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CONTENTS

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Research article

SCIENTIFIC SUBSTANTIATION OF PRIORITY CHEMICALS, OBJECTS FOR SETTING QUOTAS AND TRENDS IN MITIGATING AIRBORNE PUBLIC HEALTH RISKS WITHIN ACTIVITIES PERFORMED BY THE SANITARY SERVICE OF THE RUSSIAN FEDERATION

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The study was conducted due to the necessity to streamline management of ambient air quality in large industrial cities in the country. The relevant tasks were set within the 'Clean Air' Federal project and the system for setting emission quotas.

The aim was to develop scientific-methodical approaches that would support Rospotrebnadzor in performing its functions and duties as regards management of ambient air quality, including those accomplished within the 'Clean Air' Federal project.

We took into account that initial data for the whole system for setting emission quotas were represented by aggregated calculation of pollutant dispersion. The study relied on input and output data provided by the 'Ekolog-Gorod" software package for calculating ambient air pollution. This software employs methods for calculating emission diffusion in ambient air that are applied as standards in Russia. Calculations were accomplished at points located within residential areas in the analyzed cities and covered not less than 20 major contributions made by emission sources to levels of each chemical at each calculation point. Airborne health risks were assessed in accordance with the valid methodical documents. We applied the following criteria for permissible (acceptable) risks: carcinogenic ones should not exceed $1.0 \cdot 10^{-4}$; non-carcinogenic chronic and / or acute risks should be at a level of a hazard index for chemicals with the same effects equal to 3.0. The brunch and bound method of linear programming was applied to substantiate optimal regulatory impacts aimed at minimizing health risks by reducing emissions into ambient air.

We developed a fundamental algorithm for identifying a list of priority pollutants and a list of objects for setting emission quotas, as well as for substantiating optimal regulatory impacts to mitigate airborne public health risks. We suggest ranking chemicals as priority pollutants in case their registered levels are higher than the established hygienic standards and they in total account for not less 95 % of contributions to unacceptable health risks for critical organs and systems at least at one calculation point. Priority objects are those that are responsible for not less than 95 % of unacceptable health risks and violations of the established hygienic standards. The study describes a developed and tested instrument for selecting optimal regulatory impacts as per relevant hygienic indicators, including levels of public health risks.

The suggested approaches support the Sanitary Service in its effort to provide proper quality of ambient air. They make it possible to identify priority chemicals and objects for setting emission quotas on the unified methodical basis for any city on the country, including those listed within the 'Clear Air' Federal project as priority ones. They also allow estimating whether environmental protection activities are relevant to the essence and levels of public health risks.

***Keywords**: health risk, emissions into ambient air, regulation, 'Clear Air' Federal project, priority substances, priority objects for setting emission quotas, airborne risks, Linear programming.*

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The major strategic task in the country development is to provide such quality of ambient air that means absence of any unacceptable public health risks, cases of health harm or any other violations of sanitary-epidemiological welfare of the population. Finding solution to this task requires wide interactions between authorities at all levels of public administration.

The 'Clean Air' Federal project aims to achieve a fundamental decrease in ambient air pollution in large industrial centers (when the project started, 12 cities were included into it[1]; in 2022, 29 cities were added to the list[2])

To achieve the target decrease in ambient air pollution, an experiment on setting emission quotas has been accomplished since January 01, 2021 in accordance with the Federal Law issued on July 26, 2019 No. 195-FZ[3]. This experiment is to be completed by December 31, 2026, considering the alterations made by the Federal Law issued on March 26, 2022 No. 71-FZ[4]. Therefore, the 'Clean Air' project and the experiment on setting emission quotas are closely connected [1, 2].

Quotas are viewed as a specific procedure for regulating emissions considering their targeted decrease; within this procedure, quotas are to be set for participating enterprises to regulate their emissions as per priority ambient air pollutants based on aggregated calculations[5].

Introducing such a concept as 'priority pollutants' into the regulatory base is an extremely important step in developing the whole system for regulation of emissions. It is primarily due to the fact that industrial enterprises, motor transport (and railways in some cities as well) and autonomous heat sources jointly emit dozens or even hundreds of chemicals into ambient air in cities. Thus, for example, more than 130 chemicals are emitted annually by 211 industrial enterprises into ambient air in Chita [3]; in Nizhnii Tagil, approximately 166 chemicals [4]; in Norilsk, 107 varied chemicals are emitted [5] etc. Given that, an important research and methodical task has always been to substantiate the most effective actions aimed at reducing pollution levels.

Variable approaches have been suggested and applied to rank emissions and identify priority ones on a territory within public administration. In some cases, regardless of actual emission masses, chemicals were considered priority ones if they were included into various international or Russian regulatory, reference

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[1] Federal'nyi proekt «Chistyi vozdukh» ['Clean Air' Federal project]. *The Ministry of Natural Resources and Environment of the Russian Federation*. Available at: https://www.mnr.gov.ru/activity/clean-air/ (September 15, 2022) (in Russian).

[2] O rasprostranenii eksperimenta po kvotirovaniyu vybrosov zagryaznyayushchikh veshchestv na gorodskie poseleniya i gorodskie okruga s vysokim i ochen' vysokim zagryazneniem atmosfernogo vozdukha: Protokol soveshchaniya u Zamestitelya Predsedatelya Pravitel'stva RF V.V. Abramchenko ot 18 noyabrya 2021 g. № VA-P11-77pr [On including urban settlements and districts with high and extremely high levels of ambient air pollution into the experiment on setting emission quotas: The proceedings of the meeting headed by V.V. Abramchenko, the Deputy to the RF Government Chairman, dated November 18, 2021 No. VA-P11-77pr] (in Russian).

[3] O provedenii eksperimenta po kvotirovaniyu vybrosov zagryaznyayushchikh veshchestv i vnesenii izmenenii v otdel'nye zakonodatel'nye akty Rossiiskoi Federatsii v chasti snizheniya zagryazneniya atmosfernogo vozdukha: Federal'nyi zakon ot 26.07.2019 № 195-FZ [On performing an experiment on quoting emissions of pollutants and making alterations into some legislative acts existing in the Russian Federation as regards reduction in ambient air pollution: the Federal Law issued on July 26, 2019 No. 195-FZ]. *The official Internet portal for legal information*. Available at: http://publication.pravo.gov.ru/Document/View/0001201907260064 (September 15, 2022) (in Russian).

