utv. postanovleniem Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 27 oktyabrya 2020 goda № 32; vved. v deistvie s 01.01.2021 g. [Sanitary Rules and Standards SanPiN 2.3/2.4.3590-20. Sanitary-epidemiological requirements to organizing catering provided for population (edited as of August 22, 2024), approved by the Order of the RF Chief Sanitary Inspector on October 27, 2020 No. 32; came into force on January 01, 2021]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/566276706 (April 11, 2025) (in Russian).

use of relevant data from the reference book[4] (Appendix No. 10 SanPiN 2.3/2.4.3590-20[3]).

Socioeconomic statuses of schoolchildren's families, involvement in additional educational activities, daily routines, physical and digital activity were examined by conducting a survey using the authors' questionnaire.

Air quality inside classrooms and ambient air quality in areas near the analyzed schools as well as manganese, nickel, chromium, formaldehyde, benzene and toluene levels in blood were examined by chemical-analytical tests conducted by experts from the Department of Analytical Chemistry Analysis, Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, in 2019–2024 in accordance with valid methodical guidelines[5].

ADs such as allergic rhinitis (AR), bronchial asthma (BA), and atopic dermatitis (AD) were diagnosed based on data taken from Medical Case Histories for Schools (Official Form No. 026/y-2000) and results of profound clinical and laboratory examinations. Anti-infection immunity disorders were established per clinical-laboratory indicators including levels of IgG to herpesvirus infection caused by Herpes simplex virus 1 (HSV-1 infection)

---

[4] Tutelyan V.A. Khimicheskii sostav i kaloriinost' rossiiskikh produktov pitaniya: spravochnik [Chemical structure and caloric contents of Russian food products: reference book]. Moscow, DeLi plus Publ., 2012, 284 p. (in Russian).

[5] MUK 4.1.1045-01. VEZhKh opredelenie formal'degida i predel'nykh al'degidov (S2-S10) v vozdukhe: metodicheskie ukazaniya, utv. Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii – Pervym zamestitelem Ministra zdravookhraneniya Rossiiskoi Federatsii G.G. Onishchenko 5 iyunya 2001 g. [MUK 4.1.1045-01. HPLC determination of formaldehyde and saturated aldehydes (C_2-C_{10}) in ambient air: methodical guidelines, approved by G.G. Onishchenko, the RF Chief Sanitary Inspector and the First Depute to the RF Minister of Health on June 5, 2001]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200029341 (April 11, 2025) (in Russian); MUK 4.1.3167-14. Gazokhromatograficheskoe opredelenie geksana, geptana, benzola, toluola, etilbenzola, m-,o-,p-ksilolov, izopropilbenzola, n-propilbenzola, stirola, metilstirola, benzal'degida v atmosfernom vozdukhe, vozdukhe ispytatel'noi kamery i zamknutykh pomeshchenii: metodicheskie ukazaniya, utv. Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii 16 iyunya 2014 g. [MUK 4.1.3167-14. Gas chromatography identification of hexane, heptane, benzene, toluene, ethylbenzene, м-, o-, p-xylene, isopropyl benzene, n-propyl benzene, styrene, a-methyl styrene, benzaldehyde in ambient air, air inside test chamber and closed premises: methodical guidelines, approved by the Order of the RF Chief Sanitary Inspector on June 16, 2014]. *GARANT: information and legal portal*. Available at: https://base.garant.ru/72079584/ (April 11, 2025) (in Russian); MUK 4.1.3481-17. Izmerenie massovykh kontsentratsii khimicheskikh elementov v atmosfernom vozdukhe metodom mass-spektrometrii s induktivno svyazannoi plazmoi: metodicheskie ukazaniya, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom RF 15.06.2017 [MUK 4.1.3481-17. Measurement of mass concentrations of chemicals in ambient air by inductively coupled plasma mass-spectrometry: methodical guidelines, approved by the Order of the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on June 15, 2017]. *MEGANORM: the system for regulatory documents*. Available at: https://meganorm.ru/mega_doc/norm/metodicheskie-ukazaniya/0/muk_4_1_3481-17_4_1_metody_kontrolya_khimicheskie_faktory.html (April 11, 2025) (in Russian); MUK 4.1.2111-06. Izmerenie massovoi kontsentratsii formal'degida, atsetal'degida, propionovogo al'degida, maslyanogo al'degida i atsetona v probakh krovi metodom vysokoeffektivnoi zhidkostnoi khromatografii: metodicheskie ukazaniya, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 9 avgusta 2006 g. [MUK 4.1.2111-06. Identification of mass concentrations of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and acetone in blood samples by using high performance liquid chromatography: methodical guidelines, approved by G.G. Onishchenko, the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing and the RF Chief Sanitary Inspector on August 9, 2006]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200065243 (April 11, 2025) (in Russian); MUK 4.1.765-99. Gazokhromatograficheskii metod kolichestvennogo opredeleniya aromaticheskikh uglevodorodov (benzol, toluol, etilbenzol, o-,m-,p-ksilol) v biosredakh (krov'): metodicheskie ukazaniya, utv. Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 6 iyulya 1999 g. [MUK 4.1.765-99. Quantification of aromatic hydrocarbons (benzene, toluene, ethyl benzene, o-,m-,p-xylene) in biological media (blood) by using gas chromatography: methodical guidelines, approved by G.G. Onishchenko, the RF Chief Sanitary Inspector on July 6, 1999]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200039012 (April 11, 2025) (in Russian); MUK 4.1.3230-14. Izmerenie massovykh kontsentratsii khimicheskikh elementov v biosredakh (krov', mocha) metodom mass-spektrometrii s induktivno-svyazannoi plazmoi: metodicheskie ukazaniya, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii A.Yu. Popovoi 19 dekabrya 2014 g. [MUK 4.1.3230-14. Identification of mass concentrations of chemicals in biological media (blood and urine) by using inductively coupled plasma mass spectrometry: methodical guidelines, approved by A.Yu. Popova, the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing and the RF Chief Sanitary Inspector on December 19, 2014]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/495856222 (April 11, 2025) (in Russian).

and 5 (Cytomegalovirus infection (CMVI)) (post-infection immunity) as well as intensity of a specific humoral immune response to measles, diphtheria, and pertussis vaccine antigens (post-vaccination immunity).

All obtained data were analyzed using conventional statistical methods involving establishment of absolute, relative, and median values. Odds ratio (OR) and its 95 % confidence interval were determined to assess risk levels. Logistic regression was applied for estimating likelihood of ADs, disorders of humoral immunity to infections managed with specific prevention means, as well as changes in immunological indicators upon exposure to relevant risk factors.

We employed building of artificial neural networks to reveal deeper, mutual influence taken into account, cause-effect relations between likelihood of a decline in humoral, post-vaccination, and post-infection immunity and risk factors; between likelihood of changes in immunological indicators and analyzed factors; between likelihood of weakening anti-infection humoral immunity and immunity indicators. The networks were built using *neuralnet* library and R-studio software. We used the determination coefficient calculated as the square of the correlation coefficient between predicted and actual value to perform quantitative assessment of prediction quality. We estimated sensitivity of neural network models and established priority factors using elasticity coefficients, which showed a relative change in response at the output layer upon a relative change in input data at the first neuron layer. Ultimate contributions made by factors affecting levels of post-infection and post-vaccination antibodies were established based on integral elasticity coefficients.

Results and discussion. We analyzed how the educational process was organized in the examined schools; as a result, we revealed that long and short breaks were reduced against the standard duration by 1.3–2 times in some of them. On average, they equaled 16.87 ± 0.47 and 8.8 ± 0.36 minutes respectively in Type 1 schools against 18.0 ± 0.53 and 9.4 ± 0.31 in Type 2 schools (p = 0.060–0.130). Weekly educational loads were 5.5–19.2 % higher than their permissible levels in Type 1 schools. In Type 1 schools, duration of using a personal computer (PC) and interactive whiteboards (IWB) during a lesson was 1.2–2.2 times longer than its permissible length and 1.4–2.6 times longer than in Type 2 schools. Total daily duration of ETAs use was 1.4 times longer than established by safe standards and 2.5 times longer than in Type 2 schools.

Our study of schoolchildren's diets gave evidence of negative trends in daily rations of all examined children. In Type 1 schools, milk, sour milk products, curds, butter, meat and fish were consumed in quantities 1.5–8.4 times lower than the norm; fresh fruits and vegetables, 1.3–4.7 times; eggs, 5.2–10.0 times; cereals and beans, up to 1.6 times; wheat bread, up to 4.9 times (p < 0.001–0.007). We established excessive consumption of macaroni (1.2–1.7 times higher than the norm) and confectionary products (6.1–12.3 times) (p < 0.001–0.022). The chemical structure of average daily diet consumed by schoolchildren from Type 1 schools did not contain many nutrients in quantities meeting physiological needs; thus, contents of proteins, fats, and carbohydrates were 1.2–2 times lower than necessary; calcium, phosphor and magnesium contents, 1.2–1.4 times lower; vitamins B1 and A were introduced with food in quantities up to 1.2 times lower than needed; there was an age-specific decline in vitamin B2 consumption; caloric contents were 1.3 times lower than in the reference group (Type 2 schools) (p < 0.001).

We analyzed socioeconomic statuses of the examined schoolchildren's families; as a result, we established that average monthly incomes did not exceed 15,000 rubles per one family member in each second family in Type 2 schools. The proportion of families with such incomes was only 26.8 % in Type 1 schools; 30.4 % had monthly incomes varying between 15,001 and 30,000 rubles per a family member (p < 0.001). The proportion of schoolchildren involved in additional educational activities was 1.4 times higher in Type 1 schools against Type 2 schools (85.6 against 60.3 %, p < 0.001). Night sleep not shorter than

8 hours was mentioned in the questionnaire by 17.3 % of the schoolchildren from Type 1 schools and 18.4 % of their peers from Type 2 schools ($p = 0.057$). One or less days a week and less than 6 hours a week were spent doing sports or physical exercises by 11 and 66.7 % of the schoolchildren from Type 1 schools respectively and 18.6 and 77.6 % of their peers from Type 2 schools ($p = 0.002–0.04$). Our analysis of digital activity revealed that the proportion of children who operated three or more devices and spent more than 2 hours every day on screen time amounted to 33.1 and 77.6 % respectively in Type 1 schools; 22.1 and 66.5 % respectively in Type 2 schools ($p = 0.011–0.045$).

Hygienic assessment of ambient air quality in areas near the analyzed schools established that average manganese and formaldehyde levels were up to 1.8–8.5 times higher than reference concentrations upon chronic inhalation exposure ($p < 0.005$). Nickel and chromium levels were found to be up to 3.6 times higher than RfC for chronic exposure in sporadic ambient air samples taken in areas near the analyzed schools ($p < 0.005$).

Our analysis of air samples taken inside classroom in the analyzed schools revealed average manganese levels in some schools and average formaldehyde levels in most schools to be up to 1.9–6.4 times higher than RfC for chronic inhalation exposure ($p < 0.005$). Nickel and chromium were detected in levels 1.1–1.5 times higher than RfC for chronic exposure in sporadic air samples taken inside classrooms in Type 1 and Type 2 schools ($p < 0.005$). Benzene and toluene levels did not exceed single maximum and average daily MPC and were below RfC for chronic exposure in ambient air in areas near the schools and in air samples taken inside them.

Chemical blood tests found benzene and toluene in blood of 46.0 % of the examined children; toluene was found 1.2 times more frequently in blood of schoolchildren from Type 1 schools and average group concentrations of aromatic hydrocarbons were significantly higher in them against their peers from Type 2 schools ($p = 0.0001–0.002$). Formaldehyde contents were higher than the background level in blood of 97.9–98.6 % of the examined children and average group levels were 3.4–4.3 times higher than the background level ($p < 0.005$). In the observation group (Type 1 schools), three fourths of the schoolchildren had nickel and chromium levels in blood higher than the background level; the proportion of schoolchildren with elevated manganese contents in blood was 1.2 times higher in this group against the reference one (Type 2 schools) ($p = 0.006–0.013$).

Significant relationships between blood manganese, nickel, chromium, formaldehyde, toluene and benzene levels and a dose of chemicals introduced with ambient air were established by modeling methods ($0.16 \le R^2 \le 0.50$; $31.97 \le F \le 214.88$; $p < 0.005$). This allowed us to consider elevated blood levels of these chemicals as markers of exposure.

We comparatively analyzed ADs prevalence and anti-infection immunity disorders using the results of clinical and laboratory research. The analysis did not establish any significant intergroup differences within three levels of school education (primary, middle and high school; $p = 0.146–0.971$). Still, ADs prevalence tended to grow with age by 1.4 times among schoolchildren from Type 1 schools (from 162 to 228 cases per 1000 examined, $p = 0.042$; OR = 1.33; CI = 1.02–1.74); we did not find any significant positive dynamics in prevalence of anti-infection immunity disorders ($p = 0.084$) whereas there was a decline in the reference group, by 2.4 times (from 124 to 51 cases per 1000 examined, $p = 0.029$).

Our assessment of post-infection immunity against herpesviruses found that the high stress of serological response (IgG titers: 1:800–3200) to CMVI and HSV-1 infection was detected 1.2–1.6 times less frequently in schoolchildren from Type 1 schools against their peers from the reference group (26 against 43 % and 53.5 against 63.2 % respectively, $p = 0.002–0.068$); insufficient production of IgG (titers: 1:100–400) to CMVI was detected 1.9 times more frequently against the reference group (32.6 against 17.5 %, $p = 0.002$; OR = 2.30; CI = 1.76–2.99).

Our analysis of age-specific immune response to vaccine antigens revealed that the proportion of schoolchildren seropositive to the measles virus went down by 1.2 times in high school against primary school in the observation group (Type 1 schools) (from 74.4 to 60.8 %, p = 0,03; in Type 1 schools, from 77.2 to 75.4 %, p = 0.790); the number of schoolchildren with the negative result grew by 2.5 times (from 12.3 to 30.5 %, p < 0.005; OR = 3.11; CI = 1.88–5.36; in Type 2 schools, from 11.9 to 13.8 %, p = 0,720). The proportion of schoolchildren who did not have protective antibodies to diphtheria grew fivefold in middle school against primary school in the observation group (Type 1 schools) (from 2.7 to 13.4 %, p = 0.003; OR = 5.40; CI = 2.79–11.14; in Type 2 schools, from 1.5 to 3.7 %, p = 0.120). The proportion of schoolchildren with proper protective levels declined 1.25 times more intensively in high school against primary school in Type 1 schools (from 79.5 to 40.0 %, p < 0.005; in Type 2 schools, from 64.2 to 40.3 %, p = 0.007). The proportion of schoolchildren seronegative to the pertussis agent declined 1.2 times less intensively in high school against primary school in Type 1 schools than in Type 2 schools (from 34.5 to 23.8 %, p = 0.090 against from 32.9 to 19.3 %, p = 0.040).

Previously published works reported some results obtained by regression analysis with its aim to establish negative impacts of various factors not conforming to safe standards including the educational process, diets, lifestyles, chemical contamination of biological media on likelihood of allergic rhinitis and declining humoral immunity to diphtheria and pertussis [21, 22]. Table 1 provides data about relationships between the analyzed factors and BA and AD incidence established by building one-factor regression logistic models.

Table 1

Parameters of one-factor linear regression models describing relationships between BA and AD and priority risk factors

Factor		Response	*b0*	*b1*	R^2	*F*	*p*
Educational process	Weekly educational loads	AD	-8.98	0.22	0.47	59.4	<0.001
	Duration of IWB use in class	AD	-2.84	0.05	0.73	210	<0.001
		BA	-1.68	0.02	0.58	53.5	<0.001
	Total IWB use in school per day	AD	-2.69	0.04	0.82	108	<0.001
	Duration of PC use in class	AD	-2.63	0.02	0.30	5.81	<0.001
		BA	-2	0.02	0.79	143.7	<0.001
	Total PC use in school per day	AD	-2.77	0.04	0.36	44.7	<0.001
Diet	Wheat bread consumption	AD	-0.16	0	0.27	78.9	<0.001
	Confectionary products consumption	BA	-3.22	0.01	0.55	111	<0.001
	Poultry consumption	BA	-2.40	0.01	0.39	77.3	<0.001
Lifestyle	Periodicity of doing sports or physical exercises	AD	-0.26	-0.13	0.68	369.37	<0.001
		BA	-1.93	-0.09	0.63	303.54	<0.001
	Duration of doing sports or physical exercises	AD	-0.54	-0.06	0.25	56.90	<0.001
	The number of devices in use	AD	-0.69	0.04	0.10	29.3	<0.001
Chemical exposures	Benzene levels in blood	BA	-2.31	99.68	0.17	68.47	<0.001
		AD	-0.80	309.70	0.35	197.06	<0.001
	Toluene levels in blood	BA	-2.47	364.55	0.15	28.6	0.005
		AD	-1.65	118.65	0.15	31.7	0.0002
	Manganese levels in blood	BA	-3.08	43.37	0.10	45.80	<0.001
		AD	-2.34	83.11	0.31	61.17	<0.001
	Nickel levels in blood	AR	-0.70	56.55	0.18	89.89	<0.001
		BA	-2.96	102.70	0.33	203.19	<0.001
		AD	-1.60	45.06	0.19	65.0	<0.001
	Formaldehyde levels in blood	BA	-3.14	18.96	0.67	848.95	<0.001

Weekly educational loads ($R^2_{AR, AD}$ = 0.19–0.47), duration of IWB and PC use in one class and total weekly use of these devices ($R^2_{AR, AD, BA}$ = 0.30–0.82) are priority factors of the educational process, which influence ADs incidence. We also established a dose-dependent impact exerted by duration of small breaks on levels of IgG to the pertussis agent (R^2 = 0.17).

Consumption of milk (R^2_{AR} = 0.64; $R^2_{diphtheria}$ = 0.65), wheat bread ($R^2_{AR, AD}$ = 0.27–0.66), meat ($R^2_{diphtheria, pertussis}$ = 0.21–0.34), fish, eggs, sour milk products ($R^2_{pertussis}$ = 0.60–0.96), confectionary products (R^2_{BA} = 0.55), and poultry ($R^2_{BA, AR}$ = 0.39; $R^2_{diphtheria}$ = 0.86) are significant diet-related factors as regards post-vaccination immunity and allergic reactivity modification in schoolchildren.

Periodicity ($R^2_{AR, BA, AD}$ = 0.19–0.68) and duration of doing physical exercises or sports (R^2_{AD} = 0.25) as well as the number of devices in use (R^2_{AD} = 0.10) were the most significant lifestyle aspects out of all analyzed ones with the greatest influence on changes in incidence of allergic diseases and post-vaccination humoral immunity.

Levels of toluene ($R^2_{BA, AD, AR}$ = 0.15–0.64), benzene ($R^2_{BA, AD, AR}$ = 0.17–0.35), manganese ($R^2_{BA, AR, AD}$ = 0.10–0.31; $R^2_{pertussis, diphtheria}$ = 0.19–0.40), nickel ($R^2_{AR, AD, BA}$ = 0.18–0.33; $R^2_{diphtheria}$ = 0.78), chromium (R^2_{AR} = 0.58; $R^2_{diphtheria}$ = 0.12), and formaldehyde (R^2_{BA} = 0.67) were priority chemical factors, impacts of which on post-vaccination immunity and allergic reactivity modification were confirmed by building the above-mentioned regression models.

In addition, the artificial neural network method was employed to establish deeper relationships and to quantify contributions made by environmental factors, the modern educational process, diets, and lifestyles to formation of anti-infection (post-infection and post-vaccination) immunity. Machine learning of artificial neural networks is being widely used both in Russia and abroad; it seems an effective method for establishing qualitative and quantitative regularities of immunologic reactivity formation considering mutual influence exerted by multiple factors [23, 24]. Our created neural network models have an external input layer proportionate to the number of analyzed factors, internal layers, and an output layer corresponding to likelihood of a decline in levels of IgG to herpesvirus infection HSV-1 and CMVI (post-infection immunity) as well as to the measles virus, diphtheria toxin and pertussis antigen (post-vaccination immunity). Orientation of influence was determined by a numeric experiment, which involved consequent 1 % growth in each risk factor and subsequent prediction of changes in likelihood of post-vaccination and post-infection immunity disorders (herpesvirus infection HSV-1 and cytomegalovirus infection). Table 2 provides ranking of risk factors, which determine additional likelihood of a decline in levels of IgG specific to herpesvirus infection HSV-1 and CMVI.

According to neural network modeling results, likelihood of changes in production of specific antibodies to herpesvirus infection HSV-1 and CMVI is modified upon 1 % changes in factors related to the modern educational process, up to 26.75 and 17.68 % respectively; diets, up to 5.37 and 3.05 %; environmental factors, up to 20.08 and 23.82 %; lifestyle-related factors, up to 60.43 and 6.22 % respectively (Table 2).

The most pronounced modifying effects on likelihood that production of specific antibodies to the measles virus will deviate from the proper protective level are produced by changes in such factors related to the educational process as weekly educational loads (up to 99.38 %); the pertussis agent, duration of short breaks (up to 70.5 %); to the diphtheria toxin, duration of long breaks (up to 82.71 %) (Table 3).

Likelihood of a decline in production of specific antibodies to the measles virus is modified by diets, up to 797.65 % (consumption of confectionaries); to the pertussis agent, up to 45.6 % (consumption of fish); to the diphtheria toxin, up to 58.75 % (consumption of meat). Priority chemical environmental risk factors that modify likelihood of weakening post-vaccination humoral immunity include

Table 2

Results of neural network modeling to describe influence of priority risk factors on formation of humoral post-infection immunity, %

Factor		Changes in likelihood of declining IgG to HSV-1 (layers 9; 5, $R^2 = 0.144$)	Changes in likelihood of declining IgG to CMVI (layers 14; 7, $R^2 = 0.233$)
Educational process	Duration of long breaks	26.75	17.68
	Duration of short breaks	3.99	16.58
	Weekly educational loads	14.95	2.43
	Duration of PC use in class	3.21	3.45
	Duration of IWB use in class	1.44	2.30
Diets	Milk in typical daily diet	4.77	2.12
	Confectionaries in typical daily diets	3.69	0.73
	Cereals and beans in typical daily diet	3.66	0.26
	Macaroni in typical daily diet	3.64	0.26
	Sour milk products in typical daily diet	3.61	0.90
	Fish in typical daily diet	3.08	0.36
	Fresh vegetables in typical; daily diet	3.06	1.32
	Proteins in typical daily diet	5.73	0.40
	Vitamin B1 in typical daily diet	4.18	0.10
	Vitamin B2 in typical daily diet	1.50	1.67
	Caloric contents in typical daily diet	3.29	3.05
Environment	Manganese in ambient air	20.08	15.68
	Nickel in ambient air	3.76	6.97
	Chromium in ambient air	3.62	0.78
	Toluene in ambient air	13.79	0.93
	Benzene in ambient air	2.04	10.15
	Formaldehyde in ambient air	9.75	0.30
	Nickel in air inside classrooms	5.90	0.51
	Toluene in air inside classrooms	19.63	2.16
	Benzene in air inside classrooms	3.15	1.48
	Formaldehyde in air inside classrooms	6.97	2.54
	Manganese in air inside classrooms	2.31	23.82
	Chromium in air inside classrooms	2.36	2.02
Lifestyle	Duration of night sleep	60.43	0.56
	Involvement in additional education	4.55	6.22
	Monthly income per a family member	5.06	3.76
	Total time spent on using devices a day	3.72	0.33
	Duration of doing sports or physical exercises	0.37	2.25

Table 3

Results of neural network modeling to describe influence of priority risk factors on formation of humoral post-vaccination immunity, %

Factor		Changes in likelihood of declining IgG to measles (layers 5; 2, $R^2 = 0.152$)	Changes in likelihood of declining IgG to pertussis (layers 15; 10, $R^2 = 0.175$)	Changes in likelihood of declining IgG to diphtheria (layers 10; 8, $R^2 = 0.180$)
Educational process	Duration of long breaks	97.27	2.4	82.71
	Duration of short breaks	56.52	70.5	55.39
	Weekly educational loads	99.38	55.2	38.66
	Duration of PC use in class	50.38	17.4	5.89
	Duration of IWB use in class	22.15	10.5	9.05
	Total PC use in school per day	58.52	13.5	2.0
	Total IWB use in school per day		12.5	2.33

End of the Table 3

Factor		Changes in likelihood of declining IgG to measles (layers 5; 2, $R^2 = 0.152$)	Changes in likelihood of declining IgG to pertussis (layers 15; 10, $R^2 = 0.175$)	Changes in likelihood of declining IgG to diphtheria (layers 10; 8, $R^2 = 0.180$)
Diets	Milk in typical daily diet	60.55	34.5	1.67
	Confectionaries in typical daily diets	797.65	0.2	14.55
	Cereals and beans in typical daily diet	95.82	11.8	1.62
	Macaroni in typical daily diet	636.25	16.3	12.08
	Sour milk products in typical daily diet	435.74	5.2	15.0
	Fish in typical daily diet	720.21	45.6	15.82
	Fresh vegetables in typical; daily diet	70.36	9.9	14.93
	Fresh fruits in typical; daily diet	80.87	9.8	9.29
	Meat in typical daily diet	309.51	1.2	58.75
	Wheat bread in typical daily diet	71.78	10.6	16.4
	Eggs in typical daily diet	58.93	8.7	5.69
	Proteins in typical daily diet	410.12	12.0	5.84
	Vitamin B1 in typical daily diet	10.63	0.1	6.19
	Vitamin B2 in typical daily diet	11.55	3.2	3.49
	Vitamin A in typical daily diet	92.39	3.6	2.5
	Caloric contents in typical daily diet	30.85	7.4	11.62
	Iron in typical daily diet	115.76	3.0	23.86
	Magnesium in typical daily diet	27.96	6.6	21.78
Environment	Manganese in ambient air	70.71	42.2	34.89
	Nickel in ambient air	85.79	3.9	6.9
	Chromium in ambient air	59.73	7.4	14.96
	Toluene in ambient air	19.22	12.6	2.73
	Benzene in ambient air	68.78	25.5	7.2
	Formaldehyde in ambient air	37.84	20.3	4.69
	Nickel in air inside classrooms	19.22	5.2	8.7
	Toluene in air inside classrooms	110.17	3.40	12.19
	Benzene in air inside classrooms	31.13	13.1	0.21
	Formaldehyde in air inside classrooms	109.31	28.8	14.56
	Manganese in air inside classrooms	378.36	24.4	13.57
	Chromium in air inside classrooms	156.09	8.5	30.01
Lifestyle	Duration of night sleep	89.01	101.5	26.12
	Involvement in additional education	58.36	8.6	26.75
	Monthly income per a family member	72.25	3.6	62.08
	Total time spent on using devices a day	9.92	3.3	6.93
	Duration of doing sports or physical exercises	25.26	27.3	3.7
	Periodicity of doing sports or physical exercises	19.7	12.9	7.1

the following: for measles, manganese in air inside classrooms (378.36 %); for pertussis, benzene in ambient air (25.5 %); for diphtheria, manganese in ambient air (34.89 %). Shorter duration of night sleep modifies likelihood of insufficient production of specific antibodies to the measles and pertussis agents by 89.01 and 101.5 % respectively whereas monthly incomes per a family member have turned out to be the most significant lifestyle-related factor for anti-diphtheria immunity (62.08 %).

Intergroup comparison of the results obtained by immunological studies and subsequent mathematical modeling has made it possible to establish negative effects on the immune system and pathogenetic regularities of anti-infection immunity and allergic reactivity modification in children. It is associated with specific features of the modern educational process, the environment, diets and lifestyles.

Under combined exposure to the analyzed factors, weakened reactivity of humoral immunity is evidenced by 1.2 times lower relative levels of CD19+-lymphocytes in children from Type 1 schools against their peers form Type 2 schools, (12 (10; 14) against 14 (11; 16) %, $p = 0.046$) and levels of antibodies of the late phase in immune response (IgG) below physiological ranges in 30.5–54.7 % of children in the observation group.

Activation of a cellular adaptive immune response in children from Type 1 schools is evidenced by higher relative levels of CD3+-lymphocytes (68 (64; 73) against 66 (62; 70), $p = 0.040$) and high levels of activated T-cells (CD3+CD25+-lymphocytes) in 20.3 % of them.

Prevalence of a cytotoxic effector pathway of immune response in the children from the observation group is accompanied with higher relative levels of CD3+CD8+-lymphocytes (25 (22; 23) against 23 (21; 26) %, $p = 0.023$).

Elevated quantities of lymphocytes with CD95 receptor are observed in each third schoolchild both in their relative (35.7 %) and absolute values (21.4 %). This indicates that programmed cell death pathways have intensified and immune reactions have become less active.

Weakening activity of the non-specific protection system is accompanied with low absolute phagocytosis values in 15.6 % of the children from the observation group (against 2.0 % in Type 2 schools, $p = 0.03$), phagocytic number (PN) in 40.6 % (against 33.3 %, $p = 0.236$), phagocytic index (PI) in 4.4 % (against 0.9 %, $p = 0.157$), and phagocytosis percentage in 12.5 % (against 2.0 %, $p = 0.080$). Natural killers (CD16+CD56+-lymphocytes), which are conventionally considered a significant component of innate immunity and not only are responsible for a prompt effector response to presence of infected cells but also coordinate interaction between innate and adaptive immunity, are also registered in lower levels in the observation group against the reference one (10 (6; 14) against 12 (9; 17) %, $p = 0.060$).

Our analysis of levels of the immune system cytokines responsible for intercellular cooperation, positive or negative immune regulation, has established 1.2 times higher levels of gamma-interferon in the children from Type 1 schools against their peers from Type 2 schools (2.02 (1.49; 2.55) against 1.75 (1.28; 2.54) pg/ml, $p = 0.595$) and 2.5 times lower levels of its antagonist IL-4 (0.88 (0.55; 1.32) against 2.24 (0.58; 2.7) pg/ml, $p = 0,036$), which indicates that an adaptive response primarily develops per Th1 pathways.

IL-6 and IL-10, which, just as IL-4, promote transition of B-lymphocytes to antibodies-producers, are also detected in the children from Type 1 schools in lower levels (1.48 (1.18; 2.19) against 1.58 (0.85; 2.27) pg/ml and 3.18 (1.73; 4.9) against 3.27 (1.97; 4.75) pg/ml respectively).

IL-8 is able to support anti-inflammatory activity of monocytes / macrophages and promote development of immune reactions mediated by Type 1 T-helpers. Its levels have been found to be higher than their physiological range in 21.4 % of the children from Type 1 schools.

Levels of the tumor necrosis factor (TNF) that promotes formation of antibodies proliferation and differentiation of T-helpers and B-lymphocytes and simulates phagocytosis, are 1.2 times lower in the children from Type 1 schools against their peers from Type 2 schools (1.94 (1.28; 2.29) against 2.31 (1.74; 2.73) pg/ml, $p = 0.008$).

Interleukin 1-beta (IL-1ß) is able to stimulate T- and B-cells; IL-17 ensures activation and migration of neutrophils, stimulates production of IL-1ß, tumor necrosis factor and IL-6 by peripheral blood monocytes and is also able to negatively and positively regulate IgE synthesis and influence development and clinical course of allergic diseases. Upon exposure to the analyzed factors, their levels are 1.8 times lower in the observation group against the reference one (1.53 (0.88; 2.63) against 2.75 (1.44; 3.23) pg/ml, $p = 0.049$ and 1.13 (0.72; 4.02) against 1.67 (1.05; 3.18) pg/ml, $p = 0.543$ respectively).

Relationships between negative laboratory indicators of the immune system and relevant factors of the modern educational process and the environment have been established by creating one-factor regression logistic models (Table 4).

We have established the relationship between relative levels of CD16+56+-lymphocytes and all analyzed factor groups: diet (R^2 = 0.53), chemical factors (R^2 = 0.10–0.24), lifestyle (R^2 = 0.16), and the educational process (R^2 = 0.10). Some lifestyle-aspects, chemical exposures, and diet are priority risk factors affecting levels of IgG (R^2 = 0.65, R^2 = 0.10–0.32 and R^2 = 0.10–0.13 respectively) and relative levels of CD3+-lymphocytes (R^2 = 0.10, R^2 = 0.10–0.87 and R^2 = 0.46 respectively). Relative levels of CD3+CD8+- (R^2 = 0.13–0.69), CD3+CD25+-lymphocytes (R^2 = 0.10–0.79) and IL-8 level (R^2 = 0.71–0.87) depend on occurrence of chemicals in blood.

The next stage in neural network modeling has been performed to establish additional relationships between likelihood of negative responses by the immune system and the analyzed factors (Table 5 and 6).