[4] O vnesenii izmenenii v otdel'nye zakonodatel'nye akty Rossiiskoi Federatsii: Federal'nyi zakon ot 26.03.2022 № 71-FZ [On making alterations into certain legal acts of the Russian Federation: The Federal Law issued on March 26, 2022 No. 71-FZ]. *The official Internet portal for legal information*. Available at: http://publication.pravo.gov.ru/Document/View/0001202203260008 (September 15, 2022) (in Russian).

[5] Ob utverzhdenii pravil kvotirovaniya vybrosov zagryaznyayushchikh veshchestv (za isklyucheniem radioaktivnykh veshchestv) v atmosfernyi vozdukh: Prikaz Minprirody Rossii ot 29.11.2019 № 814 [On Approval of the rules for setting quotas of pollutant emissions (radioactive substances excluded) in ambient air: The Order by the RF Ministry of Natural Resources and Environment dated November 29, 2019 No. 814]. *The official Internet portal for legal information*. Available at: http://publication.pravo.gov.ru/Document/View/0001201912260045 (September 15, 2022) (in Russian).

or instructive documents. These documents are represented, for example, by lists of priority substances issued by the Agency for Toxic Substances[6], The Order by the RF Ministry of Natural Resources and the Environment 'On the Procedure for identifying emission sources of harmful substances (pollutants) into ambient air that are subject to the state regulation and standardization, and on the List of harmful substances (pollutants) subject to the state regulation and standardization'[7], The Letter of the RF Public Healthcare Ministry about the list of priority chemicals that can occur in the environment and their influence on public health[8], the Guide on control over ambient air pollution RD 52.04.186-89[9] and others.

Applied approaches included selecting priority substances as per their contributions to the total gross mass of emissions; as per a rank of a numeric value calculated for ratios between masses of emissions and maximum permissible concentrations or complex indexes of ambient air pollution [6, 7]; as per substances being able to produce mutagenic, carcinogenic or teratogenic effects [8]. Since the Guide entitled 'Human Health Risk Assessment from Environmental Chemicals' was published in Russia[10], it has become quite a common practice to identify priority chemicals as per a hazard index value. This value is determined considering reference levels of a given substance and preset weight factors to estimate carcinogenic and / or non-carcinogenic effects [9, 10].

The Federal Law 195-FZ '*On performing an experiment …*' has a clear definition that '*priority pollutants are those emissions of which facilitate violation of the hygienic standards for ambient air quality and create risks for human health on the territories included into the experiment'*. Therefore, it is necessary to develop a strict procedure for identifying priority substances as per health risk indicators.

Since reduction in emissions of priority substances should take place at specific objects, another important task is to identify priority emission sources of these substances and their contributions to ambient air pollution.

Finding solutions to both tasks, identifying priority chemicals and participating in substantiating lists of priority objects, is enlisted in the Clause 4 of the Federal Law dated July 26, 2019 No. 195-FZ among responsibilities borne by a federal executive

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[6] Support Document to the 2022 Substance Priority List (Candidates for Toxicological Profiles). *Agency for Toxic Substances and Disease Registry Division of Toxicology and Human Health Sciences*. Atlanta, USA, 2022, 12 p. Available at: https://www.atsdr.cdc.gov/spl/resources/ATSDR-2022-SPL-Support-Document-508.pdf (October 02, 2022).

[7] O Poryadke ustanovleniya istochnikov vybrosov vrednykh (zagryaznyayushchikh) veshchestv v atmosfernyi vozdukh, podlezhashchikh gosudarstvennomu uchetu i normirovaniyu, i o Perechne vrednykh (zagryaznyayushchikh) veshchestv, podlezhashchikh gosudarstvennomu uchetu i normirovaniyu: Prikaz Minprirody RF ot 31.12.2010 № 579 (zaregistrirovano v Minyuste RF 09.02.2011 № 19753) [On the Procedure for identifying emission sources of harmful substances (pollutants) into ambient air that are subject to the state regulation and standardization, and on the List of harmful substances (pollutants) subject to the state regulation and standardization: the Order by the RF Ministry of Natural Resources and Environment dated December 31, 2010 No. 579 (registered by RF Ministry of Justice on February 09, 2011 No. 19753)]. *KonturNormativ*. Available at: https://normativ.kontur.ru/document?moduleId=1&documentId=229990 (August 18, 2022) (in Russian).

[8] O spiske prioritetnykh veshchestv, soderzhashchikhsya v okruzhayushchei srede, i ikh vliyanii na zdorov'e naseleniya: Pis'mo Departamenta Gossanepidnadzora Minzdrava RF ot 07.08.1997 № 11/109-111 [On the list of priority substances in the environment and their influence on public health: The Letter by the Department of the State Sanitary and Epidemiological Surveillance of the RF Public Healthcare Ministry dated August 07, 1997 No. 11/109-111]. *The library for regulatory documentation*. Available at: https://files.stroyinf.ru/Data2/1/4293737/4293737491.htm (September 03, 2022) (in Russian).

[9] RD 52.04.186-89. Rukovodstvo po kontrolyu zagryazneniya atmosfery [Guide RD 52.04.186-89. The Guide on control over ambient air pollution]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200036406 (September 03, 2022) (in Russian).

[10] The Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals (approved and introduced by G.G. Onishchenko, the First Deputy to the RF Public Healthcare Minister and RF Chief Sanitary Inspector on March 5, 2004). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200037399 (November 22, 2022) (in Russian).

authority conducting the federal state sanitary-epidemiological surveillance[11].

Still, it is noteworthy that the Federal Law 'On sanitary-epidemiological welfare of the population'[12] gives chief state sanitary inspectors and their deputies the power to introduce suggestions on implementation of activities aimed at improving a sanitary-epidemiological situation and on public health protection to executive authorities at any level in public administration. It is advisable and important to use this power, including activities conducted within the 'Clean Air' Federal project. The latter is also associated with the fact that regulatory and methodical documents on the system for setting quotas do not envisage assessment of residual health risks or estimation whether any implemented activity has been effective as per health risk indicators. Absence of such estimation can result in insufficient or excessive expenses borne by economic entities and wasted on activities that hardly yield any results and / or lower social and medical-demographic significance of decisions made by authorities [11, 12]. At the same time, it seems advisable not only to estimate effectiveness of activities that have already been implemented but also to perform predictive analysis of action plans and programs in order to identify whether they are relevant to the structure, levels and spatial distribution of public health risks on a given territory.