Table 4

Parameters of one-factor regression logistic models for describing relationships between immunological indicators and relevant risk factors

Factor		Immunological indicator	Trend	*b0*	*b1*	R^2	F	*p*
Educational process	Duration of short breaks	CD16+56+-lymph., rel.	Lower	-1.88	-0.064	0.10	14.33	0.002
Diet	Eggs in daily diet	IgG	Lower	-1.03	-0.01	0.13	34.50	<0.001
	Confectionaries in daily diet	IgG	Lower	-1.19	0.002	0.10	15.87	<0.001
	Poultry in daily diet	CD3+-lymph., rel.	Higher	-4.21	0.04	0.46	162.26	<0.001
		CD16+56+-lymph., rel.	Lower	-2.90	0.022	0.53	387.81	<0.001
Lifestyle	The number of devices in use	IgG	Lower	-2.36	-0.12	0.65	811.27	<0.001
		CD3+-lymph., rel.	Higher	-4.15	0.11	0.10	46.96	<0.001
		CD16+56+-lymph., rel.	Lower	-2.71	0.13	0.16	85.37	<0.001
	Periodicity of doing sports or physical exercises	CD3+-lymph., rel.	Higher	-2.95	-0.04	0.10	5.27	0.027
Chemical factors	Benzene in blood	IgG	Lower	-1.36	209.22	0.10	9.12	0.004
		IL-8	Higher	-1.79	144.20	0.87	311.78	<0.001
		CD3+CD8+-lymph., rel.	Higher	-4.01	702.28	0.66	631.46	<0.001
	Toluene in blood	IgG	Lower	-2.85	621.93	0.32	35.66	<0.001
		IL-8	Higher	-4.90	1626.11	0.71	61.30	<0.001
		CD3+-lymph., rel.	Higher	-3.05	77.81	0.79	497.88	<0.001
		CD3+CD8+-lymph., rel.	Higher	-3.52	377.31	0.69	527.79	<0.001
		CD16+56+-lymph., rel.	Lower	-2.57	81.44	0.10	11.83	0.002
	Manganese in blood	CD3+CD25+-lymph., rel.	Higher	-2.92	82.72	0.10	8.74	0.005
		CD3+-lymph., rel.	Higher	-5.20	120.36	0.43	173.60	<0.001
		CD3+CD8+-lymph., rel.	Higher	-4.4	53.47	0.13	47.45	<0.001
		CD16+56+-lymph., rel.	Lower	-3.11	52.43	0.15	62.50	<0.001
	Nickel in blood	CD3+CD25+-lymph., rel.	Higher	-2.88	282.79	0.79	342.37	<0.001
		CD3+-lymph., rel.	Higher	-4.81	247.03	0.14	18.50	<0.001
		CD3+CD8+-lymph., rel.	Higher	-3.67	118.84	0.21	84.35	<0.001
		CD16+56+-lymph., rel.	Lower	-2.98	71.99	0.11	49.18	<0.001
	Chromium in blood	CD3+-lymph., rel.	Higher	-5.01	174.01	0.87	719.84	<0.001
		CD16+56+-lymph., rel.	Lower	-3.15	129.54	0.24	129.01	<0.001
	Formaldehyde in blood	CD3+CD8+-lymph., rel.	Higher	-4.02	20.48	0.62	646.93	<0.001
		CD16+56+-lymph., rel.	Lower	-2.66	4.93	0.10	35.49	<0.001

Table 5

Results of neural network modeling to show influence of priority risk factors on immune indicators (cellular and humoral components of the adaptive immune system), %

	Risk factor	CD3+-lymph., rel. (layers 10; 4, R^2 = 0.144)	CD3+CD8+-lymph., rel. (layers 5; 2, R^2 = 0.195)	CD19+-lymph., rel. (layers 18; 8, R^2 = 0.124)	CD3+CD25+-lymph., rel.. (layers 14; 4, R^2 = 0.587)	CD16+56+-lymph., rel. (layers 15; 2, R^2 = 0.155)	CD3+CD95+-lymph., abs. (layers 11; 11, R^2 = 0.342)	CD3+CD95+-lymph., rel. (layers 10; 5, R^2 = 0.663)	IgG (layers 12; 10, R^2 = 0,146)
Educational process	Weekly educational loads	0.14		0.33		0.54			
	Duration of short breaks	0.29			0.76	0.07			1.71
	Duration of long breaks		12.16	0.04	2.03				
	Duration of IWB use in class	0.08	0.16	0.05		0.08	0.03		
	Total weekly IWB use	0.08			0.38	0.15			
	Duration of PC use in class	0.02	1.28	0.03	0.06		0.03	0.01	0.81
	Total weekly PC use in school	0.04	0.51		0.06		0.03	0.02	1.9
Lifestyle	Involvement in additional education		0.71			0.23	0.13		2.53
	Duration of night sleep			0.21		0.59	0.72		5.61
	Monthly incomes per a family member		1.7	0.18	0.18	0.08		0.13	1.08
	Total average daily time spent on using devises			0.18	0.05		0.12	0.04	2.43
	Periodicity of doing sports or physical exercises			0.17				0.05	
	Duration of doing sports or physical exercises			0.1					1.72
Diet	Milk in a daily diet	0.15		0.02	0.17			0.05	
	Eggs in a daily diet	0.04					0.05		
	Fresh vegetables in a daily diet	0.02	5.5			0.04	0.05	0.03	
	Fresh fruits in a daily diet			0.1			0.06		
	Meat in a daily diet	0.02	1.1		0.07				0.63
	Sour milk products in a daily diet	0.09	5.53	0.11	0.11	0.07			
	Confectionaries in a daily diet	0.10	0.64		0.11				0.23
	Fish in a daily diet	0.06			0.04		0.03	0.02	
	Wheat bread in a daily diet	0.06				0.08	0.06		
	Cereals and beans in a daily diet	0.03	5.81		0.11			0.01	0.48
	Macaroni in a daily diet		0.86	0.03				0.04	
	Proteins in a daily diet	0.09		0.15	0.04			0.03	
	Caloric contents in a daily diet	0.03	0.15			0.06	0.06		
	Vitamin B1 in a daily diet				0.14	0.01		0.07	0.38
	Vitamin B2 in a daily diet	0.10		0.02	0.01		0.05		0.59
	Vitamin A in a daily diet			0.05		0.08			
	Iron in a daily diet	0.02	4.47	0.02			0.05	0.01	
	Magnesium in a daily diet	0.01		0.1	0.16	0.02			1.47
Chemical factors	Manganese in ambient air	0.24	6.85	0.06	0.13		0.08		1.54
	Chromium in ambient air	0.07			0.1			0.01	
	Nickel in ambient air	0.05		0.01		0.06			
	Formaldehyde in ambient air	0.12			0.56			0.11	4.3
	Benzene in ambient air	0.02			0.25	0.09	0.03	0.11	0.38
	Toluene in ambient air	0.05	0.54		0.28	0.06	0.11	0.05	
	Manganese in air inside classrooms		3.15		0.29			0.24	2.65
	Nickel in air inside classrooms	0.09				0.04	0.01	0.01	0.27
	Chromium in air inside classrooms		1.28	0.03		0.03	0.1		
	Formaldehyde in air inside classrooms	0.21		0.21	0.26			0.02	
	Benzene in air inside classrooms	0.01			0.14	0.15		0.11	
	Toluene in air inside classrooms	0.03					0.06	0.02	

Table 6

Results of neural network modeling to show influence of priority risk factors on immune indicators (the phagocyte system and cytokines), %

	Risk factor	Absolute phagocytosis (layers 10; 4, R^2 = 0.144)	Phagocytosis percentage (layers 18; 5, R^2 = 0.313)	Phagocytic index (layers 13; 13, R^2 = 0.108)	Phagocytic number (layers 5; 3, R^2 = 0.105)	IL-4 (layers 6; 5, R^2 = 0.485)	IL-6 (layers 12; 12, R^2 = 0.137)	IL-10 (layers 12; 9, R^2 = 0.112)	IL-17 (layers 16; 11, R^2 = 0.374)	IL-1ß (layers 12; 2, R^2 = 0.306)	INFγ (layers 8; 8, R^2 = 0.202)	TNF (layers 8; 3, R^2 = 0.185)
Educational process	Weekly educational loads	0.1		0.08			1.36	0.36	0.67			
	Duration of short breaks			0.28		1.48		0.67		0.33		
	Duration of long breaks	0.56		0.83			0.3		1.42	0.64	0.01	14.96
	Duration of IWB use in class	0.01		0.05	0.01	0.2				0.13		2.46
	Total weekly IWB use			0.08		0.05	0.12	0.17	0.92			

End of the Table 6

	Risk factor	Absolute phagocytosis (layers 10; 4, $R^2 = 0.144$)	Phagocytosis percentage (layers 18; 5, $R^2 = 0.313$)	Phagocytic index (layers 13; 13, $R^2 = 0.108$)	Phagocytic number (layers 5; 3, $R^2 = 0.105$)	IL-4 (layers 6; 5, $R^2 = 0.485$)	IL-6 (layers 12; 12, $R^2 = 0.137$)	IL-10 (layers 12; 9, $R^2 = 0.112$)	IL-17 (layers 16; 11, $R^2 = 0.374$)	IL-1ß (layers 12; 2, $R^2 = 0.306$)	INFγ (layers 8; 8, $R^2 = 0.202$)	TNF (layers 8; 3, $R^2 = 0.185$)
	Duration of PC use in class	0.13	0.03		0.02		0.02			0.11	0.02	0.77
	Total weekly PC use in school	0.03			0.01			0.22		0.1		0.14
Lifestyle	Involvement in additional education		0.07	0.39		0.16		0.62				
	Duration of night sleep	0.28	0.53		0.14	3.63				0.56	0.75	
	Monthly incomes per a family member		0.14				0.75	0.92	0.34			
	Total average daily time spent on using devises		0.01		0.02				0.06			
	Periodicity of doing sports or physical exercises	0.29		1.09	0.01	0.04	0.04		0.55		0.07	1.69
	Duration of doing sports or physical exercises		0.08	0.04	0.02		1.15	0.29				2.57
Diets	Milk in a daily diet	0.08	0.02				0.05	0.01	0.1			
	Eggs in a daily diet	0.15	0.01	0.07	0.02	0.07	0.02			0.05	0.03	1.13
	Fresh vegetables in a daily diet			0.49				0.09			0.09	
	Fresh fruits in a daily diet			0.04		0.05	0.04			0.03		1.78
	Meat in a daily diet							0.01			0.02	
	Sour milk products in a daily diet	0.12		0.49	0.01	0.05			0.04	0.05	0.01	0.89
	Confectionaries in a daily diet			1.9		0.09	0.26	0.19				
	Fish in a daily diet		0.01			0.2					0.02	
	Wheat bread in a daily diet		0.03	0.58					0.17		0.01	
	Cereals and beans in a daily diet	0.04			0.01	0.05	0.01	0.09	0.18		0.01	
	Macaroni in a daily diet	0.19	0.06	0.42			0.11		0.06		0.03	
	Proteins in a daily diet	0.25				0.07				0.12	0.08	
	Caloric contents in a daily diet			0.68		0.43	0.74	0.4	2.66		0.01	
	Vitamin B1 in a daily diet	0.04	0.02						2.01		0.05	0.39
	Vitamin B2 in a daily diet		0.02			0.25	0.09	0.29	2.23		0.03	
	Vitamin A in a daily diet	0.5	0.05	0.03		0.03			0.85	0.01		
	Iron in a daily diet		0.01	0.19					0.15		0.05	
	Magnesium in a daily diet	0.01			0.01	0.03			2.01		0.05	
Chemical factors	Manganese in ambient air	0.13								0.1		1.09
	Chromium in ambient air	0.01				0.42	0.24					0.33
	Nickel in ambient air	0.15				0.21	0.19		0.01			1.88
	Formaldehyde in ambient air						0.16	0.36		0.12		
	Benzene in ambient air		0.01		0.01	0.1		0.18	0.98	0.26		
	Toluene in ambient air	0.25	0.07		0.01	0.43		0.02		0.22		
	Manganese in air inside classrooms		0.05					0.52	2.58		0.07	
	Nickel in air inside classrooms			0.71	0.02					0.07		0.51
	Chromium in air inside classrooms		0.03	0.2		0.14	0.17	0.1				
	Formaldehyde in air inside classrooms	0.2		0.57			1.03				0.07	1.08
	Benzene in air inside classrooms			0.06	0.02	0.31	0.12	0.01				1.32
	Toluene in air inside classrooms		0.02	0.1				0.16	0.01		0.02	

Table 7

Parameters of regression logistic models to describe relationships between immunological indicators and allergic diseases / post-vaccination immunity disorders

Allergic disease / post-vaccination immunity disorders	Immunologic indicator	Trend	*b0*	*b1*	R^2	F	*p*
Allergic rhinitis	CD3+-lymphocytes, rel.	Higher	-0.42	0.25	0.10	20.84	<0.001
	CD3+CD25+- lymphocytes, rel.	Higher	-1.18	0.08	0.30	27.17	<0.001
	IL-8	Higher	-2.72	0.34	0.38	19.77	<0.001
Bronchial asthma	CD16+56+- lymphocytes, rel.	Higher	-1.98	-0.04	0.17	61.40	<0.001
	CD3+CD8+- lymphocytes, rel.	Higher	-4.48	0.07	0.22	73.80	<0.001
	CD3+CD25+- lymphocytes, rel.	Higher	-4.21	0.18	0.25	20.13	<0.001
	IgG	Higher	-1.93	-0.05	0.10	16.82	<0.001
Atopic dermatitis	CD3+- lymphocytes, rel.	Higher	-1.30	0.43	0.18	69.60	<0.001
	CD3+CD25+- lymphocytes, rel.	Higher	-3.33	15.94	0.69	8.78	0.033
	CD16+56+-lymphocytes, rel.	Higher	-0.40	-0.02	0.16	59.26	<0.001
IgG to pertussis	IgG	Lower	4.15	-0.36	0.43	14.10	0.005

Data provided in Tables 5 and 6 give evidence that indicators of cellular adaptive immunity and functional activity of immune-competent cells are more frequently influenced the most by factors related to the educational process and lifestyle. Any changes in these factors raise likelihood that levels of T-lymphocytes and cytokines will deviate from their physiological norms by 12.2–15.0 and 1.7–3.6 % respectively. Quantitative assessment of additional likelihood of changes in humoral adaptive immunity has established that the studied lifestyle aspects and chemical factors most frequently have the greatest influence on the IgG level raising likelihood of its decline by up to 5.61 and 4.3 % respectively. Lifestyle factors and diets are the most significant ones for a growth in likelihood of innate immunity indicators deviating from the physiological norm. Any change in these factors by 1 % increases likelihood of a decline in the phagocytic system indicators by up to 3.63 and 1.9 % respectively.

The list of laboratory immunological indicators of risk-associated ADs and post-vaccination immunity disorders is the ultimate result of consequent regression logistic modeling. For them, a consequent relationship has been proven: factor → change in laboratory (immunologic) indicator → allergic disease / post-vaccination immunity disorder (Table 7).

The results obtained by neural network modeling of relationships between laboratory indicators of disrupted immune homeostasis and a decline in levels of specific G class antibodies to herpesvirus infection HSV-1, CMVI, measles, diphtheria, and pertussis and conformity with biological feasibility principle made it possible to enhance the list of indicators of risk-associated post-vaccination immunity disorders and to determine laboratory immunological indicators of risk-associated post-infection immunity disorders (Table 8).

Having generalized all obtained data, we have created lists containing immunological indicators of risk-associated ADs and anti-infection immunity disorders. Thus, immunological indicators of risk-associated ADs upon combined exposure to risk factors related to the educational process, environment, diets, and lifestyle include elevated relative contents of CD3+-, CD3+CD25+-lymphocytes and a decline in relative contents of CD16+56+-lymphocytes. Likelihood of anti-infection immunity disorders associated with combined exposure to the analyzed factors is estimated per the following immunological indicators: elevated relative levels of CD3+CD8+-, CD3+CD95+-lymphocytes, declining levels of IgG, absolute phagocytosis, phagocytic index, IL-17, IL-1ß, and TNF.

Table 8

Results of neural network modeling to describe the relationship between likelihood of changes in the level of specific class G antibodies to herpesvirus and vaccine-managed infections and immunological indicators, %

Immunological indicator	IgG to HSV (layers 15; 11, R^2 = 0.323)	IgG to CMV (layers 11; 5, R^2 = 0.143)	IgG to measles (layers 15; 10, R^2 = 0.323)	IgG to diphtheria (layers 5; 2, R^2 = 0.514)	IgG to pertussis (layers 5; 2, R^2 = 0.508)
CD3+CD8+-lymphocytes, rel.	1.79	4.89	37.78	0.24	7.05
CD19+- lymphocytes, rel.			1.41	4.55	25.52
CD3+CD95+- lymphocytes, abs.				1.40	277.78
CD3+CD95+- lymphocytes, rel.	3.58	34.72		2.70	
IgG	15.84		7.15	2.58	
Absolute phagocytosis	3.94			0.98	16.92
Phagocytosis percentage				2.49	
Phagocytic index	20.54			0.03	4.41
Phagocytic number		2.23			28.17
IL-4		17.26	0.81		
IL-6		16.01			
IL-10			45.89		
IL-17	1.56	36.43		0.05	43.13
IL-1β	1.11	8.54	2.10	30.94	
INFγ		0.93			13.55
TNF	2.28		37.33		23.31

Common mechanisms of anti-infection immunity and allergic reactivity modification in children, which is associated with specific features of the modern educational process and quality of the environment, include imbalance of humoral and cellular immunity components together with activation of cellular-mediated reactions of the adaptive immunity response. This process involves a growth in relative levels of CD3+- and CD3+CD25+-lymphocytes (immunological indicators of risk-associated allergic diseases) and CD3+CD8+-lymphocytes (an immunological indicator of anti-infection immunity modification). A decline in absolute phagocytosis and the phagocytic index indicate weaker activity of the non-specific protection system when anti-infection immunity modification occurs upon exposer to environmental factors, factors related to the modern educational process, diets and lifestyle. Allergic reactivity modification is indicated by a decline in levels of natural killers, which are conventionally considered cellular components of innate immunity.

The results of the accomplished multifactorial logistic modeling have established that an isolated contribution made by the modern educational process to likelihood of risk-associated AR amounts to 61.8 %; chemical exposures, 30.6 %; lifestyle factors, 7.6 %; likelihood of BA associated with the analyzed factors is contributed by the modern educational process, 35.7 %; chemical exposures, 33.1 %; lifestyle, 31.2 %. Leading contributions made to likelihood of risk-associated AD belong to the modern educational process (74.0 %), followed by lifestyle factors (18.4 %), and chemical exposures (7.6 %).

We have accomplished quantitative assessment of likelihood of a decline in IgG antibodies to herpesvirus infection HSV-1 based on neural network modeling. As a result, we have established that lifestyle and diets, with their contributions equaling 28.9 and 26.2 % respectively, have the greatest influence on humoral immunity to herpesvirus infection; contributions made by ambient air quality and peculiarities of the modern educational process amount to 13.7 and 13.5 % respectively. Too short night sleep (10.0 %) makes the greatest contribution among lifestyle factors; among diet-related factors, the greatest contributions are made by high contents of confectionaries in a typical daily diet (4.8 %) and low magnesium contents in it (2.9 %); among chemical environmental factors, elevated formaldehyde levels in ambient air (its contribution is 7.5 %). The greatest contributions to declining levels of IgG-antibodies to CMVI are made by diets (44.0 %), the modern educational process (17.9 %), chemical exposures (12.5 %, air quality inside classrooms), and lifestyle (12.3 %). Low caloric contents in a typical daily diet and insufficient consumption of cereals and beans hold a special place among other violations of a diet structure and quality as they make the greatest contributions (9.1 and 2.9 % respectively) to a disrupted humoral response to CMVI. Among factors related to the modern educational process, the greatest contribution is made by duration of long breaks (9.5 %), followed by quality of air inside classrooms per elevated manganese levels in it (9.2 %), and lifestyle as too short night sleep (5.3 %).

The greatest contributions to declining levels of specific Class G antibodies to measles are made by the modern educational process (30.8 %), including shorter duration of long breaks (26.7 %); diets (30.3 %), including insufficient contents of sour milk products (6.4 %) and iron (4.4 %) in a typical daily diet. The contribution made by ambient air quality equals 12.4 %, including elevated manganese levels (8.1 %); lifestyle, 11.4 %, including too short duration of doing sports or physical exercises (3.2 %). The greatest contribution made to changes in levels of specific antibodies to diphtheria toxin is made by lifestyle (29.4 %), the modern educational process (20.5 %), and environmental exposures (19.9 %). Among lifestyle factors, too short night sleep holds the leading place per its contribution (16.7 %); among factors related to the modern educational process, shorter duration of long breaks (11.2 %); among environmental exposures, elevated formaldehyde levels in ambient air (7.2 %). Diet has the greatest influence on declining levels of IgG-antibodies to pertussis with its contribution reaching 34.0 %. The most significant contributions within an unhealthy diet are made by low contents of sour milk products (2.6 %) and low caloric contents (5.1 %) in a typical daily diet. The second place per a contribution to declining humoral immunity to pertussis belongs to factors related to the modern educational process (21.9 %), including too short long breaks (19.1 %); the third place is occupied by lifestyle factors (18.4 %), including too short night sleep (8.5 %).

Conclusion. Therefore, innovative educational volumes according to educational programs accepted in secondary schools with profound studies of some subjects are frequently completed involving too high educational loads for schoolchildren, which typically mean violations of safe standards for the organization of the educational process. High ‘physiological costs’ of education caused by elevated educational loads, too much time spent at school, too much time spent on doing homework, and intensive involvement in additional education make a substantial contribution to changes in schoolchildren’s lifestyles and diets.

In Type 1 schools, two rest phases are not provided for schoolchildren (duration of breaks is shortened by 1.3–2 times), educational loads do not conform to age-specific capabilities (weekly loads are 5.5–19.2 % higher than their maximum permissible levels), the central nervous system is overloaded and this results in overall exhaustion (duration of work with IWB is 1.2–2.2 times longer than allowed by safe standards). Optimal environmental conditions are not provided either as some chemicals are established in ambient air near these schools and in air inside classrooms in levels higher than RfC for chronic exposure including manganese and formaldehyde, 1.8–8.5 times; in sporadic air samples, nickel and chromium in levels up to 3.6 times higher than chronic RfC; benzene and toluene have been established in levels not higher than RfC for chronic exposure. All the foregoing combined with unhealthy diets, too short night sleep, too low physical activity and too high digital activity lead to a 1.3 times growth in ADs risks over the whole period of school; insufficient production of IgG to herpesviruses indicating a threat that such infections could be activated, 2.3 times; a growth in the proportion of seronegative persons to measles and diphtheria antigens, 3.1–5.4 times (OR = 1.33–5.40; CI = 1.02–11.14).

We have established pathogenetic modification regularities and the list of immunological indicators of risk-associated ADs and anti-infection immunity disorders. Upon exposure to the analyzed factors, children with ADs tend to have more pronounced activation of the cellular adaptive immune response (↑CD3+-, CD3+CD25+-lymphocytes, relative) and declining natural cytotoxic activity (↓CD16+56+-lymphocytes, relative). The immune status of children with risk-associated anti-infection immunity disorders is characterized with a prevailing cytotoxic effector pathway of the immune response (↑CD3+CD8+-lymphocytes, relative), strengthening pathways of programmed cell death (↑CD3+CD95+-lymphocytes, relative), a decline in reactivity of the humoral immunity component (↓IgG), weakening activity of the non-specific protection system (↓absolute phagocytosis, PN, PI, and the phagocytosis percentage), deregulated intercellular cooperation (↓IL-17, IL-1ß, TNF). Activation of cellular-mediated reactions of the adaptive immunity response (↑CD3+-, CD3+CD25+-lymphocytes, relative, and ↑CD3+CD8+-lymphocytes, relative) and weakening phagocytic activity (↓absolute phagocytosis, PI and ↓CD16+56+-lymphocytes, relative) are common immunological pathways of anti-infection immunity and allergic reactivity modification associated with exposure to environmental factors and factors related to the modern educational process.

Maximum isolated contributions made to likelihood of risk-associated ADs are made by specific features of the modern educational process, 35.7–74.0 %; isolated contributions made by chemical exposures and lifestyles are comparable and amount to 7.6–33.1 and 7.6–31.2 % respectively.

Our ranking of risk factors determining likelihood of declining humoral post-infection and post-vaccination immunity has revealed that the maximum contribution to declining levels of IgG-antibodies to herpesvirus infections is made by diets (26.2–44.0 %) followed by lifestyles (12.3–28.9 %), the modern educational process (13.5–17.9 %), and chemical exposures holding the fourth rank place (9.2–13.7 %). Contributions made by the analyzed factors to declining levels of IgG-antibodies to vaccine-managed infections are ranked as follows: the modern educational process (20.5–30.8 %) and diets (14.6–34.0 %), followed by lifestyles (11.4–29.4 %) and chemical exposures (6.5–19.9 %).

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. Bogomolova E.S., Shaposhnikova M.V., Kotova N.V., Badeeva T.V., Maksimenko E.O., Kiseleva A.S., Kovalchuk S.N., Ashina M.V., Olyushina E.O. Characteristics of physical health of students of modern educational institutions. *Gigiena i sanitariya*, 2019, vol. 98, no. 9, pp. 956–961. DOI: 10.47470/0016-9900-2019-98-9-956-961 (in Russian).

2. Kuchma V.R., Rapoport I.K., Sukhareva L.M., Skoblina N.A., Sedova A.S., Chubarovsky V.V., Sokolova S.B. The health of children and adolescents in school ontogenesis as a basis for improving the system of school health care and sanitary-epidemiological wellbeing of students. *Zdravookhranenie Rossiiskoi Federatsii*, 2021, vol. 65, no. 4, pp. 325–333. DOI: 10.47470/0044-197X-2021-65-4-325-333 (in Russian).

3. Zaitseva N.V., Dolgikh O.V., Dianova D.G. Formaldehyde contamination influence on immune system in children. *Izvestiya Samarskogo nauchnogo tsentra Rossiiskoi akademii nauk*, 2014, vol. 16, no. 5 (2), pp. 702–704 (in Russian).

4. Starkova K.G., Dolgikh O.V., Kazakova O.A. Features of the immune status of middle and high school students in conditions of high blood content of a number of exogenous chemical impurities. *Gigiena i sanitariya*, 2021, vol. 100, no. 5, pp. 501–506. DOI: 10.47470/0016-9900-2021-100-5-501-506 (in Russian).

5. Basova A.V., Komkova G.N. Mechanisms of mandatory vaccination: problems and solutions. *Zdravookhranenie Rossiiskoi Federatsii*, 2023, vol. 67, no. 4, pp. 320–328. DOI: 10.47470/0044-197X-2023-67-4-320-328 (in Russian).

6. Su S.-B., Chang H.-L., Chen K.-T. Current status of mumps virus infection: Epidemiology, pathogenesis, and vaccine. *Int. J. Environ. Res. Public Health*, 2020, vol. 17, no. 5, pp. 1686. DOI: 10.3390/ijerph17051686

7. Zhang Y., Lan F., Zhang L. Advances and highlights in allergic rhinitis. *Allergy*, 2021, vol. 76, no. 11, pp. 3383–3389. DOI: 10.1111/all.15044

8. Licari A., Magri P., De Silvestri A., Giannetti A., Indolfi C., Mori F., Marseglia G.L., Peroni D. Epidemiology of Allergic Rhinitis in Children: A Systematic Review and Meta-Analysis. *J. Allergy Clin. Immunol. Pract.*, 2023, vol. 11, no. 8, pp. 2547–2556. DOI: 10.1016/j.jaip.2023.05.016

9. Solov'eva I.L. Otsenka effektivnosti parotitno-korevoi vaktsiny i ee vliyaniya na pokazateli kletochnogo immuniteta u detei s allergicheskimi zabolevaniyami [Evaluation of effectiveness of mumps-measles vaccine and its impact on cellular immunity indices in children with allergic diseases]. *Epidemiologiya i vaktsinoprofilaktika*, 2005, no. 3 (22), pp. 11–14 (in Russian).

10. Zhestkov A.V., Pobezhimova O.O., Sidorova O.S. Features of vaccination for children with allergic diseases. *Allergologiya i Immunologiya v Pediatrii*, 2020, no. 4 (63), pp. 4–11. DOI: 10.24411/2500-1175-2020-10012 (in Russian).

11. Papadopoulos N.G., Miligkos M., Xepapadaki P. A Current Perspective of Allergic Asthma: From Mechanisms to Management. *Handb. Exp. Pharmacol.*, 2022, vol. 268, pp. 69–93. DOI: 10.1007/164_2021_483

12. Perrotta F., Simeon V., Bonini M., Ferritto L., Arenare L., Nigro E., Nicolai A., Daniele A., Calabrese C. Evaluation of Allergic Diseases, Symptom Control, and Relation to Infections in a Group of Italian Elite Mountain Bikers. *Clin. J. Sport Med.*, 2020, vol. 30, no. 5, pp. 465–469. DOI: 10.1097/JSM.0000000000000678

13. Maximova A.V., Smolkin Yu.S. Herpes infections and allergic diseases in children. Child with transplanted kidney – difficult patient: peculiarities of medical observation, interaction with family. *Pediatriya. Consilium Medicum*, 2020, no. 4, pp. 44–51. DOI: 10.26442/26586630.2020.4.200542 (in Russian).

14. Tataurshchikova N.S., Letyayeva O.I., Maksimova A.V., Levkova E.A. Herpes infection and allergy as a global problem of modern medicine. *Effektivnaya farmakoterapiya*, 2023, vol. 19, no. 28, pp. 24–29. DOI: 10.33978/2307-3586-2023-19-28-24-29 (in Russian).

15. Mokronosova M.A., Mats A.N. Infektsiya i allergiya: dve storony odnoi medali [Infection and allergy: two sides of the same coin]. *Astma i allergiya*, 2015, no. 4, pp. 9–12 (in Russian).

16. Kharrazian D. Exposure to Environmental Toxins and Autoimmune Conditions. *Integr. Med. (Encinitas)*, 2024, vol. 23, no. 1, pp. 22–26.

17. Dziarzhynskaya N., Hindziuk A., Hindziuk L., Sysoeva I., Krupskaya D., Urban U., Cherniavskaya N., Vishnevskaya A. [et al.]. Airborne chemical pollution and children's asthma incidence rate in Minsk. *J. Prev. Med. Hyg.*, 2022, vol. 62, no. 4, pp. E871–E878. DOI: 10.15167/2421-4248/jpmh2021.62.4.1568

18. Kinash M.I., Boyarchuk O.R. Fat-soluble vitamins and immunodeficiency states: mechanisms of influence and opportunities for use. *Voprosy pitaniya*, 2020, vol. 89, no. 3, pp. 22–32. DOI: 10.24411/0042-8833-2020-10026 (in Russian).

19. Aleksandrova I.E. Hygienic optimization of educational process at school involving massive use of electronic learning devices. *Health Risk Analysis*, 2020, no. 2, pp. 47–54. DOI: 10.21668/health.risk/2020.2.05

20. Mortelliti C.L., Banzon T.M., Vieira C.Z., Phipatanakul W. Environmental Exposures Impact Pediatric Asthma Within the School Environment. *Immunol. Allergy Clin. North Am.*, 2022, vol. 42, no. 4, pp. 743–760. DOI: 10.1016/j.iac.2022.05.005

21. Valina S.L., Ustinova O.Yu., Shtina I.E., Maklakova O.A., Osheva L.V. Allergic rhinitis in schoolchildren under combined exposure to various environmental risk factors. *Gigiena i sanitariya*, 2023, vol. 102, no. 10, pp. 1087–1095. DOI: 10.47470/0016-9900-2023-102-10-1087-1095 (in Russian).

22. Valina S.L., Ustinova O.Yu., Zaitseva N.V., Shtina I.E., Maklakova O.A., Makarova V.G. Peculiarities of post-vaccination immunity to infections manageable by specific prevention means in schoolchildren. *Gigiena i sanitariya*, 2024, vol. 103, no. 11, pp. 1334–1343. DOI: 10.47470/0016-9900-2024-103-11-1334-1343 (in Russian).

23. Ryazanova S.V., Komkov A.A., Mazaev V.P. Russian and world experience in the application of new artificial intelligence technologies in real medical practice. *Nauchnoe obozrenie. Meditsinskie nauki*, 2021, no. 6, pp. 32–40. DOI: 10.17513/srms.1215 (in Russian).