Development of theoretical grounds for solutions to optimization tasks within emission standardization has been described in literature and their practical implementation has been addressed in research works issued by the Voeikov Main Geophysical Observatory [13, 14].

The aim of this study was to develop scientific-methodical approaches that would support Rospotrebnadzor in performing its functions and duties as regards management of ambient air quality, including those accomplished within the 'Clean Air' Federal project.

Materials and methods. Methodical approaches were developed considering that the whole system for decision making to regulate emissions through such an instrument as quotas was based on initial data represented by aggregated calculations of emission dispersion.

In this study, we relied on the structure of input and output data applied in the 'Ekolog-Gorod' unified software package for calculating ambient air pollution, version 4.60.1, with the embedded module for calculating 'Mean values'. The software employs methods for modeling emission diffusion in ambient air that are applied as standards in Russia. When considering contributions made by specific sources to pollution, we considered not less than 20 priority contributions to concentrations of each substance at each calculation point.

The developed approaches involved binding all the sources of calculation points to vector maps of territories and mandatory calculations of contributions to ground concentrations at each point made by economic entity, motor transport (at specific sections of traffic networks) and other pollution sources.

Risk assessment was performed in accordance with the valid methodical documents approved by Rospotrebnadzor as per conventional procedures[13]. We applied the following criteria for permissible (acceptable) risks: carcinogenic ones should not exceed $1.0 \cdot 10^{-4}$; non-carcinogenic chronic and / or acute risks

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[11] O provedenii eksperimenta po kvotirovaniyu vybrosov zagryaznyayushchikh veshchestv i vnesenii izmenenii v otdel'nye zakonodatel'nye akty Rossiiskoi Federatsii v chasti snizheniya zagryazneniya atmosfernogo vozdukha: Federal'nyi zakon ot 26.07.2019 № 195-FZ [On performing an experiment on quoting emissions of pollutants and making alterations into some legislative acts existing in the Russian Federation as regards reduction in ambient air pollution: the Federal Law issued on July 26, 2019 No. 195-FZ]. *The official Internet portal for legal information*. Available at: http://publication.pravo.gov.ru/Document/View/0001201907260064 (September 15, 2022) (in Russian).

[12] O sanitarno-epidemiologicheskom blagopoluchii naseleniya: Federal'nyi zakon ot 30.03.1999 № 52-FZ [On sanitary-epidemiological welfare of the population: the Federal Law issued on March 30, 1999 No. 52-FZ]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/901729631 (September 15, 2022) (in Russian).

[13] The Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals (approved and introduced by G.G. Onishchenko, the First Deputy to the RF Public Healthcare Minister and RF Chief Sanitary Inspector on March 5, 2004). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200037399 (November 22, 2022) (in Russian).

should be at a level of a hazard index (HI) for chemicals with the same effects equal to 3.0; hazard quotient (HQ) was set at 1.0 for certain chemicals[14].

The algorithm included a procedure for estimating and verifying calculated data by using field observations obtained at the posts of environmental and / or social-hygienic monitoring with the following corrections of the aggregated database on emission sources.

Identifying which direction the study had to go involved establishing specific economic entities; in case their emissions of priority chemicals were reduced, health risks would drop to their acceptable levels in a whole city. Within this study, minimal sufficient reduction in masses of emissions on a given territory was taken as an optimization indicator. The procedure was developed relying on a baseline solution to an optimization task which was to determine such a change in masses of pollutant emissions by objects chosen for setting quotas that would ensure conformity with targeted risk levels at selected reference points. The brunch and bound method of linear programming in the R-studio environment (the state registration No. for PC is 2022669645) was applied to find a solution to the task within the suggested mathematical setting.

Basic results. We developed a fundamental algorithm for identifying a list of priority pollutants and a list of objects for setting emission quotas, as well as for substantiating optimal regulatory impacts to mitigate airborne public health risks. It is given in Figure 1. The algorithm assumes aggregated calculations to be accomplished for short-term ambient air pollution (a 20-minute period, the worst possible diffusion conditions are acute population exposure) and for average annual ambient air pollution (chronic exposure).

When selecting priority chemicals, the most optimal way is to identify ground concentrations at calculation points that correspond to geometric centers of all the residential buildings and territories used by people for recreational or health-improving purposes.

Calculation points are given preference over a regular mesh considering several important aspects:

– residential areas are often not uninterrupted rows of buildings; some spots with residential buildings might be located at a significant distance from each other and are quite small zones; it is hardly correct to consider them using a mesh with its step being more than 200 × 200 m;

– we eliminate a possibility that some calculation points would be located on industrial sites adjacent to residential areas or on motorways, etc.; this allows archiving more correct health risk assessment;

– when calculation points are located directly in residential areas, it fully corresponds to goals of health risk assessment.

In future, the same calculation points are applied to select optimal trends in actions aimed at mitigating health risks.

A ground concentration of each chemical is described with several parameters:

- shares of maximum single MPC;
- shares of average annual MPC (or average daily MPC[15]);
- a level of lifetime carcinogenic risk;
- a level of detected acute health risk, shares of ARfC;
- a level of detected chronic health risk, shares of RfC.

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[14] MR 2.1.10.01156-19. Otsenka kachestva atmosfernogo vozdukha i analiz riska zdorov'yu naseleniya v tselyakh prinyatiya obosnovannykh upravlencheskikh reshenii v sfere obespecheniya kachestva atmosfernogo vozdukha i sanitarno-epidemiologicheskogo blagopoluchiya naseleniya (utv. rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom RF A.Yu. Popovoi 02.12.2019) [The Methodical Guidelines MR 2.1.10.01156-19. Assessment of ambient air quality and public health risk analysis in order to provide well-grounded decision making as regards providing ambient air quality and sanitary-epidemiological welfare of the population (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 02, 2019)]. *YuIS Legalakt: laws, codes and regulatory documents of the Russian Federation*. Available at: https://legalacts.ru/doc/mr-21100156-19-2110-gigiena-kommunalnaja-gigiena-sostojanie-zdorovja-naselenija/ (November 22, 2022) (in Russian).

[15] In case an average annual MPC for a given substance has not been identified.