24. Bhadani R., Chen Z., An L. Attention-Based Graph Neural Network for Label Propagation in Single-Cell Omics. *Genes (Basel)*, 2023, vol. 14, no. 2, pp. 506. DOI: 10.3390/genes14020506

Valina S.L., Zaitseva N.V., Ustinova O.Yu., Shtina I.E., Maklakova O.A. Common immunological pathways of anti-infection immunity and allergic reactivity modification in children associated with peculiarities of the modern educational process and environment quality. Health Risk Analysis, 2025, no. 2, pp. 126–144. DOI: 10.21668/health.risk/2025.2.11.eng

Received: 19.05.2025
Approved: 16.06.2025
Accepted for publication: 26.06.2025

UDC 614.89:614.253:[616.98-036.22:578.834.1SARS-CoV-2
DOI: 10.21668/health.risk/2025.2.12.eng

Read online

Research article

ASSESSMENT OF RELATIVE RISK OF DISCOMFORT AND ITS SUBJECTIVE PERCEPTION ASSOCIATED WITH PERSONAL PROTECTIVE EQUIPMENT: ADAPTATION DIFFERENCES AMONG STAFF IN INFECTIOUS DISEASE AND MULTIDISCIPLINARY HOSPITALS DURING THE COVID-19 PANDEMIC

K.A. Hutsich, S.L. Itpayeva-Liudchyk, K.A. Nikalayeva, I.V. Madeksha

Research Institute of Hygiene, Toxicology, Epidemiology, Virology and Microbiology of the state institution «Republican Center of Hygiene, Epidemiology and Public Health», 8 Akademicheskaya St., Minsk, 220012, Republic of Belarus

The object of the study was represented by healthcare workers from infectious disease and multidisciplinary hospitals repurposed to treat COVID-19 patients.

The aim of the study was to assess subjective perception of discomfort associated with prolonged use of personal protective equipment (PPE) and to identify differences in adaptation to such working conditions between healthcare staff in infectious disease and multidisciplinary hospitals during the COVID-19 pandemic.

A survey was conducted among healthcare workers, including questions on daily duration of PPE use (medical masks/respirators, protective goggles, coveralls) and the presence of symptoms indicating adverse effects of prolonged PPE using.

Changes in working conditions during the COVID-19 pandemic led to a significant increase in the proportion of healthcare workers regularly using PPE as well as a substantial rise in duration of its use. Prolonged PPE use during the pandemic resulted in an increased frequency of complaints related to PPE in both hospital types. Higher prevalence of complaints associated with PPE use was observed in the multidisciplinary hospital, both during routine and repurposing periods, as well as a greater change in the frequency of systemic physiological disorders while wearing respiratory PPE (difficulty breathing, sensation of shortness of breath, dizziness) during the pandemic in the multidisciplinary hospital compared to the infectious disease hospital. The identified inter-hospital differences likely reflect greater adaptability among staff in infectious disease hospitals to prolonged PPE use attributed to stringent infection control protocols and the availability of well-tested algorithms for working in routine daily practice.

These disparities in preparedness and adaptation of healthcare workers to PPE use in repurposed hospitals highlight the need for expanded implementation of occupational health risk management measures, emphasize the importance of optimizing PPE design, improving selection and usage protocols, introducing training programs on rational PPE use, and conducting regular health screenings for PPE-related adverse effects among healthcare workers.

Keywords: *COVID-19, pandemic, healthcare workers, occupational health risks, subjective complaints, personal protective equipment (PPE), medical masks, protective goggles, protective coveralls.*

Biological threats of a scale reaching an epidemic or even a pandemic pose a serious challenge for public healthcare due to the necessity to develop immediate and effective health protection measures. The necessity to repurpose in-patient hospitals to treat patients with communicable diseases requires implementing numerous administrative, manage-

Katsiaryna A. Hutsich – Candidate of Medical Sciences, Head of the Laboratory of Applied Toxicology and Medical Device Safety (e-mail: ekhutsich@gmail.com; tel.: +375 17 378-80-56, +375 29 694-06-18; ORCID: https://orcid.org/0000-0002-1910-6556).

Sviatlana L. Itpayeva-Liudchyk – Candidate of Medical Sciences, Academic Secretary (e-mail: itpaeva.ludchik@gmail.com; tel.: +375 17 320-02-17; ORCID: https://orcid.org/0000-0002-4878-2246).

Katsiaryna A. Nikalayeva – Head of the Laboratory of Occupational Health Risk Assessment (e-mail: katyanik@tut.by; tel.: +375 17 351-72-18; ORCID: https://orcid.org/0000-0002-7757-8631).

Iryna V. Madeksha – Junior Researcher at the Laboratory of Occupational Health Risk Assessment (e-mail: ira-kyz@tut.by; tel.: +375 17 351-72-18; ORCID: https://orcid.org/0000-0001-6275-4746).

ment, engineering-technical, and organizational activities and infection control measures as well as employing additional resources such as healthcare workers and personal protective equipment (PPE). The spread of SARS-CoV-2 in 2019 and the following COVID-19 pandemic have demonstrated how important it is to prepare healthcare organizations to deal with such threats [1–3].

Occupational activities involving health protection and recovery are inherently associated with serious health risks for those who perform them [4–7]. Work with patients infected with highly contagious respiratory pathogens involves additional health risks for healthcare workers; these risks are associated not only with possibility to get infected but also growing physical, intellectual, sensory, and emotional loads, the necessity to use PPE for a long time, and contacts with disinfectants and antiseptics [8]. The necessity to use PPE for long periods of time can involve damage to the skin in places where a medical mask / respirator and goggles contact it, developing symptoms of heat stress when using coveralls, as well as lower work capacity and headaches [9–13]. Physical discomfort caused by PPE use often leads to failure to comply with standards for its use: healthcare workers can consciously reduce time of its use or refuse from using it completely, especially under heavy workloads. Such breaches of infection control protocols raise risks of occupational infection considerably eliminating any protective PPE effects and creating health threats for both healthcare workers themselves and their patients [14]. In this respect, occupational risk management, including minimization of discomfort caused by PPE use, provision of PPE ergonomics and availability, as well as teaching healthcare workers how to use PPE rationally, becomes an integral part of infection prevention and control. These measures promote compliance with safety protocols thereby reducing probability of violations and creating a sustainable system of anti-epidemic measures aimed at preventing in-hospital spread of infections [15–18][1].

A necessary activity aimed at managing occupational health risks for healthcare workers involves developing and implementing specific measures on preventing negative outcomes of occupational exposures associated with considerable changes in working conditions when in-patient hospitals are repurposed in an unfavorable epidemic situation [19, 20]. In this context, we can assume that healthcare workers employed at infectious disease hospitals possess specific knowledge and skills of treating communicable diseases; therefore, they are better trained to treat patients during a pandemic than their colleagues from multidisciplinary repurposed hospitals. Healthcare workers employed at infectious disease hospitals have a better insight into specificity of infectious processes, infection control protocols and safety precautions, which can be crucially important in time of epidemics and pandemics.

Therefore, analysis of differences in preparedness and ability to adapt among healthcare workers from infectious disease and somatic hospitals during a pandemic is an important research trend, which can help develop more effective strategies aimed at responding to future biological threats. A complex approach to raising preparedness of all healthcare organizations, providing relevant training and equipment for healthcare workers can considerably increase effectiveness of the whole healthcare system when it faces new epidemic challenges.

The aim of this study was to assess subjective perception of discomfort associated with prolonged use of personal protective equipment (PPE) and to identify differences in adaptation to such working conditions between healthcare workers in infectious disease and multidisciplinary hospitals during the COVID-19 pandemic.

Materials and methods. The research was accomplished in an infectious disease hospital and multidisciplinary hospital in

[1] Keep health workers safe to keep patients safe: WHO. *World Health Organization*. Available at: https://www.who.int/news/item/17-09-2020-keep-health-workers-safe-to-keep-patients-safe-who (March 29, 2025).

Minsk (Republic of Belarus) repurposed for treating patients infected with COVID-19. We conducted a survey among healthcare workers to investigate subjective perception of discomfort. The survey included questions on frequency and duration of PPE use in routine work (medical masks / respirators, protective goggles, and coveralls) as well as having symptoms indicating presence of some negative health effects produced by prolonged PPE use. The respondents could choose several options when answering questions about subjective symptoms associated with PPE use.

One hundred and three respondents from the infectious disease hospital and 95 respondents from the multidisciplinary hospital took part in the survey. Two samples did not differ significantly per sex (p = 0.052), age (p = 0.29), or work records (p = 0.21). All participants gave their consents to personal data analysis provided that confidentiality and anonymity were secured.

This subjective assessment method has a limitation, namely, the fact that the survey data depended on accuracy of the respondents' self-reports. This may lead to information bias due to cognitive mistakes, social advisability or differences in individual interpretation of the results. This should be considered when survey results are interpreted. However, within this study context, the survey turned out to have significant advantages: the method allowed operative data collection in an epidemic emergency when it was too difficult to apply more resource-consuming approaches; subjective assessment of discomfort is an important source of information about individual perception, which determines how ready healthcare workers are to comply with infection control protocols. Use of a standardized inventory with binary and ordinal answer options minimized variability in interpreting the questions and anonymity helped reduce social advisability when answering them.

The survey data were statically analyzed using statistical software packages Excel and Statistica 13. The data analysis involved calculating absolute and relative frequencies. The confidence interval was calculated for intensive indicators per the Wilson method. Qualitative ordinal indicators in dependent groups were compared with the Wilcoxon matched pairs test. Significance of differences in data describing qualitative indicators in independent groups was established based on the χ^2test value. Frequencies of binary indicators in dependent groups were compared using the McNemar's mid-p-value binomial test; the effect size was estimated by calculating Odds Ratio (OR) and its confidence interval. OR values in two samples were compared by using Conditional Relative Odds Ratio (CROR) and its confidence interval [21]. The research results were considered authentic and differences between indicators significant at the probability value being not less than 95.5 % ($p < 0.05$).

Results and discussion. Respiratory PPE provides necessary protection against respiratory pathogens. Our study revealed a considerably higher frequency of using respiratory PPE by healthcare workers during the COVID-19 pandemic, which is consistent with global guidelines on infection prevention and control. The share of healthcare workers who used face masks or respirators in their routine work grew significantly in both in-patient hospitals, namely, from 71.0 (62.5–79.6) to 100 % (96.4–100) ($p < 0.001$) in the infectious disease hospital and from 91.6 (84.3–95.7) to 100 % (96.1–100) (p = 0.004) in the multidisciplinary hospital. This highlights the universality of taken safety precautions. Together with a growing number of healthcare workers who used PPE, there was a growth in time of PPE use during a work shift. The trend was significantly more prominent in the infectious disease hospital ($p < 0.001$): 87.4 % (79.6–92.5) of the respondents from the infectious disease hospital ($p < 0.001$) and 47.4 % (37.6–57.3) of the respondents from the multidisciplinary hospital ($p < 0.001$) mentioned longer time of using this PPE. The growth in the number of healthcare workers using face masks was also accompanied with more frequent complaints associated with their long use (Table 1).

Table 1

The results obtained by comparative analysis of frequency of complaints associated with wearing a face mask / respirator

Complaints	The share of the respondents with complaints in routine working conditions, %	The share of the respondents with complaints during the COVID-19 pandemic, %	McNemar's mid-*p*-value	OR (95 % CI)	CROR (95 % CI)
Itch in the nose or throat	19.4 / 21.1	35.0 / 23.2	0.002 / 0.581	4.2 (1.58–11.14) / 1.4 (0.44–4.41)	3.0 (0.67–13.53)
Labored breathing	20.4 / 24.2	39.8 / 42.1	< 0.001 / < 0.001	3.86 (1.68–8.86) / 18.0 (2.40–134.84)	4.66 (0.53–41.19)
Shortness of breath	15.5 / 42.1*	22.3 / 49.5*	0.115 / 0.096	2.17 (0.82–5.70) / 2.4 (0.85–6.81)	1.11 (0.27–4.59)
Dizziness	2.9 / 8.4	3.9 / 15.8*	0.688 / 0.022	1.5 (0.25–8.98) / 8.0 (1.0–63.97)	5.33 (0.34–82.83)
Discomfort on the face and / or behind the ears	41.8 / 56.8*	68.0 / 69.5	< 0.001 / 0.019	3.46 (1.77–6.76) / 2.71 (1.14–6.46)	1.28 (0.43–3.82)
Redness and / or maceration on the face, behind the ears	9.7 / 40.0*	5.8 / 50.5*	0.180 / 0.019	3.0 (0.61–14.86) / 3.5 (1.15–10.63)	1.17 (0.17–8.19)

Note: the data in the columns 2–4 are given in the following format: 'infectious disease hospital / multidisciplinary hospital'; * means significant differences in frequency of complaints in the analyzed hospitals under the same work scenario at $p < 0.05$.

Comparative analysis of frequency of complaints associated with long use of medical face masks / respirators established the following. In the infectious disease hospital, an authentic growth in frequency of complaints during the pandemic against routine work was found for the following symptoms: itch in the nose or throat, from 19.4 (12.9–28.1) to 35.0 % (26.4–44.6) ($p = 0.002$, $OR = 4.2$); labored breathing, from 20.4 (13.7–29.2) to 39.8 % (30.9–49.5) ($p < 0.001$, $OR = 3.86$); discomfort on the face and / or behind the ears, from 41.8 (32.7–51.4) to 68.0 % (58.4–76.2) ($p < 0.001$, $OR = 3.46$). In the multidisciplinary hospital, the most prominent growth was established for the following symptoms: labored breathing, from 24.2 (16.7–33.7) to 42.1 % (32.7–52.2) ($p < 0.001$, $OR = 18.0$); dizziness, from 8.4 (4.3–15.8) to 15.8 % (9.8–24.4) ($p = 0.022$, $OR = 8.0$); discomfort on the face and / or behind the ears, from 56.8 (46.8–66.3) to 69.5 % (59.6–77.8) ($p = 0.019$, $OR = 2.71$); redness and / or maceration on the face, behind the ears, from 40.0 (30.7–50.0) to 50.5 % (40.7–60.4) ($p = 0.019$, $OR = 3.5$).

The results obtained by comparing prominence of differences between the inpatient hospitals show an authentic difference in prevalence of several symptoms both in the routine work scenario and after repurposing during the pandemic. Thus, healthcare workers from the multidisciplinary hospital more often made the following complaints associated with respiratory PPE use: in the routine work scenario, shortness of breath ($p < 0.001$), discomfort on the face and / or behind the ears ($p = 0.034$), redness and / or maceration on the face, behind the ears ($p < 0.001$); when working during the pandemic, shortness of breath ($p < 0.001$), dizziness ($p = 0.005$), redness and / or maceration on the face, behind the ears ($p < 0.001$). Assessment of effects produced by a work scenario on frequency of complaints did not establish significant differences between the hospitals; however, we can still speak about certain trends associated with more prominent influence of the pandemic on frequency of complaints about itch in the nose or throat (CROR = 3.0 (0.67–13.53)) in the infectious disease hospital, labored breathing (CROR = 4.66 (0.53–41.19)) and dizziness (CROR = 5.33 (0.34–82.83)) in the multidisciplinary hospital. It is worth noting that wide

CROR confidence intervals, which include 1, indicate considerable uncertainty of these estimates, which can be partly due to the small size of the study sample.

The share of healthcare workers who used protective goggles, grew considerably both in the infectious disease hospital (from 25.2 to 91.3 %, $p < 0.001$) and in the multidisciplinary hospital (from 50.5 to 86.3 %, $p < 0.001$). The results obtained by analyzing frequency of complaints associated with wearing protective goggles (Table 2) revealed a prominent dynamic during the COVID-19 pandemic. In the infectious disease hospital, the share of healthcare workers who complained about discomfort in the area where the goggles contacted the head grew from 15.5 (9.8–23.8) to 73.8 % (64.6–81.3) ($p < 0.001$); in the multidisciplinary hospital, from 27.4 (19.4–37.1) to 56.8 % (46.8–66.3) ($p < 0.001$). High *OR* values in both hospitals (33 and 29) highlight a prominent correlation between a long time of wearing goggles during the pandemic and discomfort appearance. It is noteworthy that, despite an authentic increase in duration of using goggles in both hospitals, healthcare workers from the multidisciplinary hospital mentioned longer duration of using this PPE type more frequently (47.4 and 15.5 % of the respondents, $p < 0.001$). Comparison of dynamics between these two hospitals (CROR = 1.14, (0.07–18.84)) did not reveal any significant differences despite initially higher prevalence of such complaints in the multidisciplinary hospital under the routine work scenario (27.4 against 15.5 %; $p < 0.05$). Skin reactions such as itch, redness and maceration in the area where the goggles contact the skin also showed a pronounced growth: in the infectious disease hospital, from 1.0 (0.2–5.3) to 33.0 % (24.7–42.6) ($p < 0.001$); in the multidisciplinary hospital, from 5.3 (2.3–11.7) to 22.1 % (14.9–31.5) ($p < 0.001$, $OR = 17$). These changes can be caused by mechanical pressure, disrupted air exchange and moisture accumulation under personal protective equipment devices. Special attention should be paid to growing prevalence of headaches in the multidisciplinary hospital, from 12.6 (7.4–20.8) to 31.6 % (23.1–41.5) ($p < 0.001$, $OR = 19$), whereas similar dynamics was insignificant in the infectious disease hospital ($p = 0.25$).

A considerable growth in the share of healthcare workers who wore protective coveralls during the pandemic was established both in the infectious disease hospital (from

Table 2

The results obtained by comparative analysis of frequency of complaints associated with wearing protective goggles

Complaints	The share of the respondents with complaints in routine working conditions, %	The share of the respondents with complaints during the COVID-19 pandemic, %	McNemar's mid-*p*-value	OR (95 % CI)	CROR (95 % CI)
Discomfort in the area where the goggles contact the head	15.5 / 27.4*	73.8 / 56.8*	< 0.001 / < 0.001	33 (4.51–241.29) / 29 (3.95–212.90)	1.14 (0.07–18.84)
Itch, redness and maceration in the area where the goggles contact the head	1.0 / 5.3	33.0 / 22.1	< 0.001 / < 0.001	– / 17 (2.26–127.75)	–
Headache	1.0 / 12.6*	2.9 / 31.6*	0.25 / < 0.001	– / 19 (2.54–141.93)	–

Note: the data in the columns 2–4 are given in the following format: 'infectious disease hospital / multidisciplinary hospital'; * means significant differences in frequency of complaints in the analyzed hospitals under the same work scenario at $p < 0.05$; blank spaces with dashes mean that the indicator was not calculated due to limitations intrinsic to the employed method.

31.1 to 98.1 %; $p < 0.001$) and in the multidisciplinary hospital (from 30.5 to 92.6 %; $p < 0.001$). This was accompanied with a significant growth in the number of complaints about physical discomfort and physiological impairments (Table 3), which is explained by a longer time of using coveralls in daily work in both analyzed hospitals. Longer use of coveralls during the pandemic was mentioned by 88.4 % (80.7–93.2) of the respondents from the infectious disease hospital ($p < 0.001$) and 69.5 % (59.6–77.8) of the respondents form the multidisciplinary hospital ($p < 0.001$).

Thus, in the infectious disease hospital, the share of the respondents who complained about elevated sweating grew from 24.3 (17.0–33.4) under the routine work scenario to 63.1 % (53.5–71.8) during the pandemic ($p < 0.001$) with the estimated odds ratio (*OR*) being equal to 41 (5.64–298.07). In the multidisciplinary hospital, it grew from 17.9 to 67.4 % ($p < 0.001$, $OR = 48$ (6.63–347.76)); the CROR value equaled 1.17 (0.07–19.31), which indicates absence of any significant differences in relative influence of changes in a work routine between these two hospitals. Similarly, complaints about feeling overheated became much more frequent in both hospitals: in the infectious disease hospital, their frequency grew from 5.8 (2.7–12.1) to 44.7 % (35.4–54.3) ($p < 0.001$); in the multidisciplinary hospital, from 12.6 (7.4–20.8) to 54.7 % (44.7–64.4) ($p < 0.001$). The *OR* estimates were not accomplished due to methodological limitations; however, statistical significance of these changes indicates pronounced effects produced by changes in working conditions on subjective perception of feeling overheated. Dynamics of complaints about dizziness showed that frequency of the symptom grew from 1.0 (0.2–5.3) to 7.8 % (4.0–14.6) ($p = 0.008$) in the infectious disease hospital and from 2.1 (0.6–7.4) to 15.8 % (9.8–24.4) ($p < 0.001$) in the multidisciplinary hospital. The *OR* values calculated for the multidisciplinary hospital equaled 14 (1.84–106.47), which emphasizes a considerable impact exerted by long time of wearing coveralls on appearance of dizziness. The number of the respondents who mentioned thirst also grew considerably: in the infectious diseases hospital, frequency of this compliant grew from 1.0 (0.2–5.3) to 16.5 % (10.6–24.9) ($p < 0.001$); in the multidisciplinary hospital, from 11.6 (6.6–19.6) to 49.5 %

Table 3

The results obtained by comparative analysis of frequency of complaints associated with wearing protective coveralls

Complaints	The share of the respondents with complaints in routine working conditions, %	The share of the respondents with complaints during the COVID-19 pandemic, %	McNemar's mid-*p*-value	OR (95 % CI)	CROR (95 % CI)
Elevated sweating	24.3 / 17.9	63.1 / 67.4	< 0.001 / < 0.001	41 (5.64–298.07) / 48 (6.63–347.76)	1.17 (0.07–19.31)
Feeling overheated	5.8 / 12.6	44.7 / 54.7	< 0.001 / < 0.001	– /–	–
Dizziness	1.0 / 2.1	7.8 / 15.8	0.008 / < 0.001	– / 14 (1.84–106.47)	–
Thirst	1.0 / 11.6*	16.5 / 49.5*	< 0.001 / < 0.001	– / 37 (5.08–269.68)	–
Palpitations	3.9 / 4.2	37.9 / 21.1	< 0.001 / < 0.001	– /–	–

Note: the data in the columns 2–4 are given in the following format: 'infectious disease hospital / multidisciplinary hospital'; * means significant differences in frequency of complaints in the analyzed hospitals under the same work scenario at $p < 0.05$; blank spaces with dashes mean that the indicator was not calculated due to limitations intrinsic to the employed method.

(39.6–59.4) ($p < 0.001$). Finally, the dynamics of palpitations showed that the frequency of the symptom grew from 3.9 (1.5–9.6) to 37.9 % (29.1–47.5) ($p < 0.001$) in the infectious disease hospital and from 4.2 (1.7–10.3) to 21.1 % (14.1–30.3) ($p < 0.001$) in the multidisciplinary hospital. The comparative analysis established higher prevalence of thirst in the multidisciplinary hospital both under the routine work scenario (11.6 against 1.0 %; $p = 0.002$) and during the pandemic (49.5 against 16.5 %; $p < 0.001$).

Conclusions. The COVID-19 pandemic led to drastic changes in working conditions of healthcare workers and wide PPE implementation in healthcare organizations. This is confirmed by the fact that 100 % of the healthcare workers in the analyzed hospitals used face masks / respirators and a considerable growth in the share of those who used protective goggles and coveralls. Another universal feature of the pandemic was a longer time of using all PPE types accompanied with considerable growth of physical and physiological discomfort. Long use of respiratory PPE led to a considerable increase in the number of complaints made by healthcare workers from the infectious disease hospital about itch in the nose or throat ($p = 0.002$, $OR = 4.2$), labored breathing ($p < 0.001$, $OR = 3.86$), and discomfort on the face and /or behind the ears ($p < 0.001$, $OR = 3.46$). During the pandemic, healthcare workers from the multidisciplinary hospital had authentically more frequent complaints about such symptoms as labored breathing ($p < 0.001$, $OR = 18.0$), dizziness ($p = 0.022$, $OR = 8.0$), discomfort on the face and /or behind the ears ($p = 0.019$, $OR = 2.71$), and redness and / or maceration on the face and /or behind the ears ($p = 0.019$, $OR = 3.5$). Our comparison of frequency of complaints associated with respiratory PPE use under the same work scenario revealed that healthcare workers from the multidisciplinary hospital more often suffered from discomfort due to wearing a face mask / respiratory both in the routine work scenario and during the pandemic. Thus, in the routine work scenario, healthcare workers from the multidisciplinary hospital more often complained about shortness of breath ($p < 0.001$), discomfort on the face and /or behind the ears ($p = 0,034$), redness and / or maceration on the face and /or behind the ears ($p < 0,001$); during the pandemic, shortness of breath ($p < 0.001$), dizziness ($p = 0.005$), and redness and / or maceration on the face and /or behind the ears ($p < 0.001$). It is worth noting that changes in working conditions and respiratory PP use during the pandemic resulted in more considerable changes in frequency of systemic health disorders in the multidisciplinary hospital: labored breathing (CROR = 4.66 (0.53–41.19)), shortness of breath (CROR = 1.11 (0.27–4.59)), and dizziness (CROR = 5.33 (0.34–82.83)). In the infectious disease hospital, more prominent changes were observed for local reactions during the pandemic such as itch in the nose or throat (CROR = 3.0 (0.67–13.53)) and discomfort on the face and /or behind the ears (CROR = 1.28 (0.43–3.82)).

Longer use of protective goggles during the pandemic also led to more frequent complaints made by healthcare workers in both analyzed hospitals: in the infectious disease hospital, discomfort in the area where the goggles contact the head ($p < 0.001$, $OR = 33$), itch, redness and / or maceration in the area where the goggles contact the head ($p < 0.001$); in the multidisciplinary hospital, discomfort in the area where the goggles contact the head ($p < 0.001$, $OR = 29$), itch, redness and / or maceration in the area where the goggles contact the head ($p < 0.001$, $OR = 17$), and headaches ($p < 0.001$, $OR = 19$). We compared frequency of complaints associated with using protective goggles in both hospitals in the same work scenario; as a result, we established that, in general, healthcare workers from the multidisciplinary hospital, just as with wearing medical face masks, more often complained about physical discomfort under both work scenarios, which was manifested through such complaints as discomfort in the area where the goggles contact the head ($p = 0.042$) and headaches ($p < 0.001$) in the routine work scenario; headaches during the COVID-19 pandemic ($p = 0.023$).

Use of protective coveralls when treating COVID-19 patients was accompanied with much more frequent complaints about all outlined symptoms both in the infectious diseases hospital and repurposed multidisciplinary hospital. However, healthcare workers from the multidisciplinary hospital more often complained about being thirsty when wearing coveralls both in the routine work scenario ($p = 0.002$) and during the pandemic ($p < 0.001$).

More frequent complaints associated with PPE use under both work scenarios as well as more frequent systemic physiological disorders caused by respiratory PPE use in the multidisciplinary hospital during the pandemic may indicate that healthcare workers from the infectious disease hospital are better adapted to longer PPE use due to stricter infection control protocols and well-tested work algorithms employed in their routine practice. Wide CROR confidence intervals, however, indicate the necessity to be very careful when interpreting the detected differences between the hospitals; this might be associated with an insufficient sample size as well as variability of compliance with the rules for PPE use among healthcare workers.

Our findings may indicate that some physiological compensatory mechanisms have been developed in healthcare workers who are employed at infectious diseases hospitals; these mechanisms are associated with regular use of respiratory PPE. Long work under moderate hypoxia is likely to stimulate adaptation (greater effectiveness of oxygen utilization in tissues, respiratory rhythm optimization, and enhanced capillary blood flow). These changes can also result from behavioral skills developed in routine practices (for example, controlled breathing aimed at minimizing discomfort).

Such differences in healthcare workers' preparedness and adaptation to PPE use in times when hospitals are repurposed give evidence of the necessity to more widely implement additional activities aimed at managing occupational health risks associated with PPE use in healthcare organizations responsible for treating patients during an epidemic rise in prevalence of respiratory infections.

To reach a comparable level of adaptation, it is advisable to implement step-by-step training programs, which simulate various work scenarios involving long PPE use. To manage occupational health risks more effectively, it is also necessary to conduct regular medical screening including estimation of external respiration. Its results can be employed to identify a risk group as regards hypoxia development; to train healthcare workers to self-diagnose early symptoms of oxygen starvation such as tachycardia, dizziness, or cognitive dysfunction; as well as to develop personalized recommendations on PPE use, which consider individual adaptation resources of healthcare workers.

Therefore, for multidisciplinary hospitals, it is important to transform measures taken during pandemics into a system for continuous training and preventive medical control. Implementation of such measures in periods between pandemics will help create sufficient preparedness to future epidemic challenges associated with the necessity to use personal protective equipment for a long time.

Funding. The research was conducted within accomplishing the task 'To Develop and Implement the Method for Managing Occupational Health Risks for Healthcare Workers in In-Patient Hospitals during an Epidemic Rise in Prevalence of Respiratory Infections', the sub-program called 'Safety of the Environments for Population' of the state scientific-technical program called 'Scientific and Technical Support for Providing Quality and Availability of Healthcare' for 2021–2025, the Ministry of Health of the Republic of Belarus being the chief customer.

Competing interests. The authors declare no competing interests.

References

1. Drawing light from the pandemic: a new strategy for health and sustainable development – A review of the evidence. In: M. McKee ed. *World Health Organization. Regional Office for Europe*. Available at: https://www.who.int/europe/publications/i/item/9789289051798 (April 14, 2025).

2. European health report 2024: keeping health high on the agenda: highlights. *World Health Organization, European Region*. Copenhagen, World Health Organization, Regional Office for Europe, 2025.

3. How coronavirus disease has changed the environment and health landscape: a policy brief. *World Health Organization, European Region*. Copenhagen, World Health Organization, Regional Office for Europe, 2023.

4. Gorblyansky Y.Y., Kontorovich E.P., Ponamareva O.P., Volynskaya E.I., Krishchenko V.N. Psychosocial occupational factors and the risk of health issues in healthcare workers. *Yuzhno-Rossiiskii zhurnal terapevticheskoi praktiki*, 2020, vol. 1, no. 3, pp. 27–36. DOI: 10.21886/2712-8156-2020-1-3-27-36 (in Russian).

5. Karamova L.M., Valeeva E.T., Vlasova N.V., Galimova R.R., Basharova G.R. Analysis of occupational risk factors causing diseases of the circulatory system in medical workers: literature review. *Health Risk Analysis*, 2021, no. 4, pp. 171–177. DOI: 10.21668/health.risk/2021.4.19.eng

6. Smirnova S.S., Egorov I.A., Zhuikov N.N., Vyatkina L.G., Kharitonov A.N., Semenov A.V., Morova O.V. Risks of becoming infected with SARS-CoV-2 for medical personnel in a large industrial city during the pandemic: comparative assessment. *Health Risk Analysis*, 2022, no. 2, pp. 139–150. DOI: 10.21668/health.risk/2022.2.13.eng

7. Hutsich K.A., Kosiachenko G.E., Sychik S.I., Nikalayeva E.A., Madeksha I.V. Subjective assessment of occupational risk factors for health and psychoemotional state of health care workers under changed working conditions during the COVID-19 pandemic. *Health Risk Analysis*, 2023, no. 3, pp. 98–108. DOI: 10.21668/health.risk/2023.3.11.eng

8. Kuzmina L.P., Leskina L.M., Golovkova N.P., Tolmachev D.A., Izmerova N.I., Osmanova P.Sh. The risk of COVID-19 infection in medical workers of a clinical hospital during a pandemic. *Meditsina truda i promyshlennaya ekologiya*, 2024, vol. 64, no. 11, pp. 748–754. DOI: 10.31089/1026-9428-2024-64-11-748-754 (in Russian).

9. Yu J., Goldminz A., Chisolm S., Jacob S.E., Zippin J.H., Wu P.A., Hylwa S., Dunnick C.A. [et al.]. Facial personal protective equipment: materials, resterilization methods, and management of occupation-related dermatoses. *Dermatitis*, 2021, vol. 32, no. 2, pp. 78–85. DOI: 10.1097/DER.0000000000000699

10. Tang H., Wang H., Hamblin M.R., Jiang L., Zhou Y., Xu Y., Wen X. Contact dermatitis caused by prevention measures during the COVID-19 pandemic: a narrative review. *Front. Public Health*, 2023, vol. 11, pp. 1189190. DOI: 10.3389/fpubh.2023.1189190

11. Manookian A., Dehghan Nayeri N., Shahmari M. Physical problems of prolonged use of personal protective equipment during the COVID-19 pandemic: a scoping review. *Nurs. Forum*, 2022, vol. 57, no. 5, pp. 874–884. DOI: 10.1111/nuf.12735

12. Zarei N., Negarandeh R., Eghbali M. Prevalence of headaches in healthcare workers while using personal protective equipment during the COVID-19 pandemic: a systematic review and meta-analysis. *BMJ Open*, 2024, vol. 14, no. 5, pp. e074596. DOI: 10.1136/bmjopen-2023-074596.

13. Batov V.E., Kuznetsov S.M. Assessment of personal protective equipment for medical personnel during a new coronavirus pandemic. *Izvestiya Rossiiskoi voenno-meditsinskoi akademii*, 2022, vol. 41, no. 1, pp. 77–82. DOI: 10.17816/rmmar84027 (in Russian).

14. Figi C.E., Herstein J.J., Beam E.L., Le A.B., Hewlett A.L., Lawler J.V., Lowe J.J., Gibbs S.G. Literature review of physiological strain of personal protective equipment on personnel in the high-consequence infectious disease isolation environment. *Am. J. Infect. Control*, 2023, vol. 51, no. 12, pp. 1384–1391. DOI: 10.1016/j.ajic.2023.05.005

15. Houghton C., Meskell P., Delaney H., Smalle M., Glenton C., Booth A., Chan X.H.S., Devane D., Biesty L.M. Barriers and facilitators to healthcare workers' adherence with infection prevention and control (IPC) guidelines for respiratory infectious diseases: a rapid qualitative evidence synthesis. *Cochrane Database Syst. Rev.*, 2020, vol. 4, no. 4, pp. CD013582. DOI: 10.1002/14651858.CD013582

16. Cattelan A.M., Sasset L., Di Meco E., Cocchio S., Barbaro F., Cavinato S., Gardin S., Carretta G. [et al.]. An integrated strategy for the prevention of SARS-CoV-2 infection in healthcare workers: a prospective observational study. *Int. J. Environ. Res. Public Health*, 2020, vol. 17, no. 16, pp. 5785. DOI: 10.3390/ijerph17165785

17. George J., Shafqat N., Verma R., Patidar A.B. Factors influencing compliance with Personal Protective Equipment (PPE) use among healthcare workers. *Cureus*, 2023, vol. 15, no. 2, pp. e35269. DOI: 10.7759/cureus.35269

18. Brooks S.K., Greenberg N., Wessely S., Rubin G.J. Factors affecting healthcare workers' compliance with social and behavioural infection control measures during emerging infectious disease outbreaks: rapid evidence review. *BMJ Open*, 2021, vol. 11, no. 8, pp. e049857. DOI: 10.1136/bmjopen-2021-049857

19. Vechorko V.I., Averkov O.V., Silaev B.V., Zhenina E.A., Gorbachyova V.A., Shapsigova O.A., Tan'shina O.V., Gumenyuk S.A. Reprofiling of a multispecialty hospital into an infectious hospital and the work under coronavirus pandemic conditions. *Diagnostika i lechenie novoi koronavirusnoi infektsii. Organizatsiya raboty v usloviyakh mnogoprofil'nogo statsionara: rukovodstvo dlya vrachei [Diagnosis and treatment of the new coronavirus infection. Organization of work in a multidisciplinary hospital: a guide for doctors]*. In: V.I. Vechorko ed. Moscow, Praktika Publ., 2020, pp. 25–40 (in Russian).

20. Nikitin A.E., Znamenskiy I.A., Shikhova Yu.A., Kuzmina I.V., Melchenko D.S., Aleshenko N.L., Korvyakov S.A., Sozykin A.V. [et al.]. Reorganization of a multi-specialty hospital in an unfavorable epidemiological situation. *Mediko-farmatsevticheskii zhurnal «Pul's»*, 2020, vol. 22, no. 10, pp. 43–47. DOI: 10.26787/nydha-2686-6838-2020-22-10-43-47 (in Russian).

21. Suzuki S. Conditional relative odds ratio and comparison of accuracy of diagnostic tests based on 2 × 2 tables. *J. Epidemiol.*, 2006, vol. 16, no. 4, pp. 145–153. DOI: 10.2188/jea.16.145

Hutsich K.A., Itpayeva-Liudchyk S.L., Nikalayeva K.A., Madeksha I.V. Assessment of relative risk of discomfort and its subjective perception associated with personal protective equipment: adaptation differences among staff in infectious disease and multidisciplinary hospitals during the COVID-19 pandemic. Health Risk Analysis, 2025, no. 2, pp. 145–154. DOI: 10.21668/health.risk/2025.2.12.eng

Received: 17.03.2025
Approved: 25.04.2025
Accepted for publication: 14.06.2025

UDC 614.2
DOI: 10.21668/health.risk/2025.2.13.eng

Read online

Research article

MANAGING THE RISK OF LOW JOB SATISFACTION AND PROFESSIONAL BURNOUT OF GENERAL PRACTITIONERS

A.B. Zudin, M.A. Kuznetsova, T.P. Vasilyeva

N.A. Semashko National Research Institute of Public Health, 12 Vorontsovo Pole St., build. 1, Moscow, 105064, Russian Federation

Increased work requirements accompanied by a long period of continuous exposure are the most common predictors of burnout syndrome among healthcare workers. The great variability of Russian and foreign data on prevalence of burnout among healthcare workers indicates some unaccounted factors determining different levels of the studied phenomenon as well as unresolved evaluation and technological approaches to its early prevention within healthcare personnel management.

The aim of the study was to create a model for predicting and managing the risks of occupational burnout among general practitioners. A survey was conducted among general practitioners (n = 340) employed at healthcare institutions in Moscow in the period from 2022 to 2023. The survey relied on using the Russian version of the international psychosocial questionnaire COPSOQ III (Long version) adapted for healthcare workers. Burnout levels in doctors with low job satisfaction were determined with reliability p ≤ 0.05 by the Kruskal – Wallis test. Prediction was calculated by using linear regression analysis; models of qualitative target variables were calculated using the Decision Tree method. Relative risks and odds ratio (95 % CI) were calculated as a quantitative measure of effects.

Statistically significant differences per 38 psychosocial factors were confirmed at p < 0.0001. In a representative sample of doctors with low job satisfaction, those with the high level of burnout accounted for 1.72 %; 'Norm', 43.10 %; 'Low', 55.18 %. On the example of the Decision Tree model, the study described an algorithm for managing evaluation parameters of low job satisfaction, which was significant for managing risks of occupational burnout in general practitioners and depended on intra-organizational psychosocial factors 'Uncertainty over Working Conditions', 'Work Life Conflicts' and 'Depressive symptoms' and contributed to an increase up to 80 % or decrease down to 3.0 % depending on their impact in an occupational environment.

The study findings substantiate the fact that an increase in medical and social effectiveness of healthcare workers can be based on employing developed organizational technologies for preventing critical levels of low job satisfaction and occupational burnout in general practitioners as well as declining quality of rendered healthcare services. The risk management algorithm offers to consider levels of job dissatisfaction, occupational burnout and the factors with the greatest influence of psychosocial working conditions and individual traits of general practitioners.

***Keywords:** burnout, job satisfaction, psychosocial risk factors, general practitioners, prognosis, risk management models, human resources, evaluation prevention technologies.*

The World Health Organization (WHO) conceptualizes burnout as a syndrome resulting from chronic workplace stress that has not been successfully managed[1]. According to the International statistical classification of diseases and related health problems, eleventh revision (ICD-11)[2], three symptoms define the entity: (i) feelings of energy depletion or exhaustion; (ii) increased mental distance from one's job or feelings of negativism or cynicism towards one's job; and (iii) a sense of ineffectiveness and lack of accomplishment. The ICD-11 includes burnout among the factors influencing health status or contact with health

Alexander B. Zudin – Doctor of Medical Sciences, director (e-mail: info@nriph.ru; ORCID: http://orcid.org/0000-0002-6966-5559).

Maria A. Kuznetsova – Junior Researcher (e-mail: mascha.kuznetsova@yandex.ru; tel.: +7 (926) 878-06-67; ORCID: http://orcid.org/0000-0001-8243-5902).

Tatyana P. Vasilyeva – Doctor of Medical Sciences, Professor, Chief Researcher (e-mail: vasileva_tp@mail.ru; ORCID: http://orcid.org/0000-0003-4831-1783).

[1] Burnout an «occupational phenomenon»: International Classification of Diseases. *World Health Organization*, 2019. Available at: https://www.who.int/news/item/28-05-2019-burn-out-an-occupational-phenomenon-international-classification-of-diseases (February 27, 2025).

[2] ICD-11 for Mortality and Morbidity Statistics. QD85 Burnout. *WHO*. Available at: https://icd.who.int/browse/2025-01/mms/en#129180281 (February 27, 2025).

services, which is classified as an occupational phenomenon and not as a medical condition [1, 2]. Burnout is a serious global issue among general practitioners (GPs). According to many estimates, high prevalence of burnout among doctors varies between 6 and 33 % across the globe [3]. Some studies report the level of emotional exhaustion among doctors to vary between 37 and 88.6 %; depersonalization, between 28 and 82.8 %; it is within the range between 19.6 and 29.28 according to doctors' self-esteems [3, 4].

Healthcare workers are established to be especially susceptible to burnout due to long periods of intense work and close contacts with other people [5, 6].

Burnout can create elevated risks of medical errors involving potential damage to patient safety and is associated with developing negative outcomes including cynicism, exhaustion, and depression [7, 8]. Some studies report burnout to be associated with such medical conditions as depression, anxiety, sleep disorders, cognitive disorders, cardiovascular diseases, and metabolic disorders[3] [9].

Therefore, burnout should be considered a serious healthcare challenge, which should be investigated and assessed for developing and implementing relevant preventive measures and, if necessary, medical interventions [10–12].

At the same time, high prevalence of burnout among doctors is known to increase financial burdens for the healthcare system due to low labor productivity, absences from work, change of a specialty or even an occupation, that is, loss of human resources [13–15].

Preservation of human resources and doctors' occupational efficiency is vital; given that, mitigation of intra-organizational and individual occupational risk factors is an effective strategy for managing human resources and occupational factors determining their well-being.

Given all the above stated, **the aim of this study** was to create a model for predicting psychosocial risks of low job satisfaction and occupational burnout among general practitioners for scientific substantiation of a thesis that intra-organizational processes in healthcare institutions aimed at their prevention were quite manageable.

Materials and methods. Within this study, correlation and regression analysis were conducted, relative risk (*RR*) and odds ratio (*OR*) were calculated using the international questionnaire COPSOQ III (Long version) to establish cause-effect relations between predictors of low job satisfaction and occupational burnout among GPs. Psychosocial risks of low job satisfaction and burnout were analyzed based on calculating the cutoff points of the upper bound of the inter-quartile interval – 75 % and higher (Q3 >) (the interquartile range or IQR). To check the test accuracy, specificity (*ST*) and sensitivity (*Se*) were determined per each risk factor. We employed the analysis of binary indicators of risk classes using the Decision Tree method. Parameters of error detection tests made for ratios between multiple factors were analyzed using ROC-curves. Initial data were analyzed with Statistica 10 software package using qualitative and quantitative statistics methods.

The study was approved at a meeting of the Independent Ethics Committee of the N.A. Semashko's National Research Institute of Public Health (Protocol No. 2 dated May 17, 2022). Informed consent was received from all participants.

Results and discussion. Factors determining different levels of such indicators as 'Job Satisfaction' and 'Burnout' in GPs were investigated relying on 37 psychosocial factors included in the COSPOQ III (long version) (Table 1).

'Burnout' was shown to have a close interrelation with all factors determining 'Job Satisfaction'. A direct proportional interrelation was established between this indicator and 15 significant factors influencing development of burnout among doctors (Quantitative

[3] Melamed S., Ugarten U., Shirom A., Kahana L., Lerman Y., Froom P. Chronic burnout, somatic arousal and elevated salivary cortisol levels. *J. Psychosom. Res.*, 1999, vol. 46, no. 6, pp. 591–598. DOI: 10.1016/s0022-3999(99)00007-0

Table 1

Interrelations between psychosocial risk factors, 'Job Satisfaction' and 'Burnout'

Psychosocial factors	Job satisfaction	Burnout
Quantitative demands	-0.46**	0.39**
Work pace	-0.09	0.31**
Cognitive demands	-0.08	0.33**
Emotional demands	-0.28**	0.43**
Demands for hiding emotions	-0.17**	0.24**
Influence at work	0.34**	-0.27**
Possibilities for development	0.42**	-0.28**
Variation of work	0.17**	-0.28**
Control over working time	0.28**	-0.37**
Meaning of work	0.10	0.13*
Predictability	0.43**	-0.16**
Recognition	0.66**	-0.46**
Role clarity	0.51**	-0.22**
Role conflicts	-0.51**	0.32**
Illegitimate tasks	-0.53**	0.41**
Quality of leadership	0.68**	-0.47**
Social support from supervisors	0.58**	-0.47**
Social support from colleagues	0.46**	-0.38**
Sense of community at work	0.53**	-0.37**
Commitment to the workplace	0.7**	-0.45**
Work engagement	0.44**	-0.16**
Job insecurity	-0.2**	0.28**
Insecurity over working conditions	-0.34**	0.37**
Quality of work	0.48**	-0.2**
Job satisfaction	1	-0.5**
Work life conflicts	-0.35**	0.34**
Horizontal trust	0.22**	-0.17**
Vertical trust	0.49**	-0.3**
Organizational justice	0.67**	-0.56**
Health	0.34**	-0.34**
Self-rated health	0.38**	-0.44**
Sleeping troubles	-0.47**	0.69**
Burnout	-0.5**	1
Stress	-0.5**	0.65**
Somatic stress	-0.42**	0.59**
Cognitive stress	-0.48**	0.5**
Depressive symptoms	-0.57**	0.56**
Self-efficacy	0.38**	-0.29**

Note: * $p = 0.05$; ** $p = 0.01$.

Demands, Work Pace, Cognitive Demands, Emotional Demands, Demands for Hiding Emotions, Meaning of Work, Role Conflicts, Job Insecurity, Insecurity over Working Conditions, Work Life Conflicts, Sleeping Troubles, Stress, Somatic Stress, Cognitive Stress, Depressive Symptoms); an inverse relation was established for 21 factors with insignificant influence (labeled with the minus in the list) in healthcare institutions in Moscow ($p \leq 0.01$).

Based on the interquartile range, three groups were created with different levels of influence exerted by the analyzed psychosocial factors on 'Burnout': 'Low' (n = 77), 'Norm' (n = 205), and 'High' (n = 58) with substantiation provided for authentic differences between them (Table 2).

Table 2

Comparison between three groups for 'Burnout' per psychosocial factors

Psychosocial factors	M ± S (*N* = 77) Low	M ± S (*N* = 205) Norm	M ± S (*N* = 58) High	*P-level*			
				df = 2	Low – Norm	Low – High	Norm – High
Quantitative demands	143.18 ± 68.90	183.54 ± 65.54	214.22 ± 56.02	<0.0001	<0.0001	<0.0001	0.0097
Work pace	197.86 ± 75.70	241.05 ± 48.42	250.81 ± 56.53	<0.0001	0.0001	<0.0001	0.2389
Cognitive demands	289.52 ± 67.62	314.94 ± 54.28	340.52 ± 47.49	<0.0001	0.0192	<0.0001	0.0033
Emotional demands	165.74 ± 65.97	219.48 ± 47.80	238.53 ± 57.27	<0.0001	<0.0001	<0.0001	0.0478
Demands for hiding emotions	253.57 ± 77.56	281.10 ± 54.62	290.95 ± 48.15	0.0010	0.0102	0.0029	0.4425
Influence at work	293.51 ± 110.50	223.66 ± 66.25	225.00 ± 72.55	<0.0001	<0.0001	0.0004	0.9987
Possibilities for development	219.48 ± 50.27	195.49 ± 37.47	183.62 ± 34.90	<0.0001	0.0011	<0.0001	0.0959
Variation of work	81.17 ± 31.97	68.54 ± 25.92	62.50 ± 27.41	<0.0001	0.0017	0.0002	0.2939
Control over working time	164.94 ± 109.29	103.05 ± 69.44	84.91 ± 78.49	<0.0001	<0.0001	<0.0001	0.0490
Meaning of work	172.40 ± 35.02	178.66 ± 26.17	182.33 ± 25.22	0.2165	0.6160	0.2792	0.6170
Predictability	155.84 ± 42.32	149.76 ± 34.30	140.52 ± 28.42	0.0087	0.2696	0.0148	0.1530
Recognition	213.96 ± 52.64	175.85 ± 39.40	150.86 ± 40.00	<0.0001	<0.0001	<0.0001	0.0030
Role clarity	265.58 ± 38.05	246.83 ± 35.04	241.81 ± 31.20	<0.0001	0.0010	0.0012	0.6458
Role conflicts	69.48 ± 42.07	92.32 ± 36.95	103.02 ± 35.69	<0.0001	0.0003	<0.0001	0.0931
Illegitimate tasks	46.10 ± 29.54	70.00 ± 22.33	72.84 ± 21.60	<0.0001	<0.0001	<0.0001	0.5964
Quality of leadership	268.18 ± 80.67	217.44 ± 65.15	164.66 ± 61.96	<0.0001	<0.0001	<0.0001	<0.0001
Social support from supervisors	190.58 ± 62.44	148.17 ± 41.64	112.07 ± 44.73	<0.0001	<0.0001	<0.0001	<0.0001
Social support from colleagues	182.79 ± 56.52	150.49 ± 40.82	130.17 ± 46.54	<0.0001	<0.0001	<0.0001	0.0353
Sense of community at work	250.00 ± 41.16	215.98 ± 35.58	205.17 ± 34.01	<0.0001	<0.0001	<0.0001	0.1871
Commitment to the workplace	352.60 ± 100.62	277.32 ± 79.58	225.00 ± 87.61	<0.0001	<0.0001	<0.0001	0.0006
Work engagement	221.43 ± 51.89	212.56 ± 36.93	196.98 ± 40.31	0.0032	0.3179	0.0044	0.0446
Job insecurity	181.49 ± 81.15	220.98 ± 50.72	234.05 ± 53.33	<0.0001	0.0028	0.0001	0.1592
Insecurity over working conditions	242.53 ± 111.22	342.07 ± 82.14	346.98 ±100.34	<0.0001	<0.0001	<0.0001	0.9179
Quality of work	158.12 ± 31.53	154.51 ± 27.25	141.81 ± 29.01	0.0026	0.6300	0.0125	0.0343
Job satisfaction	343.18 ± 59.69	283.90 ± 61.06	242.24 ± 58.90	<0.0001	<0.0001	<0.0001	<0.0001

End of the Table 2

Psychosocial factors	M ± S (*N* = 77) Low	M ± S (*N* = 205) Norm	M ± S (*N* = 58) High	*P-level*			
				df = 2	Low – Norm	Low – High	Norm – High
Work life conflicts	188.64 ± 106.57	280.00 ± 91.87	276.72 ±119.56	<0.0001	<0.0001	<0.0001	0.9776
Horizontal trust	162.01 ± 63.43	142.80 ± 40.83	126.29 ± 32.92	0.0202	0.7259	0.0360	0.0718
Vertical trust	256.82 ± 67.94	225.61 ± 51.54	207.33 ± 46.36	<0.0001	0.0001	<0.0001	0.1060
Organizational justice	246.10 ± 67.26	174.27 ± 64.45	119.40 ± 56.01	<0.0001	<0.0001	<0.0001	<0.0001
Health	68.18 ± 22.45	61.59 ± 19.72	46.55 ± 18.99	<0.0001	0.1004	<0.0001	<0.0001
Self-rated health	8.17 ± 1.39	7.48 ± 1.53	6.24 ± 1.30	<0.0001	0.0055	<0.0001	<0.0001
Sleeping troubles	90.26 ± 55.33	173.17 ± 66.14	255.60 ± 72.55	<0.0001	<0.0001	<0.0001	<0.0001
Burnout	118.51 ± 45.78	243.05 ± 36.93	349.57 ± 28.29	<0.0001	<0.0001	<0.0001	<0.0001
Stress	79.55 ± 40.50	125.61 ± 47.00	181.47 ± 38.78	<0.0001	<0.0001	<0.0001	<0.0001
Somatic stress	59.09 ± 46.40	113.29 ± 60.56	176.29 ± 65.80	<0.0001	<0.0001	<0.0001	<0.0001
Cognitive stress	37.66 ± 46.70	84.15 ± 58.46	117.24 ± 55.84	<0.0001	<0.0001	<0.0001	0.0010
Depressive symptoms	48.05 ± 56.16	103.54 ± 58.60	163.79 ± 63.38	<0.0001	<0.0001	<0.0001	<0.0001
Self-efficacy	406.18 ± 136.01	364.04 ±114.26	298.14 ± 96.36	<0.0001	0.0499	<0.0001	0.0006

Table 3

Intergroup comparisons of 'Burnout' and 'Job Satisfaction' levels in GPs

Level of Job Satisfaction among GPs	Level of Burnout among GPs			*p*, (*df* = 4)
	Low (*N* = 77)	Norm (*N* = 205)	High (*N* = 58)	
Norm	47 (61.04 %)	142 (69.27 %)	25 (43.10 %)	< 0.0001
Low	3 (3.90 %)	42 (20.49 %)	32 (55.17 %)	
High	27 (35.06 %)	21 (10.24 %)	1 (1.72 %)	

Bearing in mind that 'Burnout' was authentically associated with 'Job Satisfaction' among general practitioners, we analyzed and established authentic correlations between these two indicators in the comparison groups 'Low', 'Norm' and 'High' levels (Table 3). The results are visualized in Figure 1.

Regression analysis with 'Job Satisfaction' as the leading factor and 'Burnout' as the dependent one was conducted based on the correlation between 'Job Satisfaction' and 'Burnout' (r = -0.5, $p < 0.0001$) (Figure 2).

'Burnout' was shown to decline on average by 0.59 scores as 'Job Satisfaction' grew; the regression model was able to explain 24.1 % of this dispersion ($R^2 = 24.1$, $p < 0.0001$).

The established data with the confirmed authentic correlation between 'Burnout' and 'Jib Satisfaction' factors among GPs were additionally used in multifactorial analysis to establish significance ranks of factors determining low job satisfaction among GPs (Table 4).

The analysis reliably established 35 factors and their values that increased the risk of low job satisfaction among GPs.

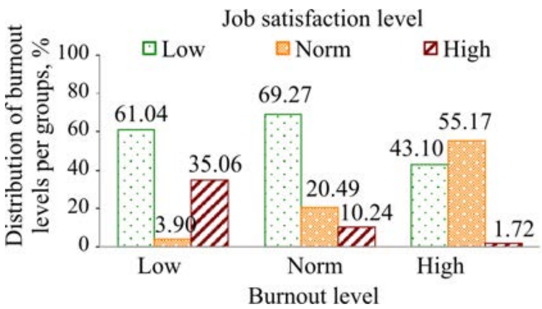

Figure 1. Distribution of 'Job Satisfaction' levels in 'Burnout' groups

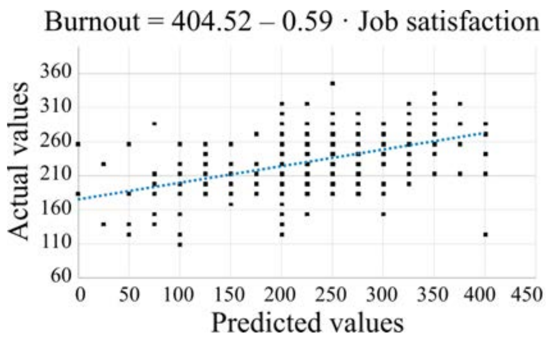

Figure 2. An average change in 'Burnout' when 'Job Satisfaction' grows

Table 4

Distribution of psychosocial factors of low job satisfaction

Psychosocial factors	*RR* (95 % CI)	*OR* (95 % CI)	AuROC	*Se*, %	*Sp*, %	*p* (*df* = 1)
Burnout ≥ 250.0	22.48 (9.41; 53.72)	56.70 (22.34; 43.87)	0.88	96.27	68.73	<0.0001
Depressive symptoms ≥ 125.0	8.31 (5.04; 13.68)	20.21 (11.08; 36.88)	0.88	88.81	71.81	<0.0001
Stress ≥ 150.0	5.67 (3.75; 8.55)	12.73 (7.49; 21.64)	0.86	83.58	71.43	<0.0001
Sleeping troubles ≥ 200.0	6.08 (3.91; 9.48)	13.30 (7.66; 23.10)	0.84	85.82	68.73	<0.0001
Cognitive stress ≥ 100.0	5.85 (3.80; 9.01)	12.75 (7.41; 21.96)	0.83	85.07	69.11	<0.0001
Job satisfaction< 275.0	4.4 (3.11; 6.24)	9.72 (5.96; 15.85)	0.83	76.87	74.52	<0.0001
Insecurity over working conditions ≥ 375.0	3.94 (2.82; 5.50)	8.26 (5.12; 13.32)	0.84	74.63	73.75	<0.0001
Organizational justice < 150.0	3.58 (2.65; 4.83)	7.85 (4.91; 12.55)	0.83	67.91	78.76	<0.0001
Quality of leadership < 200.0	3.61 (2.65; 4.92)	7.63 (4.78; 12.18)	0.78	70.15	76.45	<0.0001
Illegitimate tasks ≥ 75.0	8.81 (4.45; 17.47)	16.63 (7.81; 35.37)	0.81	94.03	51.35	<0.0001
Emotional demands ≥ 225.0	6.48 (3.71; 11.32)	12.15 (6.40; 23.07)	0.85	91.04	54.44	<0.0001
Work life conflicts ≥ 275.0	3.77 (2.61; 5.43)	6.99 (4.29; 11.39)	0.77	79.10	64.86	<0.0001
Quantitative demands ≥ 200.0	4.08 (2.73; 6.10)	7.43 (4.44; 12.41)	0.81	82.84	60.62	<0.0001
Recognition < 175.0	3.32 (2.40; 4.60)	6.20 (3.90; 9.87)	0.80	73.13	69.50	<0.0001
Somatic stress ≥ 100.0	5.63 (3.23; 9.81)	9.93 (5.23; 18.86)	0.81	91.04	49.42	<0.0001
Role conflicts ≥ 100.0	5.4 (3.03; 9.64)	9.21 (4.74; 17.89)	0.78	91.79	45.17	<0.0001
Job insecurity ≥ 225.0	5.39 (2.93; 9.88)	9.01 (4.52; 17.96)	0.80	92.54	42.08	<0.0001
Commitment to the workplace < 250.0	2.61 (1.99; 3.43)	4.79 (3.05; 7.52)	0.76	57.46	77.99	<0.0001
Work pace ≥ 250.0	2.86 (2.07; 3.95)	4.84 (3.07; 7.64)	0.75	72.39	64.86	<0.0001

End of the Table 4

Psychosocial factors	*RR* (95 % CI)	*OR* (95 % CI)	AuROC	*Se*, %	*Sp*, %	*p* (*df* = 1)
Social support from supervisors < 150.0	2.64 (1.99; 3.50)	4.66 (2.99; 7.29)	0.77	61.94	74.13	<0.0001
Sense of community at work < 225.0	2.66 (1.94; 3.64)	4.35 (2.77; 6.83)	0.75	70.90	64.09	<0.0001
Control over working time < 75.0	2.27 (1.73; 2.98)	3.72 (2.39; 5.78)	0.73	55.97	74.52	<0.0001
Demands for hiding emotions ≥ 300.0	2.39 (1.76; 3.25)	3.72 (2.39; 5.80)	0.73	68.66	62.93	<0.0001
Cognitive demands ≥ 325.0	2.62 (1.83; 3.75)	4.00 (2.48; 6.46)	0.74	78.36	52.51	<0.0001
Social support from colleagues < 150.0	2.25 (1.71; 2.98)	3.59 (2.32; 5.56)	0.73	58.96	71.43	<0.0001
Self-rated health < 8.0	2.26 (1.68; 3.04)	3.47 (2.24; 5.38)	0.67	65.67	64.48	<0.0001
Self-efficacy < 402.0	2.25 (1.62; 3.13)	3.28 (2.08; 5.17)	0.69	73.88	53.67	<0.0001
Vertical trust < 225.0	2.01 (1.50; 2.69)	2.90 (1.88; 4.46)	0.69	63.43	62.55	<0.0001
Health < 75.0	1.87 (1.40; 2.50)	2.59 (1.68; 3.98)	0.63	63.43	59.85	<0.0001
Quality of work < 150.0	1.83 (1.40; 2.40)	2.81 (1.67; 4.74)	0.68	29.10	87.26	<0.0001
Possibilities for development < 200.0	1.72 (1.30; 2.27)	2.28 (1.49; 3.50)	0.67	58.96	61.39	0.0001
Influence at work < 225.0	1.72 (1.29; 2.28)	2.28 (1.49; 3.49)	0.68	59.70	60.62	0.0001
Role clarity < 275.0	1.89 (1.33; 2.70)	2.51 (1.55; 4.05)	0.63	78.36	40.93	0.0001
Variation of work < 75.0	1.46 (1.11; 1.92)	1.79 (1.17; 2.72)	0.60	53.73	60.62	0.0066
Work engagement < 225.0	1.36 (1.03; 1.79)	1.59 (1.05; 2.42)	0.59	55.97	55.60	0.0296
Predictability < 150.0	1.3 (0.97; 1.72)	1.50 (0.95; 2.38)	0.62	32.09	76.06	0.0834
Meaning of work ≥ 200.0	1.19 (0.90; 1.57)	1.30 (0.85; 1.98)	0.58	56.72	49.81	0.2196
Horizontal trust < 150.0	0.98 (0.74; 1.30)	0.97 (0.64; 1.48)	0.59	41.79	57.53	0.8971

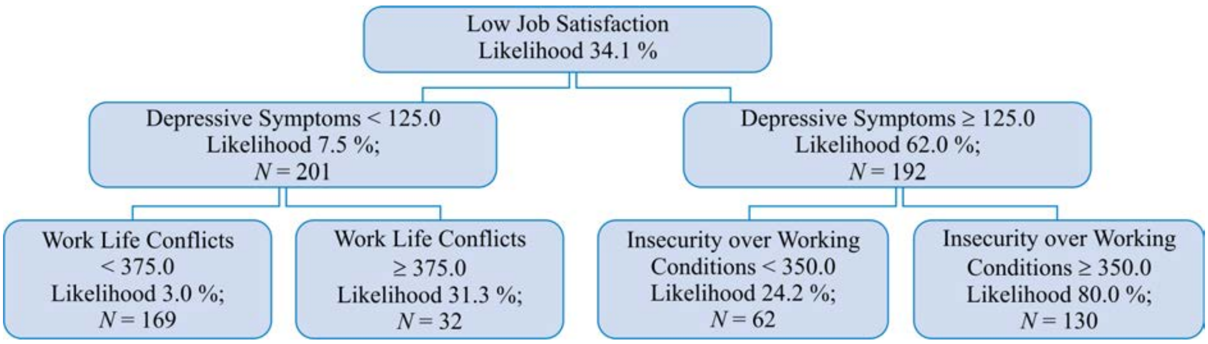

Figure 3. Risk classes in 'Low Job Satisfaction' group of GPs

Table 5

Error detection tests to check quality of the Decision Tree model per risk classes of low job satisfaction among GP

Indicator	Values
Cutoff point	24.2 %
AuROC	0.92
Sensitivity	95.9 %
Specificity	77.4 %
Effectiveness	86.6 %
Positive Predictive Value	70.7 %
Negative Predictive Value	97.0 %

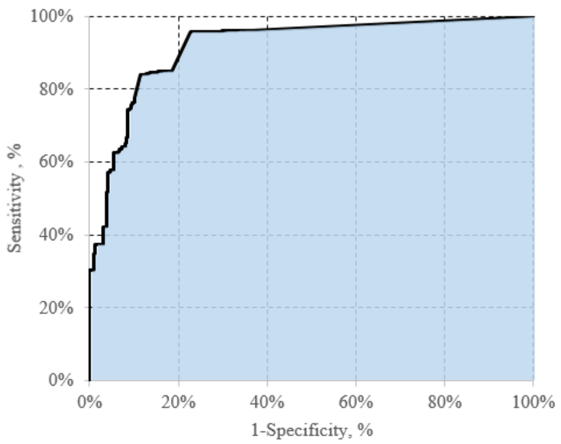

Figure 4. ROC-graph of the Decision Tree model per the calculated parameters of factors determining low job satisfaction among GPs

Using the Decision Tree model as an example, we created an algorithm for predicting low job satisfaction among GPs depending on parameters of risk classes 'Depressive Symptoms' determined by 'Work Life Conflicts' and 'Insecurity over Working Conditions' (Figure 3).

As shown by the Decision Tree model, different values of parameters of risk classes result in changes of likelihood of low job satisfaction among GPs. Thus, if 'Depressive Symptoms' factor value is (≥) 125.0 scores and 'Insecurity over Working Conditions' factor value is (≥) 350.0 scores, then likelihood of 'Low Job Satisfaction' grows up to 80 %.

When 'Depressive Symptoms' factor value is (<) 125.0 scores and 'Work Life Conflicts' factor value is (<) 375.0 scores, likelihood of 'Low Job Satisfaction' goes down to 3.0 %.