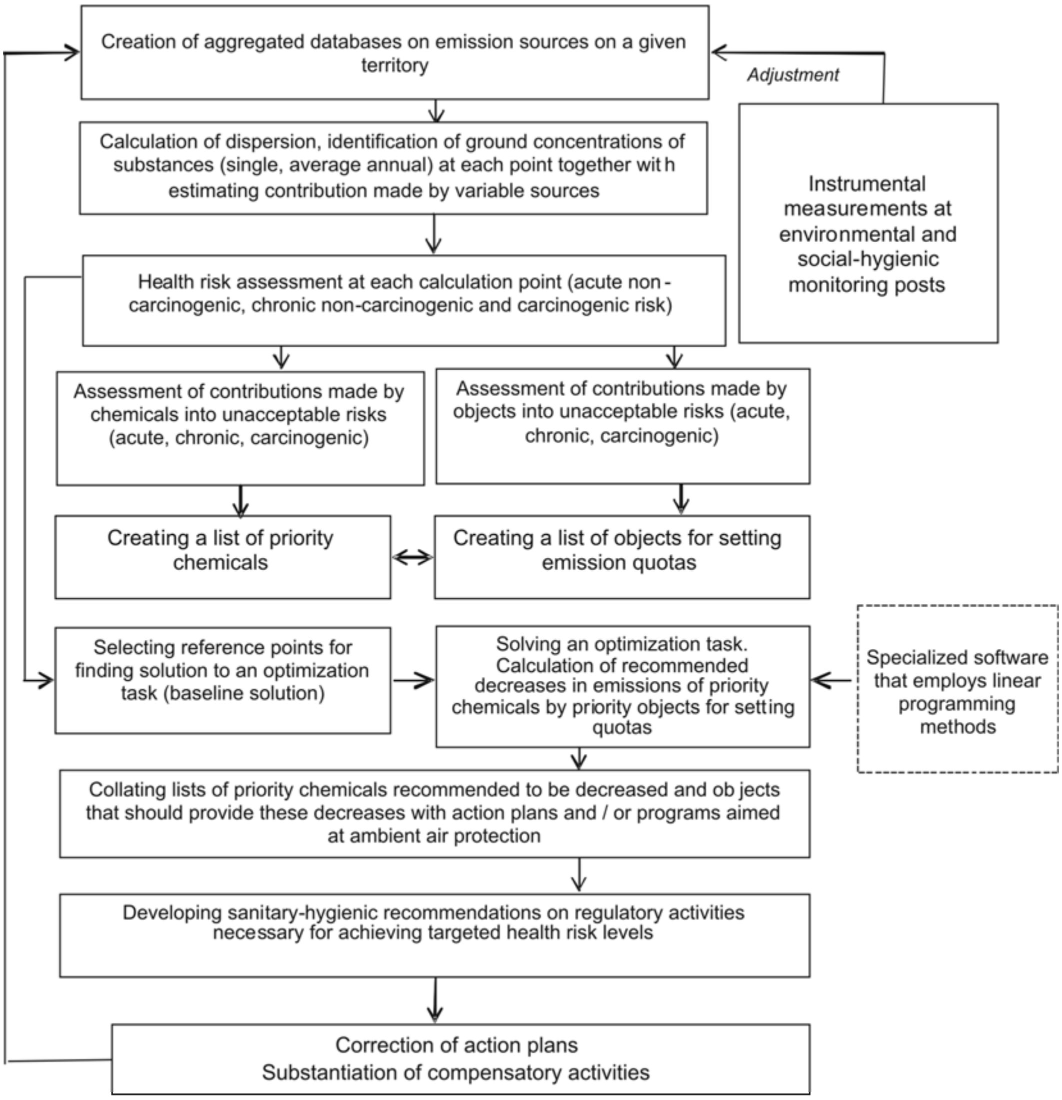

Figure 1. The fundamental algorithm for identifying a list of priority pollutants and a list of objects for setting emission quotas, as well as for substantiating optimal regulatory impacts to mitigate airborne public health risks

In case impermissible (unacceptable) health risks are detected, contributions made by specific chemicals to each health risk (carcinogenic, acute non-carcinogenic and chronic non-carcinogenic) are estimated at each point where impermissible (unacceptable) health risks have been detected. Chemicals are ranked as per their contributions to the total health risk at each calculation point where impermissible (unacceptable) health risks have been detected.

Priority substances include:

– chemicals identified in levels higher than maximum single MPC and / or average annual MPC as per results of dispersion calculation;

– chemicals with individual hazard indexes (HQ) > 1.0 or a carcinogenic risk being $1 \cdot 10^{-6}$ or higher;

– chemicals that are responsible (considering all the contributions in a descending order) for not less than 95 % of unacceptable health risks for critical organs and systems at least at one calculation point (HI > 3.0; a carcinogenic risk > $1 \cdot 10^{-4}$).

Table 1 provides an example of selecting priority chemicals at calculation points for one type of risk.

A final list of priority chemicals for a city as a whole is created based on all the aggregated data. Table 2 provides an example.

Table 1

An example how priority chemicals are identified as per such an indicator as 'Contribution to unacceptable chronic risks of respiratory diseases'

Code*	Calculation point	1	2	3	4	5	6	7	8
	Hazard index (HI) at a given point	**3.33**	**4.32**	**4.49**	**5.34**	**5.51**	**5.97**	**9.53**	**12.10**
	Total dusts, including	**69.19**	**76.33**	**69.27**	**60.63**	**71.12**	**72.96**	**77.51**	**68.95**
2908	Non-organic dust: 70–20 % SiO2	60.44	75.27	55.39	46.31	70.09	44.55	22.75	34.00
2909	Non-organic dust: up to 20 % SiO2	1.02	0.22	0.37	0.56	0.16	0.39	0.30	0.31
2902	Particulate matter	4.13	0.64	10.12	9.10	0.65	22.32	46.02	29.36
2930	Abrasive dust	0.21	0.02	0.36	0.30	0.02	0.58	1.27	0.67
2936	Wood dust	2.21	0.12	1.87	3.86	0.13	4.73	6.87	4.29
2937	Grain dust	1.15	0.05	1.14	0.47	0.06	0.35	0.19	0.27
3749	Charcoal dust	0.013	0.001	0.008	0.012	0.001	0.012	0.007	0.01
330	Sulfur dioxide (sulfuric anhydride)	14.04	17.57	12.96	10.57	16.10	10.28	5.37	7.98
301	Nitrogen dioxide (Nitrogen (IV) oxide)	10.83	5.17	13.69	18.89	11.18	11.22	7.06	16.44
150	Sodium hydroxide	0.82	0.21	1.59	3.45	0.16	3.34	3.42	1.27
304	Nitrogen (II) oxide (Nitrogen oxide)	4.47	0.55	1.45	2.54	1.19	1.19	0.75	0.75
322	Sulfuric acid (as per H2SO4 molecule)	0.08	0.04	0.08	0.06	0.03	0.12	0.09	0.23
1325	Formaldehyde	0.24	0.06	0.23	0.47	0.13	0.21	0.12	0.30
1301	Prop-2-en-1-al (Acrolein)	0.09	0.01	0.21	0.16	0.01	0.39	2.39	1.83
	The sum of contributions made by priority chemicals	98.53	99.07	95.92	96.08	98.4	97.8	95.75	96.47

Note: * means a code assigned to a chemical in the aggregated calculation system; ** means that coloring highlights substances with their ranked contribution to unacceptable health risks being more than 95 %.