Error detection tests were conducted to check quality of the Decision Tree model per risk classes of low job satisfaction among GP. They were characterized with high sensitivity equal to 95.9 % (Table 5).

The following ROC-graph visualizes the conducted error detection tests for the Decision Tree model of low job satisfaction among GPs (Figure 4).

The obtained data made it possible to substantiate low job satisfaction among up to 34.1 % of general practitioners who rendered primary medical and sanitary aid to population in healthcare institutions in Moscow (M.A. Kuznetzova with colleagues, 2024) [16, 17]. Given that, it is quite justifiable to assert that the extreme job dissatisfaction is a reliable predictor of occupational burnout.

Our findings are also consistent with results reported in other studies (K.E. Erenzhan and others, 2021), where emotional burnout was established in 35 % of general practitioners; of them, 37 % had medium level of burnout and 14 % had high level of burnout [18].

In some studies, effects produced by interventions on burnout and associated symptoms are estimated based on specific stress reduction programs (V. Minichiello et al., 2020) [19]. Other studies (S. Prentice et al., 2025) report effectiveness of psychosocial interventions (for example, a decline in depersonalization or higher personal achievements) just after their implementation (g_w = 0.243, 95 % CI [-0.042; 0.529]) [20]. However, these results were not authentically significant (p = 0.090) for the total sample and a wide prediction range made it possible to assume that in future similar interventions could have an opposite (negative) effect and only strengthen burnout symptoms among doctors [21–24].

The scientific society in general and healthcare administrators in particular are actively discussing actions able to raise doctors' medical and social effectiveness by developing psychosocial strategies aimed at managing burnout symptoms [20, 25, 26]. However, the state of this problematic research area is limited by sampling performed among specialized healthcare branches and still lacks relevant methodical and technological development of management decisions at the stage when a healthcare institution enters the first contact with patients. We have not been able to find available research publications that described predictive models on managing psychosocial risk factors causing low job satisfaction and negative outcomes of occupational burnout among GPs. This calls for further applied research on the subject.

The suggested algorithms for predictions within management of low job satisfaction risks for doctors were taken into account in development and subsequent testing of organizational technologies aimed at preventing risks of declining job satisfaction and growing burnout among GPs. They included the following consequent stages:

– recognizing an existing problem of GPs' low job satisfaction and patients' low satisfaction with quality of healthcare services rendered to them (parallel surveys among GPs (COPSOQ III questionnaire, shot version) and patients (Europep questionnaire));

– creating a schedule of meetings with GPs, where the first meeting with the leadership involved discussing goals and development strategy of healthcare institutions; GPs were offered to create a focus group and present their conceptual plan how to develop their department for discussing intermediate results;

– establishing a 'problem' zone in routine work and ways to correct it;

– developing a KPI (**Key Performance Indicators**) system based on calculating the ratio between the number of received patients and the number of patients satisfied with quality of medical aid, as well as filling in mandatory documents without errors;

– implementing a CRM-system (**Customer Relationship Management**) aimed at simplifying work with patients and documents;

– creating a rest room and organizing psychological relief trainings;

– drawing up a schedule for occupational training for doctors (skills development or seeking an academic degree), developing tutorship for young specialists as well as plans for participating in theoretical and practical conferences and professional competitions.

In addition, short breaks of 5–7 minutes were introduced in work schedules each 1.5 hours; some activities were planned involving team-building trips.

Intermediate results were to be discussed two days a week in 40-minute sessions.

Effectiveness of organizational activities was estimated per 16 psychosocial factors of job satisfaction among GPs; the estimation results showed its growth from 4.54 to 27.28 % over three months; a decline in 'Burnout' amounted to 24.09 % [27].

Our test results are similar to open data obtained by international human resources practices adopted by healthcare institutions. Thus, a study by S. Park et al. (2023) showed that doctors' job satisfaction depended on a management strategy adopted by a healthcare institution (R^2 = 13.67, p < 0.0001) and investments in education and training (R^2 = 7.96, p < 0.0001) [28]. A systemic review by B.A. Clough et al. (2017) comprises data on 23 studies, which indicate that positive effects were created by implementation of unique programs aimed at preventing influence of psychosocial factors of doctors' burnout, from d = 0.08 to d = 1.08 [29]. A later systemic review by P. Catapano et al. (2023) revealed effectiveness of organizational technologies at the individual and organizational levels with use of cognitive-psychological therapy and relaxation techniques [30].

Therefore, the suggested model for predicting risks of low job satisfaction and growing occupational burnout among general practitioners employed in healthcare institutions in Moscow has been created based on selecting, analyzing, and assessing the most significant general and unique factors related to working conditions. It makes it possible to apply a systemic approach to reducing occupational psychosocial burdens for healthcare workers by increasing medical and social effectiveness of activities performed by a healthcare institution.

Conclusion. Using the Decision Tree model as an example, we have shown an algorithm for managing estimated parameters of low job satisfaction among general practitioners; it is significant for managing risks of burnout and depends on intra-organizational psychosocial factors. Occupational factors typical for healthcare institutions in Moscow have been shown to create high scores per such components as 'Insecurity over Working Conditions', 'Work Life Conflicts' and 'Depressive Symptoms'. The developed organization technologies for preventing critical levels of low job satisfactions and burnout were used as a basis to show that intra-organizational occupational conditions were quite manageable. Depending on frequency and intensity of internal interrelations, risks were decreased by up to 3 %.

The suggested risk management algorithm considers the most significant factors related to psychosocial working conditions and GPs' individual peculiarities that determine a decline in medical and social effectiveness of human resources in a healthcare organization.

Funding. The study was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. Bianchi R., Schonfeld I.S. Examining the evidence base for burnout. *Bull. World Health Organ.*, 2023, vol. 101, no. 11, pp. 743–745. DOI: 10.2471/BLT.23.289996

2. Guseva Canu I., Marca S.C., Dell'Oro F., Balázs Á., Bergamaschi E., Besse C., Bianchi R., Bislimovska J. [et al.]. Harmonized definition of occupational burnout: A systematic review, semantic analysis, and Delphi consensus in 29 countries. *Scand. J. Work Environ. Health.*, 2021, vol. 47, no. 2, pp. 95–107. DOI: 10.5271/sjweh.3935

3. Karuna C., Palmer V., Scott A., Gunn J. Prevalence of burnout among GPs: a systematic review and meta-analysis. *Br. J. Gen. Pract.*, 2022, vol. 72, no. 718, pp. e316–e324. DOI: 10.3399/BJGP.2021.0441

4. Shen X., Xu H., Feng J., Ye J., Lu Z., Gan Y. The global prevalence of burnout among general practitioners: a systematic review and meta-analysis. *Fam. Pract.*, 2022, vol. 39, no. 5, pp. 943–950. DOI: 10.1093/fampra/cmab180

5. Jun J., Ojemeni M.M., Kalamani R., Tong J., Crecelius M.L. Relationship between nurse burnout, patient and organizational outcomes: systematic review. *Int. J. Nurs. Stud.*, 2021, vol. 119, pp. 103933. DOI: 10.1016/j.ijnurstu.2021.103933

6. The Lancet. Physician burnout: a global crisis. *Lancet*, 2019, vol. 394, pp. 93. DOI: 10.1016/S0140-6736(19)31573-9

7. Owoc J., Mańczak M., Jabłońska M., Tombarkiewicz M., Olszewski R. Association Between Physician Burnout and Self-reported Errors: Meta-analysis. *J. Patient Saf.*, 2022, vol. 18, no. 1, pp. e180–e188. DOI: 10.1097/PTS.0000000000000724

8. Zaed I., Jaaiddane Y., Chibbaro S., Tinterri B. Burnout Among Neurosurgeons and Residents in Neurosurgery: A Systematic Review and Meta-Analysis of the Literature. *World Neurosurg.*, 2020, vol. 143, pp. e529–e534. DOI: 10.1016/j.wneu.2020.08.005

9. Saleh P., Shapiro C.M. Disturbed sleep and burnout: implications for long-term health. *J. Psychosom. Res.*, 2008, vol. 65, no. 1, pp. 1–3. DOI: 10.1016/j.jpsychores.2008.05.028

10. Azzopardi-Muscat N., Zapata T., Kluge H. Moving from health workforce crisis to health workforce success: the time to act is now. *Lancet Reg. Health Eur.*, 2023, vol. 35, pp. 100765. DOI: 10.1016/j.lanepe.2023.100765

11. Woo T., Ho R., Tang A., Tam W. Global prevalence of burnout symptoms among nurses: a systematic review and meta-analysis. *J. Psychiatr. Res.*, 2020, vol. 123, pp. 9–20. DOI: 10.1016/j.jpsychires.2019.12.015

12. Dubale B.W., Friedman L.E., Chemali Z., Denninger J.W., Mehta D.H., Alem A., Fricchione G.L., Dossett M.L., Gelaye B. Systematic review of burnout among healthcare providers in sub-Saharan Africa. *BMC Public Health*, 2019, vol. 19, no. 1, pp. 1247. DOI: 10.1186/s12889-019-7566-7

13. Fargen K.M., Arthur A.S., Leslie-Mazwi T., Garner R.M., Aschenbrenner C.A., Wolfe S.Q., Ansari S.A., Dabus G. [et al.]. A survey of burnout and professional satisfaction among United States neurointerventionalists. *J. Neurointerv Surg.*, 2019, vol. 11, no. 11, pp. 1100–1104. DOI: 10.1136/neurintsurg-2019-014833

14. Shakir H.J., Cappuzzo J.M., Shallwani H., Kwasnicki A., Bullis C., Wang J., Hess R.M., Levy E.I. Relationship of grit and resilience to burnout among U.S. neurosurgery residents. *World Neurosurg.*, 2020, vol. 134, pp. e224–e236. DOI: 10.1016/j.wneu.2019.10.043

15. Thielmann B., Schwarze R., Böckelmann I. A Systematic Review of Associations and Predictors for Job Satisfaction and Work Engagement in Prehospital Emergency Medical Services – Challenges for the Future. *Int. J. Environ. Res. Public Health*, 2023, vol. 20, no. 5, pp. 4578. DOI: 10.3390/ijerph20054578

16. Kuznetsova M.A., Zudin A.B., Gorbacheva N.A. Consequence analysis: Assessment of psychosocial risk factors for burnout in general practitioners as a predictor of patient dissatisfaction with health care in the post-pandemic era. *ZNiSO*, 2024, vol. 32, no. 9, pp. 83–90. DOI: 10.35627/2219-5238/2024-32-9-83-90 (in Russian).

17. Kuznetsova M.A., Vasilyeva T.P., Zudin A.B., Gruzdeva O.A., Kuznetsova K.Yu. Study of factors influencing psychosocial and professional characteristics of general practitioners (family doctors): validation of the international questionnaire COPSOQ III (Long version). *Zdravookhranenie Rossiiskoi Federatsii*, 2024, vol. 68, no. 4, pp. 315–322. DOI: 10.47470/0044-197X-2024-68-4- 315-322 (in Russian).

18. Yerenzhan K.E., Assukhanova M.M., Dilmagambetova G.S., Moldasheva Z.B., Tleuova A.S. General practitioners' emotional burn-out. *West Kazakhstan Medical Journal*, 2021, no. 2 (63), pp. 117–122. DOI: 10.24412/2707-6180-2021- 63-117-122 (in Russian).

19. Minichiello V., Hayer S., Gillespie B., Goss M., Barrett B. Developing a mindfulness skills-based training program for resident physicians. *Fam. Med.*, 2020, vol. 52, no. 1, pp. 48–52. DOI: 10.22454/FamMed.2020.461348

20. Prentice S., Patel D.N., Dorstyn D.S. Wellbeing interventions in family medicine and general practice trainees: a preliminary meta-analysis. *Educ. Prim. Care*, 2025, vol. 36, no. 3, pp. 82–93. DOI: 10.1080/14739879.2025.2469494

21. Nathan J., McCray L., Feldman N. The text effect: stress management and resiliency training pilot for resident physicians. *Fam. Med.*, 2021, vol. 53, no. 2, pp. 139–144. DOI: 10.22454/FamMed.2021.847102

22. Halista C.E., Dalton H.A., Thornton J.A. Structured curricular change fails to combat resident burnout. *Mil. Med.*, 2024, vol. 189, no. 1–2, pp. 370–373. DOI: 10.1093/milmed/usac188

23. Johnson E., Roth E. Improving physician wellness through electronic health record education. *Int. J. Psychiatry Med.*, 2021, vol. 56, no. 5, pp. 327–333. DOI: 10.1177/00912174211034633

24. Villarreal M., Hanson P., Clarke A., Khan M., Dale J. Feasibility, acceptability and effect of the mindful practice curriculum in postgraduate training of general practitioners. *BMC Med. Educ.*, 2021, vol. 21, no. 1, pp. 327. DOI: 10.1186/s12909-021-02747-z

25. Khalfin R.A., Smolnikova P.S., Stolkova A.S. Burnout among health care workers: a pressing problem for health care management. *Natsional'noe zdravookhranenie*, 2023, vol. 4, no. 2, pp. 40–46. DOI: 10.47093/2713-069X.2023.4.2.40-46 (in Russian).

26. Shaw E., Nunns M., Spicer S.G., Lawal H., Briscoe S., Melendez-Torres G.J., Garside R., Liabo K., Thompson Coon J. What is the volume, quality and characteristics of evidence relating to the effectiveness and cost-effectiveness of multi-disciplinary occupational health interventions aiming to improve work-related outcomes for employed adults? An evidence and gap map of systematic reviews. *Campbell Syst. Rev.*, 2024, vol. 20, no. 2, pp. e1412. DOI: 10.1002/cl2.1412

27. Zudin A.B., Kuznetsova M.A., Vasilyeva T.P., Tyranovets S.V., Kuznetsova K.Yu. The strategic choice of organizational technologies preventing burning out of general practitioners in the system of patient-oriented medicine. *Problemy sotsialnoi gigieny, zdravookhraneniya i istorii meditsiny*, 2024, vol. 32, no. 6, pp. 1234–1242. DOI: 10.32687/0869-866X-2024-32-6-1234-1242 (in Russian).

28. Park S., Kim H.-K., Lee H.-J., Choi M., Lee M., Jakovljevic M. Strategic management and organizational culture of medical device companies in relation to corporate performance. *J. Med. Econ.*, 2023, vol. 26, no. 1, pp. 781–792. DOI: 10.1080/13696998.2023.2224168

29. Clough B.A., March S., Chan R.J., Casey L.M., Phillips R., Ireland M.J. Psychosocial interventions for managing occupational stress and burnout among medical doctors: a systematic review. *Syst. Rev.*, 2017, vol. 6, no. 1, pp. 144. DOI: 10.1186/s13643-017-0526-3

30. Catapano P., Cipolla S., Sampogna G., Perris F., Luciano M., Catapano F., Fiorillo A. Organizational and Individual Interventions for Managing Work-Related Stress in Healthcare Professionals: A Systematic Review. *Medicina (Kaunas)*, 2023, vol. 59, no. 10, pp. 1866. DOI: 10.3390/medicina59101866

Zudin A.B., Kuznetsova M.A., Vasilyeva T.P. Managing the risk of low job satisfaction and professional burnout of general practitioners. Health Risk Analysis, 2025, no. 2, pp. 155–165. DOI: 10.21668/health.risk/2025.2.13.eng

Received: 10.04.2025
Approved: 09.06.2025
Accepted for publication: 26.06.2025

UDC 519.254:519.257
DOI: 10.21668/health.risk/2025.2.14.eng

Read online

Research article

RANDOM FOREST METHOD FOR INTERPRETING RESULTS OBTAINED BY BIOLUMINESCENCE ANALYSIS OF SALIVA IN PERSONALIZED DIAGNOSTICS

G.V. Zhukova[1], P.A. Martyshuk[1], E.R. Afer[1], A.N. Shuvaev[1], N.A. Rozanova[2], D.V. Sergeev[2], V.A. Kratasyuk[1, 3]

[1]Siberian Federal University, 79 Svobodnyi Av., Krasnoyarsk, 660041, Russian Federation
[2]Research Center for Neurology, 80 Volokolamskoe highway, Moscow, 125367, Russian Federation
[3]Institute of Biophysics, Siberian Department of the RAS, separate division of the Federal Research Center Krasnoyarsk Scientific Center of the Siberian Department of the Russian Academy of Sciences, 50 Akademgorodok, build. 50, Krasnoyarsk, 660036, Russian Federation

Development of personalized medicine and biotechnologies is directly linked to obtaining relevant data, which largely depend on individual characteristics of examined patients. Permissible ranges of analyzed indicators that are commonly used in conventional medicine do not always describe a patient's health adequately. It seems necessary to search for such data analysis techniques, which allow considering variable individual peculiarities of patients' bodies and their lifestyles.

The aim of this study is to determine whether it is possible to use the Random Forest method for biomedical data analysis in order to achieve correct interpretation of results obtained by personalized diagnostic tests. Bioluminescent testing is used as an example since it estimates effects produced by various characteristics of examined patients and their living conditions. The method allows minimizing risks of incorrect diagnosis and adjusting monitoring schemes for specific patients.

This study relies on using the results obtained by diagnosing workloads of railway workers using the bioluminescence method. A patient's health is assessed by examining effects produced by a patient's saliva on intensity of the bi-enzyme system luminescence: NAD(P)H:FMN oxidoreductase + luciferase. This analysis is integral and responses to many factors, each of which can influence the analysis result. Effectiveness of various methods for data analysis is assessed on an example group made of traffic controllers employed by the Krasnoyarsk Branch of Russian Railways JSC. Both statistical methods and the Random Forest machine learning algorithm were used for data analysis.

As a result, our study has revealed that it is advisable to use the Random Forest method for assessing significance of some biochemical saliva indicators to predict health of railway workers. The method makes it possible to identify the most significant factors and create graphs to show partial influence exerted by various factors on the target variable. This study allows optimizing the system for health diagnostics using integral bioluminescence analysis. The Random Forest method can become a component of a personalized bioluminescent biosensor for assessing effects produced by stress and workloads on the body.

Keywords: *personalized diagnostics, machine learning, data analysis, multifactorial analysis, saliva, bioluminescence, biosensor, signal systems.*

Galina V. Zhukova – Junior Researcher of Bioluminescent Technologies Laboratory (e-mail: gvivanova@sfu-kras.ru; tel.: +7 (913) 047-88-37; ORCID: https://orcid.org/0000-0002-8646-1224).

Polina A. Martishuk – student of the Institute of Fundamental Biology and Biotechnology (e-mail: p.martishuk@yandex.ru; tel.: + 7 (904) 896-97-34).

Egor R. Afer – student of the Institute of Fundamental Biology and Biotechnology (e-mail: egorafer@yandex.ru; tel.: +7 (902) 957-64-89).

Andrey N. Shuvaev – Candidate of Physical and Mathematical Sciences, Head of the Basic Department of Biomedical Systems and Complexes of the Institute of Fundamental Biology and Biotechnology (e-mail: ashuvaev@sfu-kras.ru; tel.: +7 (950) 997-77-92; ORCID: https://orcid.org/0000-0002-3887-1413).

Natalia A. Rozanova – graduate student, laboratory research assistant at Brain Institute's Neurobiology and Tissue Engineering Laboratory (e-mail: nataliarozanovaa@gmail.com; tel.: +7 (968) 030-47-28; ORCID: https://orcid.org/0000-0001-9619-4679).

Dmitry V. Sergeev – Candidate of Medical Sciences, Senior Researcher, Neurologist (e-mail: sergeev@neurology.ru; tel.: +7 (926) 860-08-97; ORCID: https://orcid.org/0000-0002-9130-1292).

Valentina A. Kratasyuk – Doctor of Biological Sciences, Professor, Head of Biophysical Department of the Institute of Fundamental Biology and Biotechnology; Leading Researcher (e-mail: valkrat@mail.ru; ORCID: https://orcid.org/0000-0001-6764-5231).

At present, the personalized approach in medicine is becoming more and more popular and this leads to growing volumes of individual data obtained through diagnostics. Such data are usually influenced by multiple factors including age, lifestyle, working conditions, and stress states. Permissible ranges of analyzed health indicators often give no opportunity to describe patients' health or to analyze health risks considering their individual peculiarities. Saliva is a promising biological fluid, which can be used for solving this problem. It has several advantages when used in diagnostics: its collection is fast, easy, inexpensive, and non-invasive; in addition, it can reflect the physiological and pathological state of the body [1–3]. These issues have not been resolved yet for a new bioluminescent non-invasive biotest based on assessing effects produced by saliva on the bi-enzyme system luminescence: NAD(P)H:FMN oxidoreductase + luciferase and the relationship between the saliva composition and pathological and physiological states of the body [4]. Analysis results are influenced by many factors [5] and this makes it necessary to find a method for estimating their contributions into biomedical data considering greater variety of patients; personalized characteristics; to find a way to average these data and to establish criteria that describe both the normal state and deviations from it.

There are three common groups of methods for analyzing biomedical data: statistical methods, clusterization, and machine learning[1] [6–11]. The statistical component can be substantially involved in machine learning algorithms. Statistical analysis usually works with small data volumes and is aimed at getting an insight into interrelations between variables and at testing hypotheses. Machine learning is more actively used in biotechnologies since it allows automating various processes, analyzing larger and more complicated data arrays, and is focused on prediction accuracy and classification when solving tasks without an obvious variable-result. This makes it possible to analyze diverse data types and structures without any strict assumptions [6]. Statistical analysis starts with a hypothesis about a main factor, which describes the body state, and its results are used for testing it. This analysis relies on previously defined models about data distribution (for example, normal distribution)[2].

Correlation analysis is considered the basic statistical method for saliva analysis [7]. In particular, computed Spearman's rank correlation coefficient estimates intensity and direction of a correlation between two ranked variables and provides an insight into how well this correlation is described with a monotonic function. This does not require any assumptions about distribution of attributes due to the test being non-parametric. Next, saliva analysis often involves using the non-parametric Mann – Whitney test to identify authentic statistical differences between two independent groups per the level of an attribute [7, 10] even for small samples[2]. These methods make it possible to find correlations between indicators and conduct primary analysis of a data array. However, experience gained by using basic statistical methods does not always allow establishing key factors able to influence a target indicator of saliva.

Machine learning algorithms, which are often used for detecting diseases of various body systems, can be another solution to the issue of insufficient statistical analysis stock-

---

[1] Makarova N.V. Statisticheskii analiz mediko-biologicheskikh dannykh s ispol'zovaniem paketov statisticheskikh programm Statistica, SPSS, NPSS, SYSTAT [Statistical analysis of biomedical data using Statistica, SPSS, NPSS, SYSTAT statistical software packages]: methodical guide. In: Professor S.S. Aleksanin ed. Saint Petersburg, Polygraphic Center of the Saint Petersburg University of the EMERCOM of Russia Publ., 2012, 179 p. (in Russian); Shorokhova I.S., Kislyak N.V., Mariev O.S. Statisticheskie metody analiza [Statistical analysis methods]: manual, the 2nd ed. Moscow, FLINTA Publ., 2017, 300 p. (in Russian).

[2] Makarova N.V. Statisticheskii analiz mediko-biologicheskikh dannykh s ispol'zovaniem paketov statisticheskikh programm Statistica, SPSS, NPSS, SYSTAT [Statistical analysis of biomedical data using Statistica, SPSS, NPSS, SYSTAT statistical software packages]: methodical guide. In: Professor S.S. Aleksanin ed. Saint Petersburg, Polygraphic Center of the Saint Petersburg University of the EMERCOM of Russia Publ., 2012, 179 p. (in Russian)

piles for interpreting biomedical data about saliva [12–18]. We can choose an algorithm to establish regularities per previously marked data arrays judging from specificity of a task (searching for significant factors affecting saliva indicators). Data marks provide a feedback for a model thereby adjusting the results and making prediction more accurate. To analyze data on saliva, we can use a family of algorithms based on creating decision trees such as CART, Random Forest and boosting [13, 15, 16, 19]. Random Forest is among the most popular methods for solving non-deep machine learning tasks[3]. In addition to identifying a class of an object and finding the precise value of a target variable, the Random Forest algorithm predicts significance of each model parameter for decision-making considering its level against the tree top, how frequent this parameter can be found in a decision node, and the number of objects correctly classified by using it. Parameters found closer to the tree top are the most significant [20]. The Random Forest algorithm creates a partial dependence plot (PDP) for each model parameter. For regression, the plot shows averaged relationships between an attribute and a target value. For classification, an averaged relationship is built between likelihood that a class of an object is identified correctly and an attribute used in creating a model[4].

The aim of this study was to determine whether it was possible to use the Random Forest method for biomedical data analysis in order to achieve correct interpretation of results obtained by personalized diagnostic tests in a bioluminescent biosensor for estimating impacts of stress and workloads on the human body.

Materials and methods. To estimate effectiveness of data analysis, we used the results obtained by diagnostic tests performed in a group of traffic controllers employed by the Krasnoyarsk Branch of Russian Railways JSC (n = 43). The tests were performed every day for a month according to work schedules in 2022 and 2023. Resampling was used to extend the sample; re-learning was controlled by dividing the sample into the training and testing samples. The examined indicators were established by analyzing saliva collected two times a day: prior to a work shift and after it. The following saliva indicators were estimated: pH, lactate concentration, residual luminescence (luciferase index) of the bienzyme system of luminescent bacteria: NAD(P)H:FMN oxidoreductase + luciferase (LI, %) [21], as well as biochemical blood indicators and other results obtained by periodical medical check-ups of personnel accomplished at the Clinical Hospital 'Russian Railways Medicine'. We also analyzed the results obtained by a self-survey, which included subjective assessment of stress level and work capacity prior to a work shift and after it, data about daily routines, food consumption, use of drugs and energy drinks, and tobacco smoking.

All studies were conducted in conformity with the principles of biomedical ethics and approved by the local ethics committee of the Siberian Federal University (Krasnoyarsk; the meeting protocol No. 5 dated November 11, 2019).

Each participant gave a voluntary informed written consent to take part in this research after being provided with explanation of its potential risks and advantages as well as its essence.

Machine learning for classification and regression by the Random Forest algorithm was conducted in Python 3 using the Scikit-learn library. Factor significance is established in this library per the Gini Index improvement after division per each of these factors within classification or per declining dispersion of prediction per this factor within regression. The study relied on using a possibility provided by the Scikit-learn library to visualize partial influence exerted by each factor on the target variable. Statistical analysis was accomplished with the R

[3] Géron A. Hands-On Machine Learning with Scikit-Learn and TensorFlow: Concepts, Tools, and Techniques to Build Intelligent Systems, 2nd ed. O'Reilly Media Publ., 2019, 848 p.

[4] Jain A., Fandango A., Kapoor A. Tensorflow Machine Learning Projects. Packt Publishing, 2018, 322 p.

Table 1

Factors used for testing effectiveness of the program for data analysis

Field name	Measuring units / scores	Range
Lactate concentration in saliva	mmol/l	0.2–80.7
Blood glucose	mmol/l	3.2–9.8
Total cholesterol	mol/l	3.4–6.8
pH	pH	3–8
Sampling time	Prior to work shift (morning) – 1 After work shift (evening) – 0	0.1
Stress level (attitude to work)	Very anxious – 3 Anxious – 2 Calm – 1 No anxiety – 0	0–3
A time gap between having a smoke and sampling	Minutes	0–120
Sex	Female – 1 Male – 0	0.1
Smoking	Yes – 1 No – 0	0.1
Time of a day (morning / evening)	Morning – 1; evening – 0	0.1
LI (luciferase index)	%	1.08–142.50

Table 2

Correlations between some factors and the target variable (LI)

Factors	Correlation coefficient
Attitude to work (females)	-0.618
Time gap between having a smoke and sampling (females)	-0.699
Blood glucose, mmol/l (females)	0.446
Total cholesterol, mol/l (males)	0.671

software using basic libraries and the ggplot2 library. Differences were deemed significant at *p*-values below or equal to 0.05.

Results and discussion. After preliminary processing, the database containing biomedical data consisted of 310 lines. To extend it, resampling was made allowing a four-fold increase in the number of lines by randomly creating new lines out of the already existing ones. The sample was then divided into the training sample (80 % of the initial sample) and the testing sample (20 % of the initial sample). Variables provided in Table 1 were used for further analysis.

Correlation coefficients between target parameters from Table 1 were estimated using statistical data analysis as preprocessing for identifying the most promising factors to be included in machine learning.

Correlation analysis revealed significant negative correlations between the target variable LI and the participants' attitude to work per the scale between 1 and 5 and a time gap after having a smoke for female participants. Positive significant correlations were established between blood glucose (females) and total cholesterol (males). The correlation coefficients provided in Table 2 are significant at $p < 0.05$.

Random Forest, an ensemble machine learning algorithm based on decision tree building, was selected as a promising method for analysis. Two types of tasks were solved in the study: classification (using Random Forest Classifier of the Scikit-learn library), where the algorithm predicted belonging to groups of high, medium, or low LI values, and regression (using Random Forest Regressor of the Scikit-learn library) involving prediction of the LI exact value per the set factors. The most significant factors were identified and partial dependence plots were created. In both cases, we estimated prediction accuracy and significance of each factor per its influence on LI (Figure 1) and analyzed partial influence exerted on LI by a given factor depending on its level. Factor significance and standard deviation were calculated based on the results

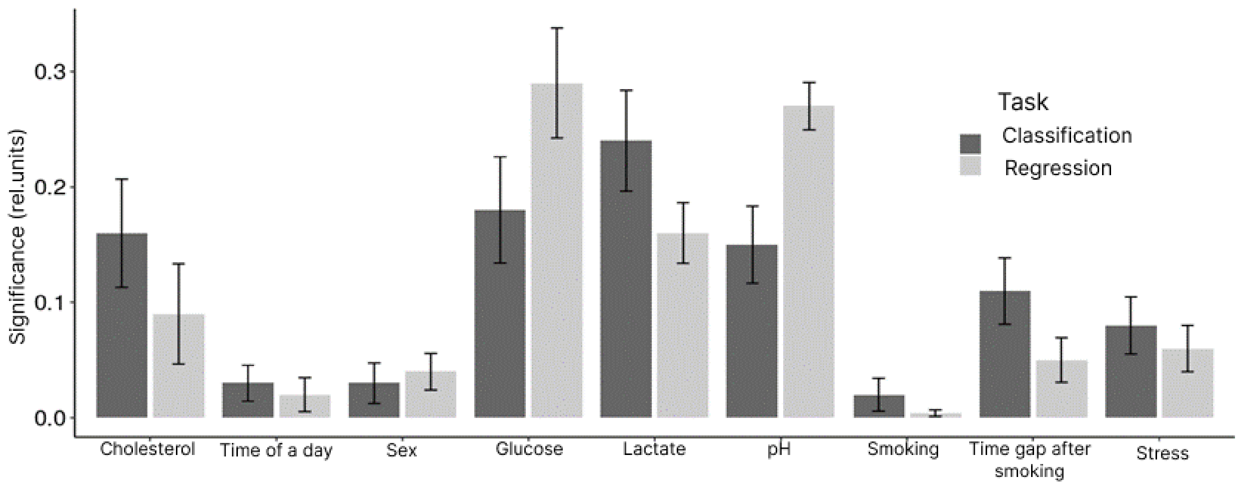

Figure 1. Distribution of factors per significance of their influence on LI in regression and classification tasks

obtained by building 2000 trees in each task and were established per the Gini Index based on a decline in uncertainty when breaking nodes: the higher a factor was in a tree, the more significant it was considered.

The hypothesis that each factor differed significantly from zero was tested using the Student's t-test with subsequent Bonferroni correction per multiple comparisons; as a result we revealed significant difference for each attribute. Three factors turned out to be the most significant per their influence on LI both in classification and regression, namely, lactate concentration, blood glucose, and pH level. In addition, total blood cholesterol can be considered significant in classification.

It is noteworthy that smoking had different significance; if the very fact of a participant being a smoker had practically zero significance, then a time gap between having a smoke and sampling was authentically higher for both tasks (*p*-values were 1.18E-07 for classification and 1.26E-06 for regression). This discrepancy can be explained by a rapid leveling of the effect produced by smoking on saliva.

Mean values of the learning metrics equaled the following: MAE = 28.73; MS = 880.99: $R^2 = 0.175$ in regression. Prediction accuracy equaled 95 % in classification. This high classification accuracy at a low R^2 value is due to a different nature of regression and classification tasks. In classification, the model recognizes classes per non-linear combinations of attributes whereas the regression model suffers from high response variance.

Partial influence was also estimated for two types of tasks (Figures 2 and 3).