Table 2

Priority substances – components in emissions from industrial facilities and motor transport

No.	Code	Chemical	Criterion for inclusion*						
			1	2	3	4	5	6	7
1	143	Manganese and its compounds (recalculated as per manganese (IV) oxide)							+
2	150	Sodium hydroxide						+	+
3	164	Nickel oxide (recalculated as per Ni)						+	+
4	301	Nitrogen dioxide	+	+			+	+	+
5	304	Nitrogen oxide							+
6	328	Carbon (Soot)							+
7	330	Sulfur dioxide						+	+
8	337	Carbon oxide	+						
9	342	Gaseous fluorides						+	+
10	602	Benzene			+	+		+	+
11	703	Benz/a/pyrene (3,4-benzpyrene)			+				
12	1301	Prop-2-el-1-al (Acrolein)						+	+
13	1325	Formaldehyde			+	+			
14	2902	Dusts (total) with priority				+	+	+	+
15	2907	*Non-organic dust > 70 % SiO2*	+			+	+	+	+
16	2909	*Non-organic dust: up to 20 % SiO2*				+	+	+	+

Note: *criteria for inclusion:
1 – levels higher than single maximum MPC are detected as per dispersion calculation;
2 – levels higher than average annual MPC are detected as per dispersion calculation;
3 – acute HQ > 1;
4 – chronic HQ > 1;
5 – a chemical is among substances responsible for 95 % of unacceptable carcinogenic risks;
6 – a chemical is among substances responsible for 95 % of unacceptable acute non-carcinogenic risks;
7 – a chemical is among substances responsible for 95 % of unacceptable chronic non-carcinogenic risks.

A priority list of chemicals for which quotas should be set seems to be dynamic and should be changed when new emission sources appear or old ones are relocated. So, an aggregated database on all the emission sources in a given city ought to be dynamic.

Results obtained by calculating contributions made by specific sources to ground concentrations of priority pollutants and unacceptable risks are applied to identify priority objects for setting emission quotas[16].

Calculation should cover all the sources that account for not less than 95 % of unacceptable health risks and the total violations of the established hygienic standards.

A contribution made to risk levels (hazards indexes) by a specific object (an industrial facility, a section in a traffic network with emissions from motor transport, or an autonomous heat source) is identified at each point as a weighted average of contributions as per the following formulas:

– for chronic risks:

$$\delta(HI_{Rfc\,j})_n^k = \frac{\sum_{i \in I_j^{Rfc}} HQ_{Rfc\,i}^{\;k} \cdot \delta aa_{in}^k}{\sum_{i \in I_j^{Rfc}} HQ_{Rfc\,i}^{\;k}}, \qquad (1)$$

where $\delta(HI_{Rfc\,j})_n^k$ is a contribution made by the n-th object to a hazard index for the j-th organ or system at the k-th point under chronic exposure;

I_j^{Rfc} is a multitude of pollutants that create a chronic health risk as per the j-th organ or system;

$HQ_{Rfc\,i}^{\;k}$ is a hazard quotient under chronic exposure to the i-th pollutant at the k-th point;

δaa_{in}^k is a contribution made by the n-th facility to average annual concentrations at the k-th point as per the i-th pollutants;

– for acute risks:

$$\delta(HI_{ARfc\,j})_n^k = \frac{\sum_{i \in I_j^{ARfc}} HQ_{ARfc\,i}^{\;k} \cdot \delta sm_{in}^k}{\sum_{i \in I_j^{ARfc}} HQ_{ARfc\,i}^{\;k}}, \qquad (2)$$

where $\delta(HI_{ARfc\,j})_n^k$ is a contribution made by the n-th object into a hazard index for the j-th organ or system at the k-th point under acute exposure;

I_j^{ARfc} is a multiplicity of pollutants creating a health risk under acute exposure as per the j-th organ or system;

$HQ_{ARfc\,i}^{\;k}$ is a hazard quotient under acute exposure to the i-th pollutant at the k-th point;

δsm_{in}^k is a contribution made by the n-th facility into single maximum concentrations at the k-th point as per the i-th substance;

– for carcinogenic risks:

$$\delta(CR)_n^k = \frac{\sum_i SF_i \; Caa_i^k \cdot \delta aa_{in}^k}{\sum_i SF_i \; Cad_i^k}, \qquad (3)$$

where $\delta(CR)_n^k$ is a contribution made by the n-th object into a carcinogenic risk at the k-th point under acute exposure;

Caa_i^k is an average annual concentration of the i-th pollutant calculated at the k-th point;

δaa_{in}^k is a contribution made by the n-th facility into average annual concentrations at the k-th point for the i-th pollutant;

SF_i is a slope factor for the i-th pollutant.

Contributions are calculated at each point concerning all critical organs and systems under chronic and acute exposure.

An integral estimation of contributions made by specific objects into health risk levels involves using weighted averaging as per all the points:

[16] In case there is no technical feasibility to calculate contributions made by sources at each point when calculating ground concentrations in accordance with the item 4.2, contributions made by enterprises, infrastructure objects or other sources are calculated at reference points.

– for chronic exposure:

$$\delta(HI_{Rfc\,j})_n = \frac{\sum_k HI_{Rfc\,j}^{\;k} \cdot \delta(HI_{Rfc\,j})_n^k}{\sum_k HI_{Rfc\,j}^{\;k}}\;, \qquad (4)$$

where $\delta(HI_{Rfc\,j})_n$ is an average weighted contribution made by the *n*-th object into a hazard index for the *j*-th organ or system under chronic exposure as per the total points;

$HI_{Rfc\,j}^{\;k}$ is a hazard index for the *j*-th organ or system under chronic exposure at the *k*-th points.