Blood glucose level higher than 6 mmol/l is the threshold, above which this factor ceases to exert any influence on the luciferase index (Figure 2). The highest likelihood of a LI value closer to the upper bound of the assumed normal range is observed when blood glucose is low. For pH, a level below 5 has practically no influence on the target value level. However, as pH grows, its effect on LI is described with ambiguous dynamics and high dispersion. Lactate concentration in saliva has effects on the luciferase index value at low levels (lower than 8 mmol/l).

For classification, blood glucose level showed a relationship opposite to that observed for regression as influence exerted by this factor grew (Figure 3).

The dynamics itself was shaped saturated and significance leveled off at the blood glucose level of approximately 5 mmol/l. The lactate and pH plots had shapes similar to that in regression. When lactate concentration reached 8 mmol/l, a clear transition appeared in the significance level, after which any influence on the luciferase index disappeared. The relationship with pH had a rather chaotic dynamic with the overall trend to grow as the factor level grew.

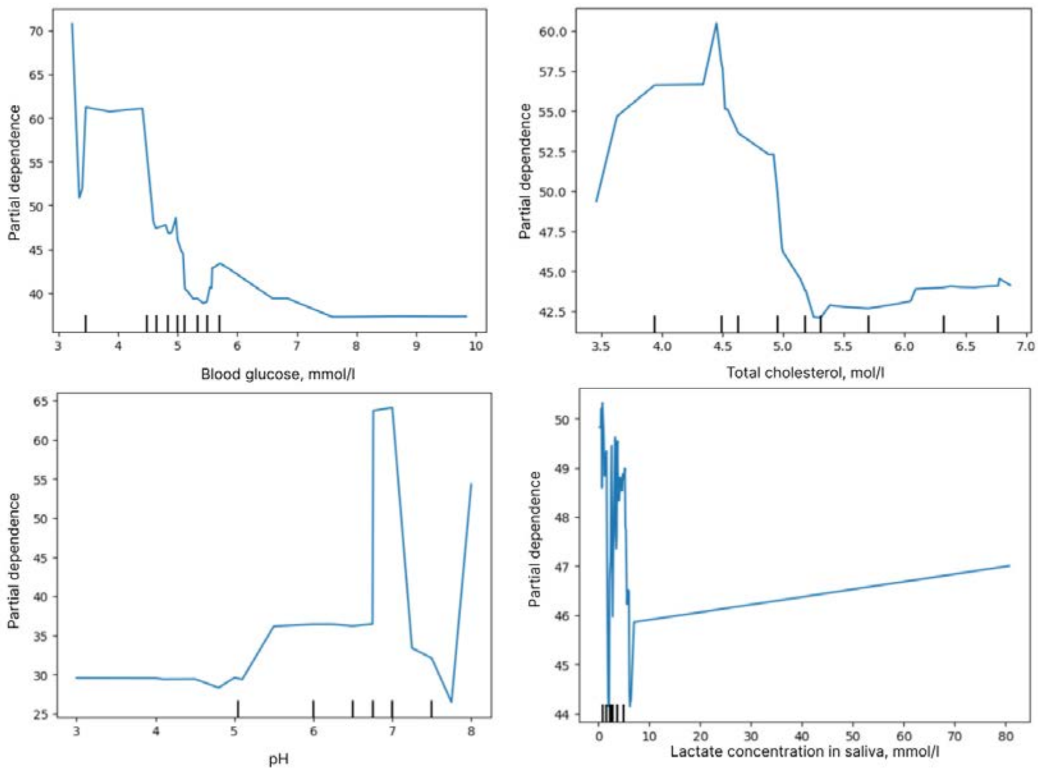

Figure 2. Partial dependence plots to show partial influence of selected factors on the target variable (LI) for regression task

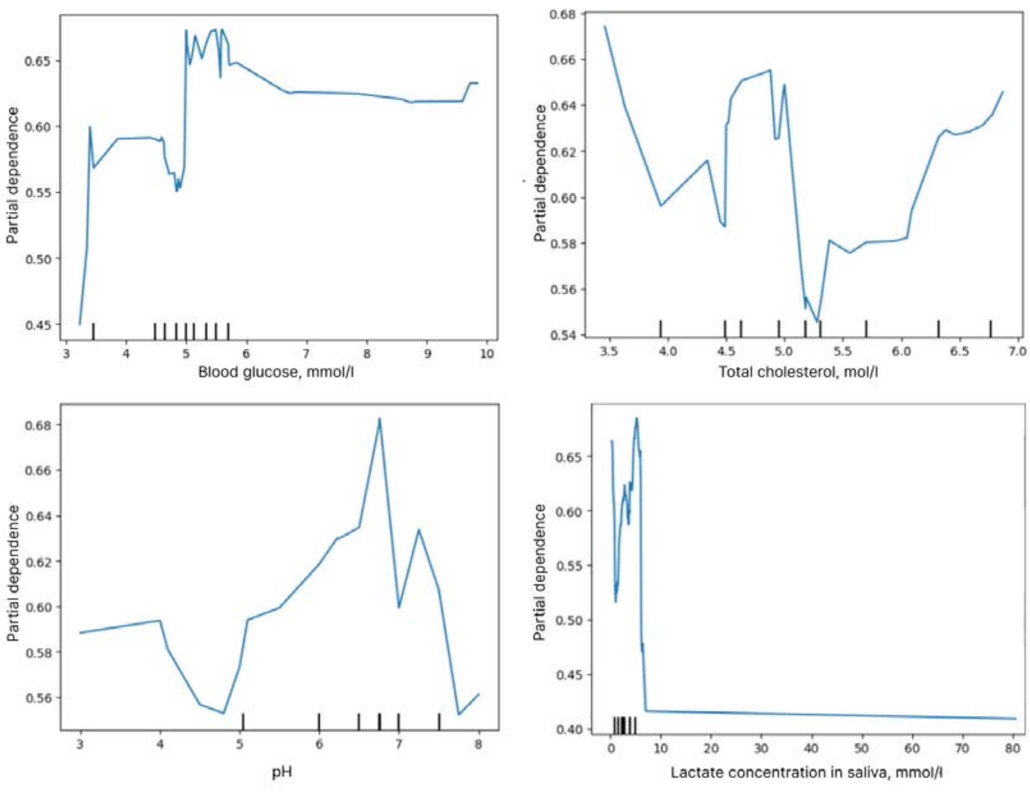

Figure 3. Partial dependence plots to show partial influence of selected factors on the target variable (LI) for classification task

Conclusion. The selected factors were analyzed using the Random Forest algorithm; the analysis showed that not all parameters were equally significant for predicting the target indicator. Blood glucose demonstrated opposite effects in the regression and classification tasks: in regression, its influence decreased when its level grew above 6 mmol/l whereas in classification the factor significance leveled off when the blood glucose level was approximately 5 mmol/l. This may indicate some complex non-linear interrelations between blood glucose and the state of the analyzed material. The pH level also had different influence depending on the task. In regression, pH effects on the luciferase index of the bioluminescence system were considerably variable, especially as it increased. In classification, dynamics of pH influence also remained uncertain and this complicated clear understanding of its role in changes of the body state despite its great significance for predicting LI levels. Lactate concentration had a precise transition value (8 mmol/l), after which its influence on the luciferase index decreased considerably. This indicates possible existence of a threshold value, after which lactate ceased to be a significant factor; this may be quite useful for practical use within workers' health monitoring. Despite the low R^2 value (0.175), the model identifies significant factors quite successfully and demonstrates high prediction accuracy. This indicates strong non-linearity and potential existence of hidden variables, which have not been covered in the current data set. Our study did not include several potentially significant factors in the analysis such as physical activity, stress situation outside workplace, and individual metabolism peculiarities although inclusion of these parameters might have considerably increased the model accuracy and usefulness. In future, we plan to compare effectiveness of the Random Forest method, conventional linear regression, logistic regression, XGBoost, and neural networks.

The Random Forest method is suitable for selecting informative factors, which describe peculiarities of personal characteristics of the body in its normal state and in case of deviations from it. It allows minimizing the number of factors influencing LI in case a test is preliminary adjusted for personalized work. The Random Forest method can become a component of a personalized bioluminescent biosensor for assessing effects produced by stress and workloads on the body. The suggested data analysis can be used for analyzing and interpreting results obtained by various research methods; it makes it possible to analyze big data volumes and reveal hidden regularities as well as to minimize risks of incorrect diagnosis and to adjust a monitoring scheme for patients, the latter being a crucial aspect in biotechnological and medical research.

Financial support. The work was supported by a grant from the Ministry of Science and Higher Education of the Russian Federation for implementation of major scientific projects in priority areas of scientific and technological development (Project No. 075-15-2024-638).

Competing interests. The authors declare the absence of obvious and potential competing interests related to the publication of this article.

References

1. Tabak L.A. Point-of-care diagnostics enter the mouth. *Ann. N Y Acad. Sci.*, 2007, vol. 1098, pp. 7–14. DOI: 10.1196/annals.1384.043

2. Kubala E., Strzelecka P., Grzegocka M., Lietz-Kijak D., Gronwald H., Skomro P., Kijak E. A Review of Selected Studies That Determine the Physical and Chemical Properties of Saliva in the Field of Dental Treatment. *BioMed Res. Int.*, 2018, vol. 2018, pp. 6572381. DOI: 10.1155/2018/657238

3. Kaczor-Urbanowicz K.E., Carreras-Presas C.M., Aro K., Tu M., Garcia-Godoy F., Wong D.T. Saliva diagnostics – Current views and directions. *Exp. Biol. Med. (Maywood)*, 2017, vol. 242, no. 5, pp. 459–472. DOI: 10.1177/1535370216681550

4. Esimbekova E.N., Torgashina I.G., Kalyabina V.P., Kratasyuk V.A. Enzymatic Biotesting: Scientific Basis and Application. *Contemp. Probl. Ecol.*, 2021, vol. 14, pp. 290–304. DOI: 10.1134/S1995425521030069

5. Kratasyuk V.A., Stepanova L.V., Ranjan R., Sutormin O.S., Pande S., Zhukova G.V., Miller O.M., Maznyak N.V., Kolenchukova O.A. A noninvasive and qualitative bioluminescent assay for express diagnostics of athletes' responses to physical exertion. *Luminescence*, 2021, vol. 36, no. 2, pp. 384–390. DOI: 10.1002/bio.3954

6. Jordan M.I., Mitchell T.M. Machine learning: Trends, perspectives, and prospects. *Science*, 2015, vol. 349, no. 6245, pp. 255–260. DOI: 10.1126/science.aaa8415

7. Bel'skaya L., Sarf E. The use of Fourier transform IR spectroscopy of saliva for rapid assessment of the level of lipid peroxidation products. *Biomedical Chemistry: Research and Methods*, 2019, vol. 2, no. 2, pp. e00094. DOI: 10.18097/BMCRM00094 (in Russian).

8. Bel'skaya L.V., Sarf E.A., Kosenok V.K. Correlation interrelations between the composition of saliva and blood plasmain norm. *Klinicheskaya laboratornaya diagnostika*, 2018, vol. 63, no. 8, pp. 477–482. DOI: 10.18821/0869-2084-2018-63-8-477-482 (in Russian).

9. Nikonorova M.L. Building optimal models for analysis of biomedical data. *Vestnik novykh meditsinskikh tekhnologii*, 2021, vol. 28, no. 1, pp. 55–59. DOI: 10.24412/1609-2163-2021-1-55-59 (in Russian).

10. Berestneva O.G., Osadchaya I.A., Nemerov E.V. Methods for studying the medical data structure. *Vestnik nauki Sibiri*, 2012, vol. 1, no. 2, pp. 333–338 (in Russian).

11. Klimenko A.V., Slashchev I.S. Klasternyi analiz dannykh [Cluster data analysis]. *Vestnik nauki*, 2019, no. 1 (10), vol. 1, pp. 159–163 (in Russian).

12. Braz D.C., Popolin Neto M., Shimizu F.M., Sá A.C., Lima R.S., Gobbi A.L., Melendez M.E., Arantes L.M.R.B. [et al.]. Using machine learning and an electronic tongue for discriminating saliva samples from oral cavity cancer patients and healthy individual. *Talanta*, 2022, vol. 243, pp. 123327. DOI: 10.1016/j.talanta.2022.123327

13. Lee E., Park S., Um S., Kim S., Lee J., Jang J., Jeong H.-O., Shin J. [et al.]. Microbiome of Saliva and Plaque in Children According to Age and Dental Caries Experience. *Diagnostics (Basel)*, 2021, vol. 11, no. 8, pp. 1324. DOI: 10.3390/diagnostics11081324

14. Kouznetsova V.L., Li J., Romm E., Tsigelny I.F. Finding distinctions between oral cancer and periodontitis using saliva metabolites and machine learning. *Oral Dis.*, 2021, vol. 27, no. 3, pp. 484–493. DOI: 10.1111/odi.13591

15. Zarrin P.S., Roeckendorf N., Wenger C. In-Vitro Classification of Saliva Samples of COPD Patients and Healthy Controls Using Machine Learning Tools. *IEEE Access*, 2020, vol. 8, pp. 168053–168060. DOI: 10.1109/ACCESS.2020.3023971

16. Murata T., Yanagisawa T., Kurihara T., Kaneko M., Ota S., Enomoto A., Tomita M., Sugimoto M. [et al.]. Salivary metabolomics with alternative decision tree-based machine learning methods for breast cancer discrimination. *Breast Cancer Res. Treat.*, 2019, vol. 177, no. 3, pp. 591–601. DOI: 10.1007/s10549-019-05330-9

17. Rabbani N., Kim G.Y.E., Suarez C.J., Chen J.H. Applications of machine learning in routine laboratory medicine: Current state and future directions. *Clin. Biochem.*, 2022, vol. 103, pp. 1–7. DOI: 10.1016/j.clinbiochem.2022.02.011

18. Li X., Zheng J., Ma X., Zhang B., Zhang J., Wang W., Sun C., Wang Y. [et al.]. The oral microbiome of pregnant women facilitates gestational diabetes discrimination. *J. Genet. Genomics*, 2021, vol. 48, no. 1, pp. 32–39. DOI: 10.1016/j.jgg.2020.11.006

19. Kuwabara H., Katsumata K., Iwabuchi A., Udo R., Tago T., Kasahara K., Mazaki J., Enomoto M. [et al.]. Salivary metabolomics with machine learning for colorectal cancer detection. *Cancer Sci.*, 2022, vol. 113, no. 9, pp. 3234–3243. DOI: 10.1111/cas.15472

20. Alam M.Z., Rahman M.S., Rahman M.S. A Random Forest based predictor for medical data classification using feature ranking. *Informatics in Medicine Unlocked*, 2019, vol. 15, pp. 100180. DOI: 10.1016/j.imu.2019.100180

21. Stepanova L.V., Kolenchukova O.A., Zhukova G.V., Sutormin O.S., Kratasyuk V.A. The Use of the Bioluminescent Enzyme Bioassay for the Analysis of Saliva of Railway Transport Workers to Monitor the Functional State of the Body in the Conditions of Labor Activity. *Biofizika*, 2024, vol. 69, no. 3, pp. 674–683. DOI: 10.31857/S0006302924030224 (in Russian).

Zhukova G.V., Martyshuk P.A., Afer E.R., Shuvaev A.N., Rozanova N.A., Sergeev D.V., Kratasyuk V.A. Random Forest method for interpreting results obtained by bioluminescence analysis of saliva in personalized diagnostics. Health Risk Analysis, 2025, no. 2, pp. 166–174. DOI: 10.21668/health.risk/2025.2.14.eng

Received: 22.12.2024
Approved: 07.05.2025
Accepted for publication: 14.06.2025

ANALYTICAL REVIEWS

UDC 57.042+57.049+ 612.1+614
DOI: 10.21668/health.risk/2025.2.15.eng

Read online

Review

SYSTEMIC EFFECTS OF RADIOFREQUENCY ELECTROMAGNETIC FIELDS (REVIEW). PART 1. SECRETION GLANDS

N.I. Khorseva[1], P.E. Grigoriev[2,3]

[1]Institute of Biochemical Physics of the Russian Academy of Sciences, 4 Kosygina St., Moscow, 119334, Russian Federation
[2]Sevastopol State University, 33 Universitetskaya St., Sevastopol, 299053, Russian Federation
[3]Academic Research Institute of Physical Methods of Treatment, Medical Climatology and Rehabilitation named after I.M. Sechenov, 10/3 Mukhina St., Yalta, Republic of Crimea, 298603, Russian Federation

Among publications on systemic effects of radio frequency electromagnetic fields (RF EMF), primarily those inherent in cellular communication devices (most often from 900 MHz to 2.5 GHz), as well as Wi-Fi, special attention should be paid to their influence on structural changes in the secretory glands, which are often direct targets for the impact of the corresponding RF EMF (for example, the thyroid gland).

Various pathohistological effects of chronic exposure to RF EMF in different modes on the glands of external secretion have been established both in experimental studies on animals and in epidemiological ones. The parotid gland, salivary glands, sweat glands are among those mentioned in them.

The endocrine glands can also be affected by RF EMF, which is confirmed by the results of numerous studies on the pineal gland, pituitary gland, thyroid gland, and adrenal glands, in which changes in their structure and functions have been recorded in both experimental animals and humans. At the same time, there is fairly pronounced dependence between resulting effects and exposure and other characteristics of RF EMF.

At present, biological effects of RF EMF produced by various frequency ranges (cellular devices and telecommunication masts) have been reliably established. Various histopathological changes have been registered in the glands of mixed secretion such as the liver, pancreas, testicles and ovaries. Serious disorders in the testicles and ovaries revealed in experimental animals are particularly relevant since they undoubtedly lead to reproductive dysfunction.

Particular concern is raised by the fact that cellular users of different ages, primarily children and adolescents, carry mobile devices in their trouser pockets, i.e. in close proximity to the sex glands.

The present period is also characterized by accumulation of comparative epidemiological data as well as non-invasive measurements of structural and functional changes in the secretory glands in humans. Based on them, an unambiguous conclusion should be made about the need to limit and take precautions when using cellular devices, which is also indicated by some of the works considered in this review.

Keywords: *electromagnetic fields of the radio frequency range, cellular devices, Wi-Fi, endocrine glands, exocrine glands, mixed secretion glands, histological changes, young animals.*

The glands of external secretion. Negative effects produced by radio frequency electromagnetic fields (RF EMF) on brain structures can be considered quite proven [1]. Given that, we can assume that RF EMF radiation of mobile phones can also 'affect' secretion glands, the ***parotid gland*** in particular.

Natalia I. Khorseva – Candidate of Biological Sciences, Senior Researcher at the Laboratory of Physical and Chemical Problems of Radiobiology and Ecology (e-mail: sheridan1957@mail.ru; tel.: +7 (905) 782-87-17; ORCID: https://orcid.org/0000-0002-3444-0050).

Pavel E. Grigoriev – Doctor of Biological Sciences, Associate Professor, Professor of the Department of Psychology; Leading Researcher of the Research Department of Physiotherapy, Medical Climatology and Resort Factors (e-mail: mhnty@ya.ru; tel.: +7 (978) 767-22-10; ORCID: https://orcid.org/0000-0001-7390-9109).

Although very few studies have been published on the subject, a work by S. Sadetzki et al. reported an increased risk of the parotid gland cancer in mobile phone users [2]; experiments conducted by Z. Ozergin Coskun et al. established histopathological changes in rats' parotid upon exposure to 1800 MHz for 6 and 12 hours a day, namely, an increase in the apoptotic index, which was more prominent upon longer exposure [3]. Dependence between the level of histopathological changes and duration of exposure to 2100 MHz RF EMF (6 hours/day, 5 days/week, for 10 or 40 days) was established in a study by F. Aydogan et al. Histological changes in salivary glands (acinar epithelial cells, interstitial space, ductal system, vascular system, nucleus, amount of cytoplasm and variations in cell size) were found to be more prominent upon longer exposure (40 days) [4].

Moreover, salivary dysfunction (lesser salivary secretion) was established both in people aged 18–30 years and 30–60 years, who lived approximately one kilometer away from mobile base stations for not less than 8 years [5].

The fifth generation mobile communication 5G potentially employs two frequency bands, 600–60 000 MHz and 24–100 GHz (4.8–4.99 GHz and 24.25–24.65 GHz in Russia). Yu.G. Grigoriev believes that active implementation of such communication networks "…can create much higher hazards associated with constant radiation exposure of new critical organs, such as skin and eyes" [6]. Underestimation of these health risks can be fatal.

Thus, K. Karipidis et al. claim in their review [7] that available results, in particular those obtained by epidemiological studies, provide little evidence of a relationship between low-level millimeter wave band and any hazardous health effects since the related exposures are well below their permissible limits stipulated by the International Commission on Non-Ionizing Radiation Protection (ICNIRP). However, this point of view is strongly criticized by S. Weller et al. since it sends the wrong messages regarding safety assessment and public health [8].

Since eye sclera and skin are considered new critical organs upon exposure to the millimeter band, we can assume multiple ***sweat glands*** to also be affected by RF EMF.

In this respect, studies by S.R. Tripathi et al. [9] and N. Betzalel et al. [10] are of interest. By using optical coherence tomography, the authors established the tips of the sweat ducts to have a helical structure so they could be considered a helical antenna. The resonance frequency of this antenna in the axial mode of operation lies in the THz wave region with a center frequency of 0.44 ± 0.07 THz [9], which may lead to a high specific absorption rate (SAR) of the skin in extremely high frequency band [10].

In addition, it was established through experiments that human skin reflectance in sub-terrahertz (sub-THz) band, which is being actively promoted on the market by mobile operators (5G in particular), depends on perspiration intensity and correlates not only with levels of stress in a given person but also ECG parameters. In this relation, it is necessary to consider possible outcomes for public health due to unrestricted use if sub-THz technologies such as 5G [10] as well as new 6G bands.

We believe that all foregoing evidence should be taken into account when new mobile communication technologies are widely implemented into practice since human skin becomes a new critical organ in this case. Considering the exposure area (1.5–2 m^2 in an adult person), this can become not a potential but rather an obvious health threat for humans, primarily children and adolescents as a population cohort, which is the most sensitive to any external exposures.

The endocrine glands. As stated above, negative effects of RF EMF on brain structures can be considered well proven [1]; therefore, such endocrine glands as ***epiphysis*** (the tectal plate in the midbrain) and ***hypophysis*** (the interbrain) can also be affected by them. The results obtained by studies outlined below are evidence of this impact.

Effects on the ***epiphysis*** are reported in a study by S.G. Yashchenko and S.Yu. Rybalko conducted on young rats. They investigated impacts exerted by RF EMF of up-to-date communication devices such as personal computers (PC) (4h/day, for 26 weeks) and mobile phones (MP) (925 MHz, each 5 minutes for 12 hours for 26 weeks, power flux densities (PFD) reached 97.8 $\mu W/cm^2$ in the cell center with the mean integral value being 22 $\mu W/cm^2$). The authors established changes in the epiphysis tissues ultrastructure involving development of general specific morphological changes for each exposure type, PC and MP. Their findings give evidence of negative effects produced by RF EMF on the epiphysis [11].

Use of electrophotography image or gas discharge visualization after 15-minute exposure to MP for students (aged 17.40 ± 0.24 years) established significant reductions in subtle energy levels in several organs (pancreas, thyroid gland, cerebral cortex, cerebral vessels, left ear and left eye, liver, right kidney, spleen and the immune system), ***hypophysis*** included [12].

At present, smartphones are being commonly used as a new mobile phone generation; the gadget antenna is located at its bottom and it has created a new exposure type that involves greater electromagnetic effects on the neck. It is evidenced by estimated distribution of absorbed doses in this area and the ***thyroid gland*** becomes a new critical organ in this case [13, 14].

Various changes in the thyroid structure and function as well as changes in hormone levels upon exposure to RF EMF have been summarized in several reviews [15, 16], including those focusing on children and adolescents [14].

In addition, ***experimental studies*** established histological and biochemical changes in thyroid cells in rats and mice upon exposure to RF EMF within band between 900 MHz and 2.45 GHz. Experiments on young rats revealed a decline in cuboid cells, follicular colloid fluid and follicle sizes upon exposure to MP RF EMF (900 MHz, 10 minutes, 12 times a day for 1 month) [17]; formation of apoptotic bodies and increased caspase-3 and caspase-9 activities in thyroid cells of the rats were found upon exposure to impulse-modulated RF fields (900 MHz, modulation equal to 217 MHz, 20 min/day, for 2 months) [18].

Exposure to RF EMF created by MP with double transceiver 900/1800 MHz (50 calls 30 sec each with 15-second breaks between them for 8 weeks) led to a decrease in colloid content in microfollicles, wider connective tissue septa and more dilated blood capillaries [19].

A diathermy model was employed by M.J. Misa-Agustiño et al. to investigate effects of 2.45 GHz on rats' thyroid with maximum exposure in the left front leg. Exposure to RF EMF was single (30 min/day, 3 and 12 W) or repeated (30 min/day, 3 W, for 2 weeks). Morphometric measurements in thyroid tissue were conducted 90 minutes and 24 hours after the last exposure in all experimental groups. The authors did not find any signs of apoptosis in thyroid cells or any changes in septa width in the central part of connective tissue; however, they established certain changes in the size of central and peripheral follicles, which indicate developing hypertrophy of the gland related to exposure intensity and / or number of exposures [20].

Studies with participating children and adolescents as well as people younger than 25 years have revealed a wide range of changes in the thyroid gland upon exposure to RF EMF.

At present, RF EMFs created by base mobile stations are known to be able to produce negative effects on hormone levels in people living in close proximity to them. In particular, a study by E.F. Eskander reported changes in hormone levels in dynamics established for young males/men and young females/women (aged 14–22 years and 25–60 years respectively) as an exposure period grew longer (1, 3, and 6 years); all participants lived not farther than 500 meters away from a base station. The greatest changes were found for the 6-year exposure such as a significant decline in the thyroid hormones, both serum thyroxin (T4) and triiodothyronine (T3), and adrenocorticotrophic hormone (ACTH) in all groups, and in the sex hormones (declining testosterone levels in young males/men, declining serum prolactin levels in young females but growing levels of the hormone in adult women) [21].

To investigate effects of MP RF EMF on the human thyroid gland, researchers analyze hormone levels [22], conduct clinical examinations [23] and US-examinations with simultaneous surveys involving use of various inventories.

Thus, a survey conducted among 77 students with different MP use, 5–20 min/day and more than 120 min/day together with hormone level analysis established a higher than normal TSH level, low mean T4 and normal T3 concentrations in mobile users; the changes were the most prominent in active users [22].

Similar studies were conducted by N.M. Baby et al. with 83 participating students (aged 18–25 years). On average, 53 % of the respondents talked on the mobile phone for 0.5 hours/day; 28.9 %, 1.5 hours/day; 10.8 %, 3.5 hours/day. Clinical examination of the thyroid revealed that 13.6 % of the participants had thyroid swelling, 3.6 % had symptoms of thyroid dysfunction and 3.6 % had both thyroid swelling and symptoms of thyroid dysfunction. A significant correlation was also found between total radiation exposure and an increase in TSH in both groups [23].

A prospective study aimed at investigating MP RF EMF effects on the thyroid was done by N.M. Elsayed et al. on 180 participants, 110 females and 70 males, ranging in age from 15 to 65 years. US examination revealed 46.7 % of the participants to have abnormal findings of the thyroid gland, more frequently in smartphone users and in females. Multinodular goiter was the most common abnormality (54 %) [24].

Statistical reports on the population incidence of endocrine, nutritional and metabolic disorders in the Russian Federation give evidence of a significant increase in the number of children and adolescents suffering from these diseases since 2000: 2.8 times for the age group of 0–14 years and 5.5 times for the age group of 15–17 years [14].

Basic structures of the ***thymus***, in particular in rats, are known to be still forming in the postnatal period [25]. Therefore, RF EMF effects can be quite drastic in this period as evidenced by sporadic studies conducted on young rats.

Thus, exposure to 900 MHz (1 hour/day, for 22–59 days after birth) led to histopathological changes in the thymus tissue: extravascular erythrocytes were observed in the medullary/corticomedullary regions [26]. A study involving acute (1 week) and repeated (10 weeks) exposure to RF EMF of young rats in two frequency bands (900 and 2100 MHz, 2 hours/day) established greater expression of caspase 3 and 12, glucose-regulated protein 78 kDa (Grp78), C/EBP and homologous protein, which indicates increased levels of ER stress pathway proteins and could cause apoptosis of the thymus cells [27].

M.J. Misa-Agustiño et al., just as they did when investigating RF EMF effects on the thyroid, used a diathermy model to examine effects of 2.45 GHz (power equal to 1.5, 3.0 or 12.0 W, 30 min/day for 1 and

10 weeks) on young rats' thymus. The study found increased distribution of blood vessels (more prominent in the medulla) along with the appearance of red blood cells and hemorrhagic reticuloepithelial cells in animals exposed to 12.0 W (SAR per 1 gram of the thymus tissue is 0.482 ± 12.10^{-3} W/kg). The effect here depended solely on the exposure power and not its duration. Moreover, the authors found a relationship between exposure to RF EMF and greater endothelial permeability and vascularization of the thymus [28].

Established histopathological changes in experimental animals' thymus upon exposure to RF EMF may well be found in people who are active mobile phone users and tend to keep their phones in breast pockets. Still this assumption requires further investigation.

Hormones released by the ***adrenals*** are known to maintain homeostasis and support adaptation to various physiological and emotional situations. However, there are only sporadic studies that concentrate on investigating RF EMF effects on the adrenals.

We managed to find only two available publications, A. Kocaman et al. and S. Shahabi et al. Both studies were conducted on young Wistar rats exposed to 900 MHz EMF.

A. Kocaman et al. detected a significant increase in the mean volume of the adrenal gland upon exposure to RF EMF 60 minutes/day fir 15 days [29]. The study by S. Shahabi et al. involved more intensive RF EMF exposure (6h/day, for 4 and 8 weeks respectively); as a result, it was established that the fasciculata layer of adrenal cortex eventually thickened. While the number of cells in zona fasciculata remained constant, the cell size and perimeter increased during RF EMF exposure. The outlined changes were more prominent upon the 8-week exposure [30].

Since negative changes were detected both in the hypophysis [12] and adrenals as stated above [29, 30], we can assume that RF EMF affects the hypothalamic-pituitary-adrenal axis, a neuroendocrine system responsible for maintaining homeostasis, adaptation to external factors and survival under stress. This, in its turn, can lead to various systemic responses to RF EMF exposure.

The glands of mixed secretion. This group includes such glands as the liver, pancreas, and sex glands, namely, testicles in males and ovaries in females.

However, very few publications are focused on investigating RF EMF effects on the liver and testicles and only sporadic ones concentrate on effects on the pancreas and ovaries.

In particular, upon exposure to 900 MHz (1, 2 and 4 h/day for 30 days), M. Sepehrimanesh et al. did not establish any significant histopathological changes in the ***liver*** [31]. However, such changes were detected by H.R. Ma et al. [32] and D.Ö. Okatan et al. [33] even upon shorter exposure to RF EMF.

In the first case (4 h/day for 18 days), the histopathology examination showed diffuse hepatocyte swelling and vacuolization, small pieces and focal necrosis [32]; in the second case (1 h/day for 24 days), occasional irregularities in the radial arrangement of hepatocytes, cytoplasmic vacuolization, hemorrhage, sinusoid expansion, hepatocyte morphology and edema [33].

Daily exposure of adult mice to RF EMF created by the two-band Nokia 1112 (900/1800 or 950/1900 MHz) 1 h/day for 10 days induced inflammatory cellular infiltration and the hepatocytes appeared vacuolated and contained denser nuclei. Upon exposure duration being 12 h/day, liver sections of group three showed more intensive inflammatory response around the central vein whereas hepatocytes were swollen and their cytoplasm appeared to be highly vacuolated [34].

Effects of exposure to 2.45 GHz are different from those produced by exposure to 900 MHz. Histopathological studies by

K. Holovská et al. (3 h/day for 3 weeks) did not established any changes in hepatocyte structure; however, electron microscopy of hepatocytes revealed vesicles of different sizes and shapes, lipid droplets, and proliferation of smooth endoplasmic reticulum as well as presence of necrotic hepatocytes. In addition, the authors found moderate hyperemia, dilatation of liver sinusoids, and small inflammatory foci in the center of liver lobules [35]. Completely distorted liver architecture and elongated nuclei with cytoplasm of some hepatocytes were found in a study by P. Chauhan et al. (2 h/day for 35 days) [36].

Later, E.A. Adebayo et al. established pronounced dilated sinusoids, distorted architecture, hyperchromatic nuclei, and congested central vein with change of hepatocytes structure in rats kept 24 meters away from the base of two different telecommunication network masts (1800 MHz) for 5 weeks (1.40 W/cm^2) [37].