Similar to (4), an integral estimation is performed to calculate a contribution made by a specific object into levels of an acute and / or carcinogenic risk.

All the objects with their contributions accounting for 95 % of unacceptable (carcinogenic, acute and / or chronic) risks are included into a list of objects for which it is advisable to set emission quotas if we want to reduce health risks down to their safe levels. Factors (chemicals) emissions of which are subject to immediate reduction should be identified for each object. Table 3 provides an example how to substantiate a list of priority objects.

Table 3

Substantiating a list of priority objects as sources of impermissible health risks on a given territory (a fragment)

	A contribution made by a specific object to a certain impermissible health risk, %						Chemicals making contributions to an impermissible health risk
	Chronic, respiratory diseases	Chronic, impacts on development	Chronic, diseases of the nervous systems	Chronic, diseases of blood	Acute, respiratory diseases	Acute, diseases of the immune system	
Facility 1	38.51		56.71	57.90	2.1	67.0	Nickel oxide, manganese and its compounds, benzene
Motor transport	21.57			27.87	1.92		Nitrogen dioxide, nitrogen oxide
Facility 2	17.26	96.35	8.11		13.10		Gaseous fluorides, non-organic fluorides, sulfur dioxide, non-organic dusts, nitrogen dioxide, benz(a)pyrene, benzene
Autonomous heat sources	8.19				5.50		Sulfur dioxide, non-organic dusts, nitrogen dioxide, particulate matter
Facility 3	3.62		11.56	4.33	37.2		Nitrogen dioxide, nitrogen (II) oxide non-organic dusts, manganese and its compounds, carbon (soot), sulfur dioxide
Facility 4	3.08				20.4		Nitrogen dioxide, sulfur dioxide, non-organic dusts, nitrogen (II) oxide
Facility 5	1.92						Non-organic dusts, sodium hydroxide, copper oxide
Facility 6	1.29			5.20	8.70		Prop-2-en-1-al (acrolein), sulfur dioxide, nitrogen dioxide, sodium hydroxide, nickel oxide
Facility 7			7.68				Manganese and its compounds
Facility 8			6.30				Manganese and its compounds
Facility 9						12.3	Manganese, benzene
Facility 10						8.20	Benzene
Facility 11						5.60	Benzene
… others (9 facilities overall)			4.90		8.10	2.30	Sulfur dioxide, non-organic dusts, nitrogen dioxide, benzene, particulate matter
Total	95.44	96.35	95.23	95.30	96.62	95.4	

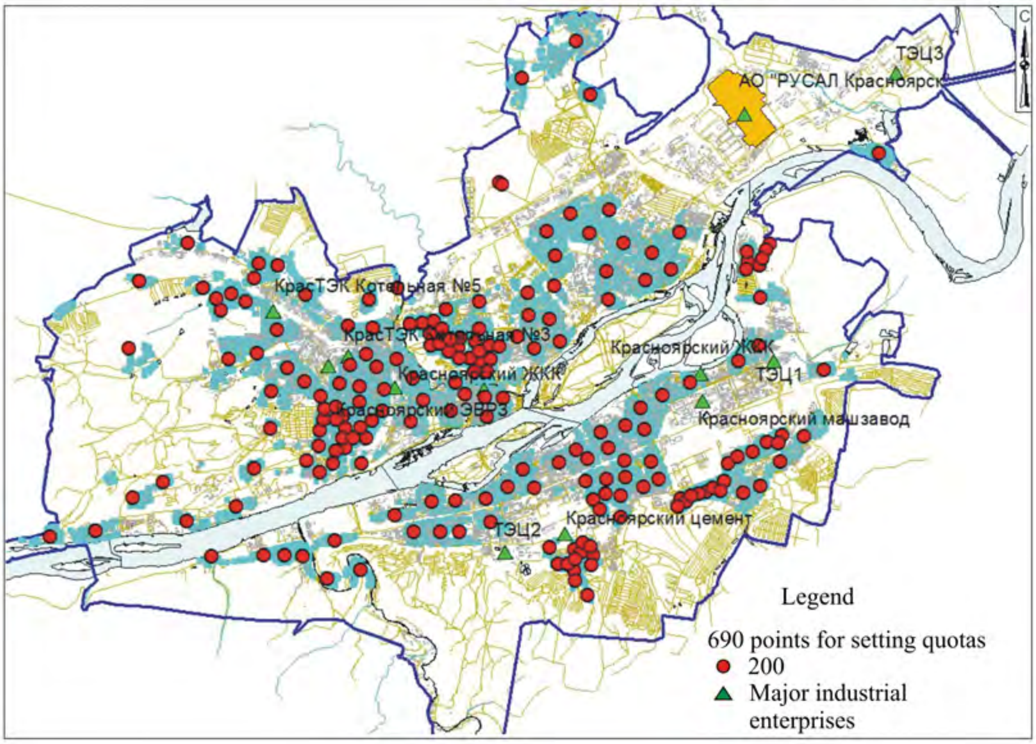

Figure 2. The electronic map of Krasnoyarsk with marked local maximum points applied as reference ones when solving the task of setting quotas

This example illustrates that 20 industrial facilities, motor transport and autonomous heat sources are to be enlisted as priority ones on the examined territory. Contributions made by these sources and lists of priority chemicals emissions of which should be reduced are different and specific for each object. Therefore, it is hardly advisable to reduce an emission from each object by a certain share since this obviously does not guarantee ultimate mitigation of health risks down to their acceptable (permissible) levels.

The Sanitary Service needs reliable tools that do not replace the order and methodology for setting emission quotas but make it possible to estimate whether any accomplished activities on environmental protection are relevant to the existing risk levels. To do that, we suggest this method for selecting optimal trends in regulatory impacts as per hygienic indicators.

A system of reference points is applied to reduce time required for calculations and volumes of output information necessary to solve the task. These points are those where local maximums are established in compact residential areas as per indicators describing conformity to hygienic standards (average annual and single maximum MPC), hazard quotients for acute and chronic exposure, hazard indexes for acute and chronic exposure and its effects on critical organs and systems, and carcinogenic risks.

Use of local maximums as reference points for setting quotas makes it possible to reduce dimensionality of the task by several orders without any substantial losses in precision of obtained estimates thereby reducing needs in computational resources.