Therefore, exposure to RF EMF created by both mobile phones and base stations can induce histopathological changes in liver tissues of experimental animals.

Negative histopathological changes have also been established in ***testicle*** tissues.

Thus, grossly distorted seminiferous tubules and epididymis with loss of cellular structure and an area of inflammatory changes with complete absence of spermatozoa, which may lead to low fertility, were found in rats kept 24 meters away from telecommunication network masts (1800 MHz) for 5 weeks [37].

Upon exposure to 900 MHz RF EMF (1 h/day for 30 days), exposed male rats had vacuoles in seminiferous tubules basal membrane and edema in the inter-tubular space. Seminiferous tubule diameters and germinal epithelium thickness were both smaller, and apoptotic index was higher [38].

M. Saygin et al. detected a declining quantity of Leydig cells, testis cell degeneration and apoptosis in rat testicle tissue upon exposure to 2.45 GHz (60 min/day for 28 days) [39]. P. Chauhan et al., having simulated repeated RF EMF exposure (2.45 GHz, 2 h/day for 35 days) revealed epididymis epithelium degeneration and tube necrosis, lumen occlusion, and smaller testicular tubule size [36].

In our opinion, special attention should be paid to investigating outcomes of exposure to Wi-Fi (2.24 GHz, 1 h/day and 7 h/day for 2 months). As a result, the number of apoptosis-positive cells and caspase-3 activity increased in the seminiferous tubules of exposed rats and this was more prominent upon daily 7 h/day exposure. Upon 1h/day exposure, the testicular tubule architecture and interstitial tissue remained unchanged and germinal testis epithelium was undamaged. On the contrary, 7-hour exposure led to both fewer layers of embryo cells and lower mean testis score [40].

Histopathological changes in female rats' ovaries were established upon exposure to 1800 MHz (2 h/day for 30 and 60 days respectively). After 30 days, stagnation and fewer quantities of follicles were found in the ovaries; after 60 days, degeneration of pre-ovulatory follicle cells and macrophage infiltration were added and vacuolization of interstitial cells and granulose cell layers was activated [41].

Based on all foregoing, we can state that exposure to RF EMF of various frequency bands induces considerable histopathological changes in testis and ovary tissues. We believe the matter deserves special attention since many mobile phone users (especially often children and adolescents) tend to keep their mobile phones / smartphones in trousers' pockets.

The only study that reported negative effects on RF EMF on several organs and tissues, the ***pancreas*** included, was published by H. Bhargav et al. In this study, a statistically significant decline in subtle energy levels was detected using electrical photonic visualization in ***61 adolescents*** (the average age was 17.40 ± 0.24 years) after 15-minute exposure to mobile phone EMF on the right year [12].

Conclusion. To sum up, we can state that most analyzed studies reported not only functional but also variable histopathological changes in secretion glands, their tissues and cells. We believe that these results obtained by investigating exposure to RF EMF of various frequency bands on the glands of external, internal and mixed secretion provide convincing evidence of their negative effects including those produced on children and adolescents.

Funding. The study was accomplished with the state task given to Institute of Biochemical Physics of the Russian Academy of Sciences named after N.M. Emanuel (44.1, the state registration No. 0084-2019-004).

Competing interests. The authors declare no competing interests.

Acknowledgments. This publication is in memory of Yury G. Grigoriev, an outstanding Soviet and Russian scientist, prominent expert in radiobiology, Doctor of Medical Sciences, Professor, President of the Russian National Committee on Non-Ionizing Radiation Protection, permanent member of the WHO Advisory Committee on Electromagnetic Fields and Public Health international program.

References

1. Khorseva N.I., Grigoriev P.E. Effects of Radio Frequency Electromagnetic Fields on the Nervous System. In vivo Experiments (Review). *Zhurnal mediko-biologicheskikh issledovanii*, 2024, vol. 12, no. 3, pp. 383–398. DOI: 10.37482/2687-1491-Z195 (in Russian).

2. Sadetzki S., Chetrit A., Jarus-Hakak A., Cardis E., Deutch Y., Duvdevani S., Zultan A., Novikov I. [et al.]. Cellular phone use and risk of benign and malignant parotid gland tumors – a nationwide case-control study. *Am. J. Epidemiol.*, 2008, vol. 167, no. 4, pp. 457–467. DOI: 10.1093/aje/kwm325

3. Ozergin Coskun Z., Tumkaya L., Yilmaz A., Dursun E., Mercantepe T., Kalkan Y., Ersoz S. Does Radiofrequency Radiation From Mobile Phones Affect the Formation of Parotid Gland Malignancy? An Experimental Study. *Ear Nose Throat J.*, 2024, vol. 103, suppl. 3, pp. 75S–82S. DOI: 10.1177/01455613241287295

4. Aydogan F., Unlu I., Aydin E., Yumusak N., Devrim E., Samim E.E., Ozgur E., Unsal V. [et al.]. The effect of 2100 MHz radiofrequency radiation of a 3G mobile phone on the parotid gland of rats. *Am. J. Otolaryngol.*, 2015, vol. 36, no. 1, pp. 39–46. DOI: 10.1016/j.amjoto.2014.10.001

5. Singh K., Nagaraj A., Yousuf A., Ganta S., Pareek S., Vishnani P. Effect of electromagnetic radiations from mobile phone base stations on general health and salivary function. *J. Int. Soc. Prev. Community Dent.*, 2016, vol. 6, no. 1, pp. 54–59. DOI: 10.4103/2231-0762.175413

6. Grigoriev Yu.G., Samoylov A.S. 5G-cellular standards. Total radiobiological assessment of the danger of planetary electromagnetic radiation exposure to the population. Moscow, SRC – FMBC Publ., 2020 (in Russian).

7. Karipidis K., Mate R., Urban D., Tinker R., Wood A. 5G mobile networks and health-a state-of-the-science review of the research into low-level RF fields above 6 GHz. *J. Expo. Sci. Environ. Epidemiol.*, 2021, vol. 31, no. 4, pp. 585–605. DOI: 10.1038/s41370-021-00297-6

8. Weller S., May M., McCredden J., Leach V., Phung D., Belyaev I. Comment on "5G mobile networks and health-a state-of-the-science review of the research into low-level RF fields above 6 GHz" by Karipidis et al. *J. Expo. Sci. Environ. Epidemiol.*, 2023, vol. 33, no. 1, pp. 17–20. DOI: 10.1038/s41370-022-00497-8

9. Tripathi S.R., Miyata E., Ishai P.B., Kawase K. Morphology of human sweat ducts observed by optical coherence tomography and their frequency of resonance in the terahertz frequency region. *Sci. Rep.*, 2015, vol. 5, pp. 9071. DOI: 10.1038/srep09071

10. Betzalel N., Ben Ishai P., Feldman Y. The human skin as a sub-THz receiver – Does 5G pose a danger to it or not? *Environ. Res.*, 2018, vol. 163, pp. 208–216. DOI: 10.1016/j.envres.2018.01.032

11. Yashchenko S.G., Rybalko S.Yu. Morphological structure of rat epiphysis exposed to electromagnetic radiation from communication devices. *Gigiena i sanitariya*, 2016, vol. 95, no. 10, pp. 977–979. DOI: 10.18821/0016-9900-2016-95-10-977-979 (in Russian).

12. Bhargav H., Srinivasan T.M., Bista S., Mooventhan A., Suresh V., Hankey A., Nagendra H.R. Acute effects of mobile phone radiations on subtle energy levels of teenagers using electrophotonic imaging technique: A randomized controlled study. *Int. J. Yoga*, 2017, vol. 10, no. 1, pp. 16–23. DOI: 10.4103/0973-6131.186163

13. Grigoryev Yu.G., Vorontsova Z.A., Ushakov I.B. Evaluation of the Hazards of Electromagnetic Fields on the Morphofunctional Condition of the Thyroid Gland. *Radiatsionnaya biologiya. Radioekologiya*, 2020, vol. 60, no. 6, pp. 622–626. DOI: 10.31857/S0869803120050112 (in Russian).

14. Grigor'ev Yu.G., Horseva N.I., Grigor'ev P.E. The Thyroid – a New Critical Body for Impacting Electromagnetic Fields Mobile Communications: Assessment of Possible Effects for Children and Adolescents. *Medical Radiology and Radiation Safety*, 2021, vol. 66, no. 2, pp. 67–75 (in Russian).

15. Asl J.F., Larijani B., Zakerkish M., Rahim F., Shirbandi K., Akbari R. The possible global hazard of cell phone radiation on thyroid cells and hormones: a systematic review of evidences. *Environ. Sci. Pollut. Res. Int.*, 2019, vol. 26, no. 18, pp. 18017–18031. DOI: 10.1007/s11356-019-05096-z

16. Alkayyali T., Ochuba O., Srivastava K., Sandhu J.K., Joseph C., Ruo S.W., Jain A., Waqar A., Poudel S. An Exploration of the Effects of Radiofrequency Radiation Emitted by Mobile Phones and Extremely Low Frequency Radiation on Thyroid Hormones and Thyroid Gland. *Histopathology. Cureus*, 2021, vol. 13, no. 8, pp. e17329.

17. Hajioun B., Jowhari H., Mokhtari M. Effects of cell phone radiation on the levels of T3, T4 and TSH, and histological changes in thyroid gland in rats treated with Allium sativum extract. *Afr. J. Biotechnol.*, 2014, vol. 13, no. 1, pp. 163–169. DOI: 10.5897/AJB2013.12471

18. Eşmekaya M.A., Seyhan N., Ömeroğlu S. Pulse modulated 900 MHz radiation induces hypothyroidism and apoptosis in thyroid cells: a light, electron microscopy and immunohistochemical study. *Int. J. Radiat. Biol.*, 2010, vol. 86, no. 12, pp. 1106–1116. DOI: 10.3109/09553002.2010.502960

19. Shaukat F., Qamar K., Ahmed Butt S. Effects of mobile phone induced electromagnetic field on height of follicular cells in thyroid gland of mice. *J. Rawalpindi Med. Coll.*, 2011, vol. 15, no. 1, pp. 27–29.

20. Misa-Agustiño M.J., Jorge-Mora T., Jorge-Barreiro F.J., Suarez-Quintanilla J., Moreno-Piquero E., Ares-Pena F.J., López-Martín E. Exposure to non-ionizing radiation provokes changes in rat thyroid morphology and expression of HSP-90. *Exp. Biol. Med. (Maywood)*, 2015, vol. 240, no. 9, pp. 1123–1135. DOI: 10.1177/1535370214567611

21. Eskander E.F., Estefan S.F., Abd-Rabou A.A. How does long term exposure to base stations and mobile phones affect human hormone profiles? *Clin. Biochem.*, 2012, vol. 45, no. 1–2, pp. 157–161. DOI: 10.1016/j.clinbiochem.2011.11.006

22. Mortavazi S., Habib A., Ganj-Karami A., Samimi-Doost R., Pour-Abedi A., Babaie A. Alterations in TSH and Thyroid Hormones following Mobile Phone Use. *Oman Med. J.*, 2009, vol. 24, no. 4, pp. 274–278. DOI: 10.5001/omj.2009.56

23. Baby N.M., Koshy G., Mathew A. The effect of electromagnetic radiation due to mobile phone use on thyroid fun3ction in medical students studying in a medical college in South India. *Indian J. Endocrinol. Metab.*, 2017, vol. 21, no. 6, pp. 797–802. DOI: 10.4103/ijem.IJEM_12_17

24. Elsayed N.M., Jastaniah S.D. Mobile phone use and risk of thyroid gland lesions detected by ultrasonography. *Open Journal of Radiology*, 2016, vol. 6, no. 2, pp. 140–146. DOI: 10.4236/ojrad.2016.62021

25. Breusenko D.V., Dimov I.D., Klimenko E.S., Karelina N.R. Modern concepts of thymus morphology. *Pediatr*, 2017, vol. 8, no. 5, pp. 91–95. DOI: 10.17816/PED8591-95 (in Russian).

26. Kulaber A., Kerimoğlu G., Ersöz Ş., Çolakoğlu S., Odacı E. Alterations of thymic morphology and antioxidant biomarkers in 60-day-old male rats following exposure to a continuous 900 MHz electromagnetic field during adolescence. *Biotech. Histochem.*, 2017, vol. 92, no. 5, pp. 331–337. DOI: 10.1080/10520295.2017.1312525

27. Kaya E., Kırımlıoğlu E., Er H., Okan Oflamaz A., Özen Ş., Demir N. Can 900 MHz and 2100 MHz radiofrequency radiation exposure induce endoplasmic reticulum stress and apoptosis in rat thymus? *Eur. Res. J.*, 2023, vol. 9, no. 1, pp. 29–38. DOI: 10.18621/eurj.1049381

28. Misa-Agustiño M.J., Leiro-Vidal J.M., Gomez-Amoza J.L., Jorge-Mora M.T., Jorge-Barreiro F.J., Salas-Sánchez A.A., Ares-Pena F.J., López-Martín E. EMF radiation at 2450 MHz triggers changes in the morphology and expression of heat shock proteins and glucocorticoid receptors in rat thymus. *Life Sci.*, 2015, vol. 127, pp. 1–11. DOI: 10.1016/j.lfs.2015.01.027

29. Kocaman A., Gül M., Yurt K.K., Altun G., Zayman E., Kıvrak E.G. Does omega-3 have a protective effect on the rat adrenal gland exposed to 900 MHz electromagnetic fields? *J. Microsc. Ultrastruct.*, 2017, vol. 5, no. 4, pp. 185–190. DOI: 10.1016/j.jmau.2017.08.003

30. Shahabi S., Hassanzadeh Taji I., Hoseinnezhaddarzi M., Mousavi F., Shirchi S., Nazari A., Zarei H., Pourabdolhossein F. Exposure to cell phone radiofrequency changes corticotrophin hormone levels and histology of the brain and adrenal glands in male Wistar rat. *Iran. J. Basic Med. Sci.*, 2018, vol. 21, no. 12, pp. 1269–1274. DOI: 10.22038/ijbms.2018.29567.7133

31. Sepehrimanesh M., Azarpira N., Saeb M., Nazifi S., Kazemipour N., Koohi O. Pathological changes associated with experimental 900-MHz electromagnetic wave exposure in rats. *Comp. Clin. Path.*, 2014, vol. 23, pp. 1629–1631. DOI: 10.1007/s00580-013-1835-0

32. Ma H.R., Ma Z.H., Wang G.Y., Song C.M., Ma X.L., Cao X.H., Zhang G.H. Impacts of exposure to 900 MHz mobile phone radiation on liver function in rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*, 2015, vol. 31, no. 6, pp. 567–571.

33. Okatan D.Ö., Kulaber A., Kerimoglu G., Odacı E. Altered morphology and biochemistry of the female rat liver following 900 megahertz electromagnetic field exposure during mid to late adolescence. *Biotech. Histochem.*, 2019, vol. 94, no. 6, pp. 420–428. DOI: 10.1080/10520295.2019.1580767

34. Al-Glaib B., Al-Dardfi M., Al-Tuhami A., Elgenaidi A., Dkhil M. A technical report on the effect of electromagnetic radiation from a mobile phone on mice organs. *Libyan J. Med.*, 2008, vol. 3, no. 1, pp. 8–9. DOI: 10.4176/080107

35. Holovská K., Almášiová V., Cigánková V., Beňová K., Račeková E., Martončíková M. Structural and ultrastructural study of rat liver influenced by electromagnetic radiation. *J. Toxicol. Environ. Health A*, 2015, vol. 78, no. 6, pp. 353–356. DOI: 10.1080/15287394.2014.979272

36. Chauhan P., Verma H.N., Sisodia R., Kesari K.K. Microwave radiation (2.45 GHz)-induced oxidative stress: Whole-body exposure effect on histopathology of Wistar rats. *Electromagn. Biol. Med.*, 2016, vol. 36, no. 1, pp. 20–30. DOI: 10.3109/15368378.2016.1144063

37. Adebayo E.A., Adeeyo A.O., Ogundiran M.A., Olabisi O. Bio-physical effects of radiofrequency electromagnetic radiation (RF-EMR) on blood parameters, spermatozoa, liver, kidney and heart of albino rats. *J. King Saud Univ. – Sci.*, 2019, vol. 31, no. 4, pp. 813–821. DOI: 10.1016/j.jksus.2018.11.007

38. Odacı E., Özyılmaz C. Exposure to a 900 MHz electromagnetic field for 1 hour a day over 30 days does change the histopathology and biochemistry of the rat testis. *Int. J. Radiat. Biol.*, 2015, vol. 91, no. 7, pp. 547–554. DOI: 10.3109/09553002.2015.1031850

39. Saygin M., Caliskan S., Karahan N., Koyu A., Gumral N., Uguz A. Testicular apoptosis and histopathological changes induced by a 2.45 GHz electromagnetic field. *Toxicol. Ind. Health*, 2011, vol. 27, no. 5, pp. 455–463. DOI: 10.1177/0748233710389851

40. Shokri S., Soltani A., Kazemi M., Sardari D., Mofrad F.B. Effects of Wi-Fi (2.45 GHz) Exposure on Apoptosis, Sperm Parameters and Testicular Histomorphometry in Rats: A Time Course Study. *Cell J.*, 2015, vol. 17, no. 2, pp. 322–331. DOI: 10.22074/cellj.2016.3740

41. Alchalabi A.S.H., Rahim H., Aklilu E., Al-Sultan I.I., Aziz A.R., Malek M.F., Ronald S.H., Khan M.A. Histopathological changes associated with oxidative stress induced by electromagnetic

waves in rats' ovarian and uterine tissues. *Asian Pac. J. Reprod.*, 2016, vol. 5, no. 4, pp. 301–310. DOI: 10.1016/j.apjr.2016.06.008

Khorseva N.I., Grigoriev P.E. Systemic effects of radiofrequency electromagnetic fields (review). Part 1. Secretion glands. Health Risk Analysis, 2025, no. 2, pp. 175–184. DOI: 10.21668/health.risk/2025.2.15.eng

Received: 06.03.2025
Approved: 25.04.2025
Accepted for publication: 14.06.2025

UDC 613.632.2; 615.916
DOI: 10.21668/health.risk/2025.2.16.eng

Read online

Review

UPDATE ON PULMONO-, HEPATO-, AND CARDIOTOXICITY OF NANOPARTICLES *IN VIVO:* A LITERATURE REVIEW

N.A. Gertan[1], M.P. Sutunkova[1,2], L.V. Shabardina[1], T.V. Makhorina[1], K.M. Nikogosyan[1], R.F. Minigalieva[1,3]

[1]Yekaterinburg Medical Research Center for Prevention and Occupational Health of Industrial Workers, 30 Popov St., Yekaterinburg, 620014, Russian Federation
[2]Ural State Medical University, 3 Repin St., Yekaterinburg, 620028, Russian Federation
[3]Ural Federal University, 51 Lenin Ave., Yekaterinburg, 620000, Russian Federation

A wide use of nanoparticles (NPs) in various industries, agriculture, science, medicine and cosmetology, as well as their omnipresence in the environment necessitate a comprehensive study of their effects on living systems to predict health risks and develop preventive measures. In this study, we aimed to study and systematize available scientific evidence of toxic effects of nanoparticles on the lungs, liver, and heart.

The search for publications issued in 2022–2024 was carried out in Russian (eLIBRARY.RU) and foreign (PubMed, Google Scholar) databases and electronic libraries. Articles containing information on health effects of particles in the 1–100 nanometer range were eligible for inclusion in the review while descriptions of in vitro, in silico, and epidemiological studies were excluded. Of more than 150 articles screened, we selected 31 full-text in vivo study publications (including one preprint) and 18 articles describing the identified effects.

Toxic effects of nanoparticles are attributed to their unique properties and depend on numerous factors, including chemical composition, size, and shape of nanoparticles, their concentration, exposure duration, and ability to cross internal barriers of the body. Adverse effects of nanoparticles are observed at all structural levels of the organism. Nanoparticles mainly induce inflammatory, dystrophic and necrotic changes. Closely interrelated inflammation and oxidative stress are the main mechanisms of toxicity.

Assessment and analysis of an array of experimental studies on potential risks of nanoparticle exposure at various structural levels make it possible to identify minute changes in organs for further development of a system of preventive measures aimed at increasing resistance to such NP-mediated pathological effects.

Keywords: *nanotoxicity, nanoparticles, intoxication, review, in vivo studies, lung, liver, heart.*

Nanoparticles (NPs) generated in the course of natural and, to a greater extent, anthropogenic processes enter the body through various environmental media and objects, including air, water, soil, food, and cosmetics, or directly by medical manipulations (diagnostics, nanoparticle-based drug delivery), all representing inhalation, oral, percutaneous or parenteral routes of exposure. This leads to structural and functional disorders in the human body, thereby increasing the risks to human health and necessitating measures to prevent negative effects. The risk of nanoparticle-related diseases and their severity depend on many factors, including particle size and migration

---

Natalia A. Gertan – Junior Researcher of the Department of Toxicology and Bioprophylaxis (e-mail: gertan00@mail.ru; tel.: +7 (343) 371-11-05; ORCID: https://orcid.org/0009-0008-3239-5998).

Marina P. Sutunkova – Doctor of Medical Sciences, director; Head of the Department of Occupational Hygiene and Medicine (e-mail: sutunkova@ymrc.ru; tel.: +7 (343) 371-20-87; ORCID: https://orcid.org/0000-0002-1743-7642).

Lada V. Shabardina – Junior Researcher of the Department of Toxicology and Bioprophylaxis (e-mail: lada.shabardina@mail.ru; tel.: +7 (343) 371-11-05; ORCID: https://orcid.org/0000-0002-8284-0008).

Tatyana V. Makhorina – Candidate of Medical Sciences, Senior Researcher of the Department of Toxicology and Bioprophylaxis (e-mail: lisa430@yandex.ru; tel.: +7 (343) 214-11-31; ORCID: https://orcid.org/0000-0001-6126-9706).

Karen M. Nikogosyan – Junior Researcher of the Department of Toxicology and Bioprophylaxis (e-mail: nikoghosyankm@ymrc.ru; tel.: +7 (343) 371-20-87; ORCID: https://orcid.org/0009-0003-0780-5733).

Regina F. Minigalieva – laboratory research assistant of the Department of Molecular Biology and Electron Microscopy (e-mail: regina.minigalieva@yandex.ru; tel.: +7 (343) 371-20-87; ORCID: https://orcid.org/0000-0003-0013-6111).

potential [1–3], cumulative properties [4–6], chemical composition [7], method of synthesis (chemical or biological) [8, 9], and exposure type (isolated or combined) [3, 10], level and duration [4, 11].

The ubiquity of NPs and their high biological activity accounting for their serious toxic effects on the body make it relevant to analyze and systematize recent data on toxicity of NPs and specifics of their pathogenesis at different levels of organization, which is also necessary for predicting health risks and further development of preventive measures. We have focused on the lungs, liver, and heart as the most studied of the main target organs for NP exposure; in addition, the NP-mediated disruption of their functioning poses a serious health threat.

We **aimed** to study and systematize available scientific evidence of toxic effects of nanoparticles on the lungs, liver, and heart.

Materials and methods. We applied information analysis techniques to systematize and summarize relevant findings. The bibliographic search was conducted in Russian and English in the databases and scientific electronic libraries (eLIBRARY.RU, PubMed, and Google Scholar) using the following keywords: toxicity, nanoparticles, size, pulmonary toxicity, cardiotoxicity, hepatotoxicity, *in vivo*. References contained in selected publications were screened for additional works to be included in the review. The inclusion criteria were the study of particles of various chemical compositions sized 1–100 nm, the presence or absence of a toxic effect of NPs on organs, and a publication date no earlier than 2022. The exclusion criteria were *in vitro, in silico* and epidemiological studies since their results are difficult to extrapolate to the organismal level. We selected five of 392 articles found on eLIBRARY.RU, one of nine found in PubMed, and 25 (including one preprint) of more than 17,000 search results on Google Scholar. Most of the studies reviewed were conducted in Russia, Egypt, China, and Iran, one each in Mexico, the United Kingdom, the UAE, Pakistan, Denmark, Türkiye, Japan, the United States, and the Republic of Korea. The review included studies of the following NPs: Ag – 11/43 (26 %), Si – 5/43 (12 %), Zn, Ti, and Al – 4/43 each (9 %), Cu – 3/43 (7 %), Pb, Fe, and Sn – 2/43 each (5 %), Ni, Au, Mo, Se, In, and Co – 1/43 each (2 %).

Results and discussion. Recent publications disclose various pathological manifestations of the toxic effect of NPs by various routes of entry, including inhalation, intratracheal, intranasal, intraperitoneal, oral, intravenous, and whole-body ones. Toxic effects were divided by selected organs and described from the molecular genetic to the organismal levels. Warm-blooded animals (rats and mice) and fish served as objects for *in vivo* studies.

Pulmonotoxic effects of nanoparticles. Starting from the molecular genetic level, changes in the bronchoalveolar lavage fluid (BALF) and blood were noticeable. An increase in the levels of malonic dialdehyde (MDA) and superoxide dismutase (SOD) in the BALF was noted after exposure to In/Sn NPs at 1.2, 3, 6 mg/kg b.w. [12], while $CoFe_2O_4$ NPs (5 mg/kg body weight (b.w.)), in addition to an increase in the MDA level, caused a decrease in that of reduced glutathione in the lung homogenate [13]. Such changes indicated the development of oxidative stress, which was closely associated with inflammation [14] induced by NPs. Thus, NPs (Al 20 and 40 mg/kg b.w. and In/Sn 1.2, 3, 6 mg/kg b.w.) caused a significant increase in the level of inflammatory cytokines (IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, and tumor necrosis factor-α (TNF-α)) in the BALF [12, 15]. Changes were also observed at the genetic level – the exposure to cobalt ferrite NPs ($CoFe_2O_4$) at the doses of 0.5 and 5 mg/kg b.w. increased the expression of *TNF-α* and *IL-1β* genes and decreased that of *IL-10* and the transforming growth factor beta-1 (*TGFβ-1*) [13], an important fibrogenic cytokine [16].

Exposures to nanoparticles of Al (1.70 mg/m^3) and In/Sn (1.2, 3, 6 mg/kg b.w.) induced an increase in the activity of lactate dehydrogenase (LDH) [17], aspartate aminotransferase (AST), and hydroxyproline [12] in blood. As for the BALF, the exposure to NPs of Sn (162 μg/mouse), Ti (162 μg/mouse), and In/Sn (1.2, 3, 6 mg/kg b.w.) induced a significant increase in the levels of total protein [18] and LDH [12], while that to Pb NPs (0.215 mg/m^3) in one of our previous studies accounted for an insignificant tendency towards an increase in alanine aminotransferase (ALT), AST, gamma-glutamyl transpeptidase (GGTP), and LDH [19]. LDH and total protein are known to be associated with pulmonary injury, inflammation, and permeability of the blood–air barrier [1, 12, 20]; GGT, being a membrane-bound enzyme, is involved in oxidative processes, and changes in AST and ALT in the BALF may indicate cellular injury [19]. Direct pulmonary damage and inflammation often develop into fibrotic changes, signs of which can be detected already at the molecular genetic level. An increase in hydroxyproline levels following the exposure to Si NPs (6.0 mg/kg b.w.) indicated collagen deposition and the development of fibrosis [21]. In addition, the relationship between oxidative stress and fibrosis was observed, as shown by the established positive correlation between hydroxyproline and MDA and nitric oxide (NO) and the negative one between that and glutathione, SOD, and catalase [22, 23]. It is important to note that fibrosis progression also correlated with the levels of interleukins IL-1β [24], IL-4 [25], and IL-13 [26] affected by NP exposures.

Changes at the molecular genetic level inevitably lead to changes at the cellular and tissue levels. When studying the cellular phagocytic activity of the respiratory tract after exposure to NPs of Pb (0.215 mg/m^3), Cu (4 mg/m^3, 2.6 and 12 μg/mouse), Al (54 μg/mouse, 20 and 40 mg/kg b.w.), Sn (162 μg/mouse), Zn (0.7 μg/mouse), Ti (162 μg/mouse), and In/Sn (1.2, 3, 6 mg/kg b.w.), an increase in the counts of neutrophils [2, 15, 18, 19], eosinophils [15], and alveolar macrophages [2, 12, 15] and a decrease in those of monocytes and lymphocytes [2, 19], alveolar macrophages in the BALF [19] were established. The study of Si NPs (3 and 6 mg/kg b.w.) found an increase in the proportion of alveolar and interstitial macrophages, while those of natural killers (NK cells), neutrophils, and monocytes were decreased [21]. Fluctuations in counts of phagocytic cells can be explained by their redistribution in tissues, increased activity followed by exhaustion, and different experimental conditions. Changes in cellular phagocytic activity and a decrease in the ratio of segmented neutrophils to alveolar macrophages observed in the studies indicate cytotoxic and inflammatory effects of NPs and activation of the immune response [2, 19]. In addition, a dose-dependent increase in inducible nitric oxide synthetase (iNOS) and Cox-2 ($CoFe_2O_4$ 0.5 and 5 mg/kg b.w.) [13], which are markers of modulation of the anti-inflammatory response [16, 27], was observed, also confirming activation of immune protection in response to toxic damage. Thus, activation of immune response can be added to the mechanisms of NP toxicity.

At the tissue and organ levels, post-exposure histological preparations of the rat lung showed epithelialization and proliferation of type 2 pneumocytes, hyperemia (Si 900 mg/kg b.w./day) [28] and pulmonary edema (Mo 1.84 mg/m^3, Cu 1.2–1.4 mg/m^3) [1, 2], brown pigmentation of macrophages, foci of emphysema and exudation of erythrocytes into the lumen of the alveoli, hemorrhagic infarction (Cu 1.2–1.4 mg/m^3, Al 1.70 mg/m^3) [2, 17], foci of interstitial inflammation (Mo 1.84 mg/m^3, Cu 1.2–1.4 mg/m^3, Al 20, 40, 100 mg/kg b.w., $CoFe_2O_4$ 0.5 mg/kg b.w.) [1, 2, 12, 13, 15, 29]. Besides, NP exposure induced collagen deposition in the lungs and alveolar wall thickening (Si 3 mg/kg b.w. and 6 mg/kg b.w. and In/Sn 1.2, 3, 6 mg/kg b.w.) [12, 21], which, together with changes in the levels of MDA and inflammatory cytokines, confirms the effect of NPs on the development of fibrotic changes in the lungs through inflammatory and oxidative damage.

No reported changes at the organismal level were found in the reviewed articles; some studies demonstrated no significant damage either (Si 0.125 mg/kg b.w., Ag 200 μg/kg b.w.)[1] [30]. Yet, the evidence of disturbances at other levels significantly increases the likelihood of pulmonary and other respiratory diseases. Differences in the findings may be attributed to a number of factors, such as physicochemical properties of nanoparticles, the administered dose, the route and duration of exposure, methods of detection, and the types of experimental animals.

Hepatotoxic effects of nanoparticles. Liver is an important target organ for many toxicants. It performs barrier and storage functions, which makes it more vulnerable to the damaging effects of NPs [4]. At the molecular genetic level, exposures to nanoparticles of Al (100 mg/kg b.w.), Si (500 μg/kg b.w. and 1.5, 3.0, and 6.0 mg/kg b.w.), Ni (1, 20, 150 mg/kg b.w.), Ag (50 mg/kg b.w., 200 ppb, and 5 μg/kg b.w.), Ag + Zn (50 + 30 mg/kg b.w.), and Fe (100 mg/kg b.w.) were found to increase the levels of reactive oxygen species (ROS) [7, 31–33], 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG) – the main product of DNA oxidation [34], substances that react with thiobarbituric acid [31], nitric oxide (NO) [35], MDA [7, 29, 32, 35–37], and oxidized glutathione [32], and to decrease those of reduced glutathione [32, 37], catalase [29, 31, 35–37], peroxidase [29, 31, 35, 36], and SOD [29, 31, 35, 36] in the liver. Another study, on the contrary, showed an increase in SOD and catalase levels attributed by the authors to the activation of a protective feedback mechanism [34]. An imbalance between pro-oxidants and antioxidants causing disruption of cell membrane integrity and DNA damage was clearly registered [38]. In addition to oxidative stress, endoplasmic reticulum (ER) stress was induced in liver tissues. Elevated levels of immunoglobulin heavy chain binding protein were observed in the liver following exposure to Si NPs at 1.5, 3.0, and 6.0 mg/kg b.w. The same study revealed ER abnormalities, such as expansion of the ER space, detachment of ribosomes, disruption of ER integrity and structure [32].