The suggested method was tested in Krasnoyarsk; the testing gave an opportunity to identify 35 compact residential areas with local maximum points as per all the safety indicators (Figure 2).

A minimal total change in masses of pollutant emissions as per all the objects for setting quotas can be applied as a target function (an optimization criterion) (5):

$$\sum_{n=1}^{N}\sum_{i=1}^{I}(1-q_{in})M_{in} \rightarrow \min\,, \qquad (5)$$

where q_{in} is a share by which a mass annual emission of the *i*-th pollutant from the *n*-th object is reduced;

M_{in} is a mass emission of the *i*-th pollutant from the *n*-th object per a unit of time, tons/year.

The task is to reduce mass emissions that influence average annual concentrations of pollutants and levels of chronic (carcinogenic and non-carcinogenic) health risks.

The solution assumes that the following conditions should be met at each calculation point:

– a tolerance range for reduction in mass emissions of pollutants at objects for setting quotas varies between 0 to 1.0[17] (control parameters) (6):

$$0 \le q_{in} \le 1, i = \overline{1...I}, n = \overline{1...N} \quad (6)$$

– a hazard quotient (HQ) for chemicals creating a non-carcinogenic risk should not exceed 1.0 (7):

$$HQ_{Rfci}^{\ k} = \frac{\sum_{n=1}^{N} q_{in} Caa_{in}^{k}}{Rfc_i} \le R_{HQ}, \quad (7)$$
$$i = \overline{1...I}, k = \overline{1...K},$$

where Rfc_i is a reference concentrations of the *i*-th pollutant under chronic (average annual) exposure, mg/m^3; all the other denominations are the same as in the previously described equations;

– a hazard index (HI) for affected organs and systems should not exceed 3.0 if we aim to achieve ‘acceptable risk’ level (8):

$$HI_{Rfc\,j}^{\ k} = \sum_{i \in I_j^{Rfc}} \frac{\sum_{n=1}^{N} q_{in} Caa_{i}^{k}}{Rfc_i} \le R_{HI}, \quad (8)$$
$$j = \overline{1...J}, k = \overline{1...K}$$

– a carcinogenic risk CR^k should not exceed 10^{-4} (9):

$$CR^k = \sum_{i=1}^{I} SF_i \; q_{in} Caa_i^k \le R_{cr}, k = \overline{1...K}. \quad (9)$$

The results yielded by solving the task should be considered a tool to estimate advisability and sufficiency of plans with their aim to reduce emissions of a certain chemical by a certain object. The estimation should focus on whether there was a resulting reduction in public health risks and be useful for developing recommendations on making corrections into actions plans of both economic entities and local authorities.

Table 4 provides an example of solving the task on substantiating regulatory actions aimed at minimizing health risks associated with manganese levels in ambient air in a given city.

Manganese emissions are declared by approximately 240 objects in the analyzed city. The total emissions are equal to 1.7563 tons/year. An unacceptable chronic non-carcinogenic risk of nervous diseases is established at 32 calculation points within residential areas. It varies between 3.1 to 6.15 HI.

Contributions to 95 % of this unacceptable risk are made by 14 economic entities. The targeted reduction is down to 3.0 HI at all the points where this unacceptable risk level has been detected.

The solution to the task indicates that it is advisable to develop activities aimed at reducing manganese emissions at the enterprises No. 1, 5 and 6 and to introduce control over their implementation. Major attention should be paid to activities performed at the enterprise No. 1. Any reduction in emissions from other objects might not guarantee that acceptable health risk levels are achieved.

Other optimization criteria can be used as well; it depends on targets and available initial data.

Thus, for example, economic indicators can be used as a target function (optimization criteria) apart from the total mass of emissions; they can be, for example, minimal financial expenses on environmental protection. Functional that reflects regularities in growing total expenses on activities aimed at reducing emissions (tons/year) can be applied as an optimization criterion based on an assumption

[17] There are certain limitations here such as exclusion of permissible reduction in emissions down to 0; limitations of reduction below a certain level at specific object etc. can be introduced into the task additionally.

Table 4

Substantiating regulatory actions aimed at reducing risks of the nervous diseases down to their acceptable levels under exposure to manganese and its compounds emitted into ambient air

Economic entity	Total emissions, tons/year	A contribution to total emissions, shares	A contribution to a chronic health risk (impacts on the nervous system)	Recommended targeted emissions, tons/year, that provide achieving acceptable risks	Recommended reduction, a share of the initial emission volumes
Facility 1	0.640	0.364	0.567	**0.353**	**0.551**
Facility 2	0.152	0.086	0.019	0.152	0.000
Facility 3	0.140	0.080	0.015	0.140	0.000
Facility 4	0.117	0.067	0.077	0.117	0.000
Facility 5	0.104	0.059	0.116	**0.010**	**0.096**
Facility 6	0.058	0.033	0.063	**0.003**	**0.052**
Facility 7	0.049	0.028	0.012	0.049	0.000
Facility 8	0.045	0.026	0.004	0.045	0.000
Facility 9	0.041	0.023	< 0.01	0.041	0.000
Facility 10	0.037	0.021	0.015	0.037	0.000
Facility 11	0.037	0.019	< 0.01	0.037	0.000
Facility 12	0.029	0.016	< 0.01	0.029	0.000
Facility 13	0.027	0.015	0.019	0.027	0.000
Facility 14	0.026	0.015	< 0.01	0.026	0.000

that the latter are inversely proportionate to a relative change in their mass flow (10).

$$\sum_{n=1}^{N}\sum_{i=1}^{I}\frac{M_{in}}{q_{in}} \rightarrow \min \qquad (10)$$

Inclusion of cost-related parameters into the optimization task can yield a bit different results when it comes down to selecting relevant activities aimed at reducing emissions. At the same time, decision-makers get access to new information that enables them to make necessary corrections into action plans and programs.

It is noteworthy that these instruments are universal. Other health risk indicators can be applied as safety criteria when the optimization task is solved; for example, if our task is to achieve minimal targeted carcinogenic risk levels, this indicator can be a risk level equal to $1\cdot10^{-6}$; if our target is reduction in non-carcinogenic risks, the target indicator is HI = 1.0).

Discussion. Activities performed by Rospotrebnadzor within its authorities, including those outlined by the 'Clean Air' Federal project, are aimed at making environmental protection be largely guided by public health indicators. This approach, while not being limited to reducing emissions made by specific economic entities, seems to be able to provide solutions to the whole set of strategic tasks set within the National projects such as creating a comfortable living environment, providing public health protection and an increase in life expectancy at birth in Russia [11, 12].