The inflammatory response to nanoparticle exposures (Ti 50 mg/kg b.w., Ag 50 mg/kg b.w. and 200 ppb, Ag+Zn 50 + 30 mg/kg b.w., Ni 1, 20, 150 mg/kg b.w.) was accompanied by changes in the levels of inflammatory cytokines as follows: TNF-α [7, 35, 37], IL1β [7, 36, 37], IL-6 [7, 37] increased while IL-10 [37] decreased. In addition, exposures to nanoparticles of Al (1.70 mg/m^3, 100 mg/kg b.w.), Se (0.2 mg/kg b.w.), Si (250 mg/kg b.w., 500 μg/kg b.w.), Ti (50 mg/kg b.w.), Ag (10% b.w.), Ni (1, 20, 150 mg/kg b.w.) and Fe (100 mg/kg b.w.) induced an increase in the activity of ALT [4, 7, 8, 17, 29, 31, 35–37], AST[2] [4, 7, 17, 29, 31, 35–37] and LDH [36], in the level of lysophosphatidylinositols [4] and direct bilirubin [17, 29], but a decrease in the concentrations of alkaline phosphatase (ALP) [4], succinate dehydrogenase [4], levels of bile and glycocholic acids [4], albumin [31, 35, 36], total protein [36], and creatinine [6] in the blood serum. According to other data, concentrations of ALP [17, 29, 31, 36] and albumin [11] increased. Similar changes

---

[1] Cao X., Xie B., Xu M., Li J., Dai X., Tian Y., Zhang J., Chen Y. [et al.]. Toxicity study of silica nanoparticles following 94-day repeated oral administration in Sprague Dawley rats: preprint (Version 1). *Research Square*, 2024. DOI: 10.21203/rs.3.rs-4531919/v1

[2] Ibid.

were observed in liver homogenates, e.g. a significant dose-dependent increase in the activity of hepatic LDH, ALT, and AST (due to Si NP exposures at 1.5, 3.0, and 6.0 mg/kg b.w.) [32]. Increased values of transaminases and alkaline phosphatase in the liver indicated inflammation, dysfunction of hepatocyte mitochondria, and oxidative or nitrosative stress [31].

Changes in lipid metabolism were found to be characteristic of toxic damage to the liver. Si NPs at the doses of 1.5, 3.0, and 6.0 mg/kg b.w. caused an increase in the expression of mRNA levels of the *Fasn*, *Elovl6*, and *Scd1* genes associated with fatty acid synthesis [32, 39, 40], a decrease in the expression of the *Cpt1a*, *Acox1*, and *Ppara* genes involved in beta-oxidation of fatty acids [32, 41], and *Scarb1*, *Abca1*, *Abcg1*, and *Lxra* genes involved in reverse cholesterol transport [32, 42]. Lipid metabolism disorders were also established after exposure to nanoparticles of Al (100 mg/kg b.w.), Ti (50 mg/kg b.w.), Si (1.5, 3.0, and 6.0 mg/kg b.w. and 1,000 mg/kg b.w.), Ag (5, 10, and 15 ml/L), and Ni (1, 20, 150 mg/kg b.w.). The lipid spectrum showed an increase in the levels of triglycerides [6, 11, 29, 32, 35], cholesterol [11, 32, 35, 36], and low- and very low-density lipoproteins [29, 35] with a decrease in those of high-density lipoproteins [29, 35]. Changes in beta-oxidation of fatty acids were noted after the exposure to Se NPs at doses of 0.2, 1, and 2 mg/kg b.w. as assessed by altered blood levels of acylcarnitines and their derivatives in rats [4]. Ag NPs (200 ppb) increased the production of angiotensin-converting enzyme (ACE) in the liver [34]. The accumulation of ACE and fatty acid esters in the blood, an increased level of lysophosphatidylethanolamines, and decreased levels of bile and glycocholic acids indicated suppression of the secretory function of the liver and its damage [4, 34].

Apoptosis is yet another mechanism of the toxic effect of NPs. Animal experiments demonstrated that the exposures to Si, Ni, Ag, and Zn NPs increased the expression of the *Bax* (Si 900 mg/kg b.w., Ni 1, 20, 150 mg/kg b.w.), *Bcl-2* (Si 600 mg/kg b.w.), *Caspase3* (Cas-3) (Si 900 mg/kg b.w., Ni 1, 20, 150 mg/kg b.w., Ag 50 mg/kg b.w., Ag+Zn 50 + 30 mg/kg b.w.), and *p53* (Ni 1, 20, 150 mg/kg b.w.) genes in rats [28, 36, 37]. The level of *Bcl-2* decreased following the exposure to Ni NPs [36] due to the suppression of the anti-apoptotic system. Changes in the expression of these genes confirmed the development of apoptosis [43, 44].

Oxidative stress, inflammation, and apoptosis cause changes at other levels of the body. Thus, at the cellular level, degenerative dystrophic changes in the nucleus (karyorrhexis, karyopiconosis, karyolysis) and cytoplasm (vacuolization), as well as aniokaryolysis and cytoplasmic hypertrophy were observed in rat hepatocytes after exposure to NPs of Si (500 µg/kg b.w.) and Ag (10 % b.w., 50 mg/kg b.w., 5 µg/kg b.w., 0.04 mg/L^{-1}), [8, 10, 31, 33, 37]. NP exposure also induces mitochondrial disorders. Nanoparticles of Se (2 mg/kg b.w.) and Si (1.5, 3.0, and 6.0 mg/kg b.w.), for instance, caused a decrease in the ratio of normal mitochondria [4], their deformation, rupture, and disappearance of cristae [32]. In addition, NPs of Se (1 mg/kg b.w., 2 mg/kg b.w.), Al (100 mg/kg b.w.), Si (500 µg/kg b.w.), and Ag (0.04 mg/L^{-1}) increased the proportion of degenerated hepatocytes, as well as the number of aneuploid hepatocytes and Kupffer cells [4, 10, 29, 31]. Pathological changes were manifested by hepatocyte disorganization (Al 100 mg/kg b.w., Si 500 µg/kg b.w.) [29, 31], infiltration with inflammatory cells (Si 500 mg/kg b.w., 1.5, 3.0, and 6.0 mg/kg b.w., Al 100 mg/kg b.w., 18 and 54 µg/mouse)[3] [18, 29, 32], macrophage aggregates (Sn 54 and 162 µg/mouse, Ti 162 µg/mouse, Ag 5, 10, 15 ml/L)

[3] Cao X., Xie B., Xu M., Li J., Dai X., Tian Y., Zhang J., Chen Y. [et al.]. Toxicity study of silica nanoparticles following 94-day repeated oral administration in Sprague Dawley rats: preprint (Version 1). *Research Square*, 2024. DOI: 10.21203/rs.3.rs-4531919/v1

[11, 18], lymphocytes, and foreign materials (Sn 54 and 162 μg/mouse, Ti 162 μg/mouse) [18].

Inflammation-induced dystrophies were noted at the tissue and organ levels. Thus, NP exposures (Mo 1.84 mg/m^3, Ag 5, 10, 15 ml/L) caused widespread hydropic [1], hyaline-droplet [1] and fatty dystrophies of the liver [1, 11]. Necrotic changes were registered in some cases with necrotic zones observed in the liver parenchyma (Al 100 mg/kg b.w., Si 500 μg/kg b.w., Si 900 mg/kg b.w./day and Ag 5, 10, 15 ml/L) [11, 28, 29, 31], including the bile ducts (Al 100 mg/kg b.w., Ti 50 mg/kg b.w.) [29, 35]. As in the lungs, fibrotic changes were expressed including deposition of collagen fibers in the pericentral and periportal areas (Al 100 mg/kg b.w., Ti 50 mg/kg b.w.) [29, 35], increased deposition of lipids and collagen in the liver tissue (Si 1.5, 3.0, and 6.0 mg/kg b.w.) [32]. One of the studies of liver histomorphology also showed that the degree of damage depended on the route of administration: oral administration of Ni NPs (150 mg/kg b.w.) induced congestion, cellular degeneration, and infiltration of mononuclear cells in the liver; intraperitoneal administration (20 mg/kg b.w.), in addition to infiltration of mononuclear cells, caused hemorrhage, proliferation of Kupffer cells, sinusoidal expansion, and necrosis, while intravenous administration at 1 mg/kg b.w. induced similar disorders, except for necrosis [36].

Liver toxicity of NPs is mainly manifested by inflammatory, dystrophic, fibrotic, and necrotic changes. We did not evaluate disorders at the organismal level but the suppression of liver functioning increases the risks of developing many diseases, including those of other organs and systems, owing to the great importance of the functions it performs. Only one study (Ag 200 μg/kg b.w.) [30] found no toxic effect on the liver, which might be associated with both the properties of the substance itself and the experimental conditions.

Cardiotoxic effects of nanoparticles. A number of studies, including one of ours [45], revealed cardiac toxicity of nanoparticles. At the molecular genetic level, an increase in oxidative and nitrosative stress was observed under effect of NPs (Ag 0.5 mg/kg b.w., Cu 400 mg/kg b.w., and Fe 100 mg/kg b.w.), including a significant increase in the levels of ROS, MDA [7], lipid peroxidation, reduced glutathione, oxidized glutathione [46], total NO concentration [46, 47], total thiol, thiobarbituric acid reactive substances, with decreased levels of SOD, catalase and reduced glutathione [47] in cardiac homogenates. Signs of inflammation and apoptosis were registered. In the study of Ag NP exposure (0.5 mg/kg b.w.), a significant increase in IL-6 concentrations was observed [46], while the study with Ti and Zn NPs (10 mg/kg b.w.) showed a trend towards an increase in TNF-α production. Changes in the production of apoptotic and autophagy-related proteins, i.e. a decrease in Cyt-C and Bcl-2 and an increase in LC3B, Beclin-1, Cas-3 and Cas-9, as well as an increase in calcium levels, were also detected and likely associated with mitochondrial damage [48].

Oxidative stress caused myocardial tissue damage and inflammation, which in turn led to tissue damage and aggravated oxidative stress [46]. This forms a vicious circle that begins at the cellular level – most often with mitochondrial structure disorders – and ends with histomorphological changes in the organ, which subsequently leads to cardiac dysfunction.

Under exposure to nanoparticles of Al (1.70 mg/m^3), Si (250 mg/kg b.w., 500 μg/kg b.w.), Ag (0.5 mg/kg b.w.), Pb (2.32 mg/kg b.w.), Cd (0.22 mg/kg b.w.), and Cu (400 mg/kg b.w.), a decrease in troponin [17] and AST[4] [17, 45] and an increase in LDH [17, 46, 47], myoglobin [47] and creatine kinase MB [46, 47] were observed in rats and mice. In some studies,

---

[4] Cao X., Xie B., Xu M., Li J., Dai X., Tian Y., Zhang J., Chen Y. [et al.]. Toxicity study of silica nanoparticles following 94-day repeated oral administration in Sprague Dawley rats: preprint (Version 1). *Research Square*, 2024. DOI: 10.21203/rs.3.rs-4531919/v1

on the contrary, NPs of Pb (2.5 mg/kg b.w.) and Cu (400 mg/kg b.w.) decreased the activity of creatine kinase and LDH [45] and increased AST [47]. Such changes indicate cellular damage to the heart induced by nanoparticles, and the fluctuations in the indicators can be explained by different phases of the response to damage and dissimilar experimental conditions.

At the cellular level, the exposure to Pb NPs at 2.5 mg/kg b.w. resulted in some loss of myofibrils, destruction of the internal space of mitochondria and, as a consequence, a decrease in the strength of isometric contractions of isolated myocardial preparations [45]. This is consistent with the results of assessing mitochondrial function in rats – Ti and Zn NPs at doses of 10 mg/kg b.w. accelerated oxygen consumption by cardiac mitochondria, disuniting them. In addition, areas of sarcomere disorganization with a loss of ultrastructural alignment, small disorganized mitochondria, elementary particles and lysosomes inside, as well as signs of apoptosis were observed [48].

At the tissue and organ levels, the development of dystrophy was characteristic of both the heart and the liver: initial signs of myocardial dystrophy were noted following the exposure to Pb NPs at 2.5 mg/kg b.w. In the study of Cu NPs (100 mg/kg b.w.), pronounced myocardial hypertrophy, moderate congestion and severe focal necrosis of cardiomyocytes with inflammatory cellular infiltration were observed [47]. Fibrotic changes in the form of collagen accumulations were registered in one study (Zn and Ti NPs 10 mg/kg b.w.) [48], while another found no morphological changes in the heart structures after exposure to Ag NPs (0.5 mg/kg b.w.) [46]. This can be explained by the use of a polyethyleneglycol coating of Ag NPs, which mitigates the toxic effect of the latter on the organism. Yet, the rat exposure to Si NPs (without coating) at the dose of 500 mg/kg b.w. induced no significant changes,[5] possibly due to the experimental conditions.

Even small changes in the heart can provoke its functional disorders. Thus, at the organismal level, a tendency towards an increase in the QT interval, T wave amplitude, and QRS duration was observed in rats following the exposure to Pb NPs at the dose of 2.5 mg/kg b.w. [45]. Thus, NPs contribute to the development of cardiovascular diseases, thereby increasing the risks to population health.

Conclusions. The presented review of scientific literature systematizes and summarizes data on pulmonary, hepato- and cardiotoxic effects of nanoparticles at different levels of organization revealing nanoparticle exposure as a significant risk factor for population health. The review also highlights toxic effects of NPs by routes and levels of exposure and chemical nature of the toxicant determining the degree of risk of development and severity of induced pathological conditions.

The main mechanisms of toxicity include closely related direct damage, genotoxicity, mitochondrial damage, apoptosis, inflammation and oxidative stress: as a rule, nanoparticle exposure induces changes in the levels of damage biomarkers, geno- and cytotoxic phenomena, excessive ROS production, an increase in the levels of inflammation mediators and tissue damage, which can cause diseases not only of the organs under study, but also of some body systems. Assessment and analysis of the array of experimental studies on the potential risks of NPs at various structural levels can be used to identify patterns of such exposure and develop criteria and methods for hygienic assessment of risks to public health, as well as for further development of a system of preventive measures aimed at increasing the body resistance to toxic effects of nanoparticles on the lungs, liver, and heart.

Funding. This research received no external funding.

Conflict of interest. The authors have no conflicts of interest to declare.

[5] Cao X., Xie B., Xu M., Li J., Dai X., Tian Y., Zhang J., Chen Y. [et al.]. Toxicity study of silica nanoparticles following 94-day repeated oral administration in Sprague Dawley rats: preprint (Version 1). *Research Square*, 2024. DOI: 10.21203/rs.3.rs-4531919/v1

References

1. Zaitseva N.V., Zemlyanova M.A., Stepankov M.S., Ignatova A.M., Nikolaeva A.E., Pustovalova O.V. Issledovanie i sravnitel'naya otsenka toksichnosti nanochastits oksida molibdena (VI) pri mnogokratnoi ingalyatsionnoi ekspozitsii krys [Study and comparative evaluation of toxicity of molybdenum (VI) oxide nanoparticles upon repeated inhalation exposure in rats]. *Rossiiskie nanotekhnologii*, 2023, vol. 18, no. 2, pp. 260–267. DOI: 10.56304/S1992722323020139 (in Russian).

2. Stepankov M.S. Peculiarities of bioaccumulation and toxic effects produced by copper oxide (II) nanoparticles on the respiratory organs under inhalation exposure as opposed to their microsized chemical analogue: assessment for prevention purposes. *Health Risk Analysis*, 2023, no. 4, pp. 124–133. DOI: 10.21668/health.risk/2023.4.12.eng

3. Liu Z., Liu H., Vowden R., Hughes L., Qi D., Francis W., Perino G., Pink R. [et al.]. Combination of cobalt, chromium and titanium nanoparticles increases cytotoxicity in vitro and proinflammatory cytokines in vivo. *J. Orthop. Translat.*, 2022, vol. 38, pp. 203–212. DOI: 10.1016/j.jot.2022.10.013

4. Ryabova Yu.V., Sutunkova M.P., Chemezov A.I., Minigalieva I.A., Bushueva T.V., Shelomentsev I.G., Klinova S.V., Sakhautdinova R.R. Effects of selenium oxide nanoparticles on the morphofunctional state of the liver: experimental data. *Health Risk Analysis*, 2023, no. 1, pp. 147–156. DOI: 10.21668/health.risk/2023.1.14.eng

5. Windell D.L., Mourabit S., Moger J., Owen S.F., Winter M.J., Tyler C.R. The influence of size and surface chemistry on the bioavailability, tissue distribution and toxicity of gold nanoparticles in zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety*, 2023, vol. 260, pp. 115019. DOI: 10.1016/j.ecoenv.2023.115019

6. Akagi J.-I., Mizuta Y., Akane H., Toyoda T., Ogawa K. Oral toxicological study of titanium dioxide nanoparticles with a crystallite diameter of 6 nm in rats. *Part. Fibre Toxicol.*, 2023, vol. 20, no. 1, pp. 23. DOI: 10.1186/s12989-023-00533-x

7. Wu L., Wen W., Wang X., Huang D., Cao J., Qi X., Shen S. Ultrasmall iron oxide nanoparticles cause significant toxicity by specifically inducing acute oxidative stress to multiple organs. *Part. Fibre Toxicol.*, 2022, vol. 19, no. 1, pp. 24. DOI: 10.1186/s12989-022-00465-y

8. Ziaolhagh S.J., Ardakanizadeh M., Kaveh A., Yahyaei B. Liver tissue changes induced by biological and chemical silver nanoparticles in trained male Wistar rats. *J. Trace Elem. Med. Biol.*, 2023, vol. 79, pp. 127253. DOI: 10.1016/j.jtemb.2023.127253

9. Desai A.S., Singh A., Edis Z., Haj Bloukh S., Shah P., Pandey B., Agrawal N., Bhagat N. An in vitro and in vivo study of the efficacy and toxicity of plant-extract-derived silver nanoparticles. *J. Funct. Biomater.*, 2022, vol. 13, no. 2, pp. 54. DOI: 10.3390/jfb13020054

10. Mahjoubian M., Naeemi A.S., Moradi-Shoeili Z., Tyler C.R., Mansouri B. Toxicity of silver nanoparticles in the presence of zinc oxide nanoparticles differs for acute and chronic exposures in zebrafish. *Arch. Environ. Contam. Toxicol.*, 2023, vol. 84, no. 1, pp. 1–17. DOI: 10.1007/s00244-022-00965-0

11. Azadikhah D., Yalsuyi A.M., Saha S., Saha N.C., Faggio C. Biochemical and pathophysiological responses in Capoeta capoeta under lethal and sub-lethal exposures of silver nanoparticles. *Water*, 2023, vol. 15, no. 3, pp. 585. DOI: 10.3390/w15030585

12. Liu N., Guan Y., Zhou C., Wang Y., Ma Z., Yao S. Pulmonary and systemic toxicity in a rat model of pulmonary alveolar proteinosis induced by indium-tin oxide nanoparticles. *Int. J. Nanomedicine*, 2022, vol. 17, pp. 713–731. DOI: 10.2147/IJN.S338955

13. Hassanen E.I., Abdelrahman R.E., Aboul-Ella H., Ibrahim M.A., El-Dek S., Shaalan M. Mechanistic approach on the pulmonary oxido-inflammatory stress induced by cobalt ferrite nanoparticles in rats. *Biol. Trace Elem. Res.*, 2024, vol. 202, no. 2, pp. 765–777. DOI: 10.1007/s12011-023-03700-5

14. Mokhtari-Zaer A., Norouzi F., Askari V.R., Khazdair M.R., Roshan N.M., Boskabady M., Hosseini M., Boskabady M.H. The protective effect of Nigella sativa extract on lung inflammation and oxidative stress induced by lipopolysaccharide in rats. *J. Ethnopharmacol.*, 2020, vol. 253, pp. 112653. DOI: 10.1016/j.jep.2020.112653

15. Lim J.-O., Kim W.-I., Pak S.-W., Lee S.-J., Park S.-H., Shin I.-S., Kim J.-C. Toll-like receptor 4 is a key regulator of asthma exacerbation caused by aluminum oxide nanoparticles via regulation of NF-κB phosphorylation. *J. Hazard. Mater.*, 2023, vol. 448, pp. 130884. DOI: 10.1016/j.jhazmat.2023.130884

16. Gungor H., Ekici M., Onder Karayigit M., Turgut N.H., Kara H., Arslanbas E. Zingerone ameliorates oxidative stress and inflammation in bleomycin-induced pulmonary fibrosis: modulation of the expression of TGF-β1 and iNOS. *Naunyn Schmiedebergs Arch. Pharmacol.*, 2020, vol. 393, no. 9, pp. 1659–1670. DOI: 10.1007/s00210-020-01881-7

17. Zemlyanova M.A., Zaitseva N.V., Stepankov M.S. Peculiarities of toxic effects produced by aluminum oxide nano- and microparticles under multiple inhalation exposure. *Gigiena i sanitariya*, 2023, vol. 102, no. 5, pp. 502–508. DOI: 10.47470/0016-9900-2023-102-5-502-508 (in Russian).

18. Torero Gutierrez C., Loizides C., Hafez I., Brostrom A., Wolff H., Szarek J., Berthing T., Mortensen A. [et al.]. Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part. Fibre Toxicol.*, 2023, vol. 20, no. 1, pp. 4. DOI: 10.1186/s12989-023-00514-0

19. Sutunkova M.P., Minigalieva I.A., Klinova S.V., Ryabova Yu.V., Tazhigulova A.V., Shabardina L.V., Bateneva V.A., Shelomentsev I.G., Privalova L.I. Acute Toxicity Induced by Inhalation Exposure to Lead Oxide Nanoparticles in Rats. *ZNiSO*, 2023, vol. 31, no. 9, pp. 24–30. DOI: 10.35627/2219-5238/2023-31-9-24-30 (in Russian).

20. Poitout-Belissent F., Grant S.N., Tepper J.S. Aspiration and inspiration: Using bronchoalveolar lavage for toxicity assessment. *Toxicol. Pathol.*, 2021, vol. 49, no. 2, pp. 386–396. DOI: 10.1177/0192623320929318

21. Li Y., Yao Q., Xu H., Ren J., Zhu Y., Guo C., Li Y. Lung single-cell transcriptomics offers insights into the pulmonary interstitial toxicity caused by silica nanoparticles. *Environ. Health (Wash.)*, 2024, vol. 2, no. 11, pp. 786–801. DOI: 10.1021/envhealth.4c00052

22. Nabil A., Ali I., Shiha G., Zahran F. Correlation between oxidative stress and Hydroxyproline content in Liver Fibrosis. *Biochemistry Letters*, 2021, vol. 17, pp. 22–29. DOI: 10.21608/blj.2021.180487

23. Cui Y., Robertson J., Maharaj S., Waldhauser L., Niu J., Wang J., Farkas L., Kolb M., Gauldie J. Oxidative stress contributes to the induction and persistence of TGF-β1 induced pulmonary fibrosis. *Int. J. Biochem. Cell Biol.*, 2011, vol. 43, no. 8, pp. 1122–1133. DOI: 10.1016/j.biocel.2011.04.005

24. Li J., Xue J., Wang D., Dai X., Sun Q., Xiao T., Wu L., Xia H. [et al.]. Regulation of gasdermin D by miR-379-5p is involved in arsenite-induced activation of hepatic stellate cells and in fibrosis via secretion of IL-1β from human hepatic cells. *Metallomics*, 2019, vol. 11, no. 2, pp. 483–495. DOI: 10.1039/c8mt00321a

25. Batsaikhan B., Lu M.-Y., Yeh M.-L., Huang C.-I., Huang C.-F., Lin Z.-Y., Chen S.-C., Huang J.-F. [et al.]. Elevated interleukin-4 levels predicted advanced fibrosis in chronic hepatitis C. *J. Chin. Med. Assoc.*, 2019, vol. 82, no. 4, pp. 277–281. DOI: 10.1097/JCMA.0000000000000064

26. Wong S.-W., Ting Y.-W., Yong Y.-K., Tan H.-Y., Barathan M., Riazalhosseini B., Bee C.J., Tee K.-K. [et al.]. Chronic inflammation involves CCL11 and IL-13 to facilitate the development of liver cirrhosis and fibrosis in chronic hepatitis B virus infection. *Scand. J. Clin. Lab. Invest.*, 2021, vol. 81, no. 2, pp. 147–159. DOI: 10.1080/00365513.2021.1876245

27. Zhao Y., Yang Y., Liu M., Qin X., Yu X., Zhao H., Li X., Li W. COX-2 is required to mediate crosstalk of ROS-dependent activation of MAPK/NF-κB signaling with pro-inflammatory response and defense-related NO enhancement during challenge of macrophage-like cell line with Giardia duodenalis. *PLoS Negl. Trop. Dis.*, 2022, vol. 16, no. 4, pp. e0010402. DOI: 10.1371/journal.pntd.0010402

28. Firouzamandi M., Hejazy M., Mohammadi A., Shahbazfar A.A., Norouzi R. In vivo toxicity of oral administrated nano-SiO2: Can food additives increase apoptosis? *Biol. Trace Elem. Res.*, 2023, vol. 201, no. 10, pp. 4769–4778. DOI: 10.1007/s12011-022-03542-7

29. Abo-EL-Sooud K., Abd-Elhakim Y.M., Hashem M.M.M., El-Metwally A.E., Hassan B.A., El-Nour H.H.M. Ameliorative effects of quercetin against hepatic toxicity of oral sub-chronic co-exposure to aluminum oxide nanoparticles and lead-acetate in male rats. *Naunyn Schmiedebergs Arch. Pharmacol.*, 2023, vol. 396, no. 4, pp. 737–747. DOI: 10.1007/s00210-022-02351-y

30. Edan M.S., Sultan F.I., Attallah A.H., Haider A.J., Haider M.J., Tawfeeq A.T., Hussein N.N., Husain Khalif O. Effect of silver nanoparticles synthesized by pulsed laser ablation in liquid on the

hematological, hepatic, and renal functions of albino rats. *Iraqi Journal of Science*, 2023, vol. 64, no. 12, pp. 6242–6256. DOI: 10.24996/ijs.2023.64.12.13

31. Ali A., Saeed S., Hussain R., Afzal G., Siddique A.B., Parveen G., Hasan M., Caprioli G. Synthesis and characterization of silica, silver-silica, and zinc oxide-silica nanoparticles for evaluation of blood biochemistry, oxidative stress, and hepatotoxicity in albino rats. *ACS Omega*, 2023, vol. 8, no. 23, pp. 20900–20911. DOI: 10.1021/acsomega.3c01674

32. Abulikemu A., Zhao X., Xu H., Li Y., Ma R., Yao Q., Wang J., Sun Z. [et al.]. Silica nanoparticles aggravated the metabolic associated fatty liver disease through disturbed amino acid and lipid metabolisms-mediated oxidative stress. *Redox Biol.*, 2023, vol. 59, pp. 102569. DOI: 10.1016/j.redox.2022.102569

33. Yousof S.M., Erfan H., Hosny M.M., Shehata S.A., El-Sayed K. Subacute toxic effects of silver nanoparticles oral administration and withdrawal on the structure and function of adult Albino Rats' hepatic tissue. *Saudi J. Biol. Sci.*, 2022, vol. 29, no. 5, pp. 3890–3898. DOI: 10.1016/j.sjbs.2022.02.054

34. Nayek S., Lund A.K., Verbeck G.F. Inhalation exposure to silver nanoparticles induces hepatic inflammation and oxidative stress, associated with altered renin-angiotensin system signaling, in Wistar rats. *Environ. Toxicol.*, 2022, vol. 37, no. 3, pp. 457–467. DOI: 10.1002/tox.23412

35. Sallam M.F., Ahmed H.M.S., El-Nekeety A.A., Diab K.A., Abdel-Aziem S.H., Sharaf H.A., Abdel-Wahhab M.A. Assessment of the oxidative damage and genotoxicity of titanium dioxide nanoparticles and exploring the protective role of holy basil oil nanoemulsions in rats. *Biol. Trace Elem. Res.*, 2023, vol. 201, no. 3, pp. 1301–1316. DOI: 10.1007/s12011-022-03228-0

36. Adiguzel C., Karaboduk H., Apaydin F.G., Kalender S., Kalender Y. Comparison of nickel oxide nano and microparticles toxicity in rat liver: molecular, biochemical, and histopathological study. *Toxicol. Res. (Camb.)*, 2023, vol. 12, no. 5, pp. 741–750. DOI: 10.1093/toxres/tfad062

37. Shehata A.M., Salem F.M.S., El-Saied E.M., Abd El-Rahman S.S., Mahmoud M.Y., Noshy P.A. Evaluation of the ameliorative effect of zinc nanoparticles against silver nanoparticle-induced toxicity in liver and kidney of rats. *Biol. Trace Elem. Res.*, 2022, vol. 200, no. 3, pp. 1201–1211. DOI: 10.1007/s12011-021-02713-2

38. Pisoschi A.M., Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *Eur. J. Med. Chem.*, 2015, vol. 97, pp. 55–74. DOI: 10.1016/j.ejmech.2015.04.040

39. Junjvlieke Z., Khan R., Mei C., Cheng G., Wang S., Raza S.H.A., Hong J., Wang X. [et al.]. Effect of ELOVL6 on the lipid metabolism of bovine adipocytes. *Genomics*, 2020, vol. 112, no. 3, pp. 2282–2290. DOI: 10.1016/j.ygeno.2019.12.024

40. Pecka-Kiełb E., Kowalewska-Łuczak I., Czerniawska-Piątkowska E., Króliczewska B. FASN, SCD1 and ANXA9 gene polymorphism as genetic predictors of the fatty acid profile of sheep milk. *Sci. Rep.*, 2021, vol. 11, no. 1, pp. 23761. DOI: 10.1038/s41598-021-03186-y

41. Tahri-Joutey M., Andreoletti P., Surapureddi S., Nasser B., Cherkaoui-Malki M., Latruffe N. Mechanisms mediating the regulation of peroxisomal fatty acid beta-oxidation by PPARα. *Int. J. Mol. Sci.*, 2021, vol. 22, no. 16, pp. 8969. DOI: 10.3390/ijms22168969

42. Rozhkova A.V., Dmitrieva V.G., Nosova E.V., Dergunov A.D., Limborska S.A., Dergunova L.V. Genomic variants and multilevel regulation of ABCA1, ABCG1, and SCARB1 expression in atherogenesis. *J. Cardiovasc. Dev. Dis.*, 2021, vol. 8, no. 12, pp. 170. DOI: 10.3390/jcdd8120170

43. Hussar P. Apoptosis Regulators Bcl-2 and Caspase-3. *Encyclopedia*, 2022, vol. 2, no. 4, pp. 1624–1636. DOI: 10.3390/encyclopedia2040111

44. Westphal D., Kluck R.M., Dewson G. Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis. *Cell Death Differ.*, 2014, vol. 21, no. 2, pp. 196–205. DOI: 10.1038/cdd.2013.139

45. Minigaliyeva I.A., Klinova S.V., Sutunkova M.P., Ryabova Y.V., Valamina I.E., Shelomentsev I.G., Shtin T.N., Bushueva T.V. [et al.]. On the mechanisms of the cardiotoxic effect of lead oxide nanoparticles. *Cardiovasc. Toxicol.*, 2024, vol. 24, no. 1, pp. 49–61. DOI: 10.1007/s12012-023-09814-5

46. Nemmar A., Al-Salam S., Greish Y.E., Beegam S., Zaaba N.E., Ali B.H. Impact of intratracheal administration of polyethylene glycol-coated silver nanoparticles on the heart of normotensive and hypertensive mice. *Int. J. Mol. Sci.*, 2023, vol. 24, no. 10, pp. 8890. DOI: 10.3390/ijms24108890

47. Tousson E., El-Gharbawy D.M. Impact of Saussurea lappa root extract against copper oxide nanoparticles induced oxidative stress and toxicity in rat cardiac tissues. *Environ. Toxicol.*, 2023, vol. 38, no. 2, pp. 415–421. DOI: 10.1002/tox.23688

48. Herrera-Rodríguez M.A., del Pilar Ramos-Godinez M., Cano-Martínez A., Segura F.C., Ruiz-Ramírez A., Pavón N., Lira-Silva E., Bautista-Pérez R. [et al.]. Food-grade titanium dioxide and zinc oxide nanoparticles induce toxicity and cardiac damage after oral exposure in rats. *Part. Fibre Toxicol.*, 2023, vol. 20, no. 1, pp. 43. DOI: 10.1186/s12989-023-00553-7

Gertan N.A., Sutunkova M.P., Shabardina L.V., Makhorina T.V., Nikogosyan K.M., Minigalieva R.F. Update on pulmono-, hepato-, and cardiotoxicity of nanoparticles in vivo: a literature review. Health Risk Analysis, 2025, no. 2, pp. 185–195. DOI: 10.21668/health.risk/2025.2.16.eng

Received: 21.03.2025
Approved: 05.05.2025
Accepted for publication: 14.06.2025