Use of health risks and health harm as indicators for managing ambient air quality fully corresponds to the recommendations issued by the World Health Organization [15–17] and to the best world practices [18]. Moreover, such approaches give much more social significance to implemented actions and make population much more satisfied with activities performed by authorities and businesses [19, 20].

At the same time, implementation of these approaches requires the following:

– systemic interdepartmental interaction at the stage when action plans and / or programs aimed at ambient air protection are being analyzed on a given territory;

– making businesses more socially responsible since achieving permissible (acceptable) risk levels may require more profound and effective measures aimed at reducing emissions than achieving maximum permissible concentrations of certain chemicals or established summation groups. This concerns even chemicals with their long-term (average annual) MPC established considering health risk indicators.

– constant monitoring of actual ambient air quality. The latter is due to the fact that calculated data are far from always being highly convergent with data obtained by field measurements [21, 22]. In some cases, calculated values can be higher than actual measured ones. This leads to excessive expenses on ambient air protection, which hardly brings any economic benefits. In cases, when calculated ground concentrations are lower than measured ones, quotas and reductions in emissions may fail to provide safe levels both as per MPC and permissible health risks.

– predictive analysis of plans and / or programs of environmental protection activities by Rospotrebnadzor experts in order to develop recommendations on their improvement taking into account public health risks and health harm.

Conclusions. We have suggested the fundamental algorithm for identifying a list of priority pollutants and a list of objects for setting emission quotas, as well as for substantiating optimal regulatory impacts to mitigate airborne public health risks. Its major stages involve health risk assessment relying on data of aggregated dispersion calculations; identifying contributions made by chemicals and objects and emission sources to impermissible health risks; calculating recommended values of reductions in emissions of priority pollutants by priority objects.

We suggest ranking chemicals as priority pollutants in case their registered levels are higher than average annual or single maximum MPC according to the results of dispersion calculations and they in total account for not less 95 % of contributions to unacceptable health risks for critical organs and systems at least at one calculation point on a given territory.

Priority objects are those that are responsible for not less than 95 % of unacceptable health risks and violations of the established hygienic standards.

The Sanitary Service should be provided with tools that do not replace the order and methodology for setting emission quotas but make it possible to estimate whether environmental protection activities are relevant to the existing health risks. To do that, we have suggested the described method for selecting optimal regulatory impacts as per relevant hygienic indicators, including levels of public health risks.

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Research article

LIFE EXPECTANCY AT BIRTH IN RF REGIONS WITH DIFFERENT SANITARY-EPIDEMIOLOGICAL WELLBEING AND DIFFERENT LIFESTYLES. MANAGEMENT RESERVES

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The article focuses on estimating reserves of growth in life expectancy at birth (LEB) for the RF population in regions with different sanitary-epidemiological wellbeing and people's lifestyles. The existing trends in the country development within the regional context corroborate relevance of the present study.

The methodical approach includes use of factor and cluster analysis, artificial neural networks, and scenario forecasting. Activities performed by Rospotrebnadzor within its authority produce positive modifying effects on LEB as an integral health indicator. Differentiated contribution made by these activities to achieving regional target LEB levels by 2024 (COVID-related processes excluded) amounts to 8–62 % as per the group of indicators that describes a sanitary-epidemiological situation on a given territory and 5–45 % as per the group of lifestyle-related indicators.

We identified priority factors for each of four types of regions; these factors provide the maximum positive effect on LEB. Working conditions for working population, quality of drinking water, ambient air and nonfoods are priority manageable factors in regions where the sanitary-epidemiological situation is the most unfavorable. Levels of alcohol and food consumption, balanced diets and people's physical activity are the priority manageable factors in regions with the most unfavorable lifestyle-related indicators.

The study revealed that additional LEB growth would be secured if the targets set within national projects were achieved. By 2024, this additional LEB growth would equal 6–420 days and 107–659 days accordingly given the existing trends and regional differentiation as regards improved sanitary-epidemiological situation in regions and people's lifestyles. Improved working conditions, better quality of drinking water and ambient air are reserves of LEB growth for all types of the RF regions in short and middle-term. A potential reserve of LEB growth and priority determinants were identified for each type of regions. These identified national and regional determinants should be considered when building an optimization model of LEB management allowing for reserves of its growth.

The study results develop the authors' methodical approach to estimating potential LEB growth based on scenario modeling; they are consistent with the results obtained by other relevant studies. We have identified limitations of the present study as well as prospects and trends for future research.

Keywords: *life expectancy at birth, LEB, socio-hygienic determinants, environmental factors, lifestyle factors, sanitary-epidemiological wellbeing, RF regions, forecast, artificial neural networks, cluster analysis.*

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Over the last three years (2020–2022), the medical-demographic situation has deteriorated due to the COVID-19 pandemic, a global adverse factor [1, 2]. Although the number of confirmed cases and deaths caused by to COVID-19[1] has declined due to spread of Omicron (B.1.1.529) strain, which is more contagious and less pathogenic [3, 4], global outcomes of the pandemic are only becoming apparent now and we can expect more of them in future [5]. Forced concentration of production, research and social efforts on maintaining public healthcare systems has led to growing expenses or redistribution of funds previously allocated on programs aimed at providing sustainable economic development [6]. This also concerns national programs[2] [7] aimed at satisfying the existing population needs as regards minimizing influence exerted on the environment [8]. The immediate forecasts issued by the RF Ministry of Economic Development [9] expect economic recession in developed countries and stricter sanctions; this can become additional risk factors for public health. The IHME[3] experts [10] put forward a concept of a syndemic which will combine aggravation of chronic diseases, social inequality and the COVID-19 pandemic. This combination makes population groups with the existing burden of diseases even more vulnerable. Therefore, some additional risk factors have already been described by now and more are expected to occur in future against the background of the global imperative to ease off the global burden of diseases [11–14].

The authors of the Global Burden of Diseases study analyzed 286 death causes, 369 various diseases and injuries and 87 health risk factors in 204 countries. They concluded that the greatest share of disease prevention was based on a complex approach aimed at mitigating effects produced by risk factors (environmental, occupational, behavioral and metabolic ones). They also noted that the leading role here belonged to the Social Determinants of Health (SDoH for short) [15]. The authors also highlighted that it was vital to trace influence exerted by risk factors on public health and to estimate its intensity in dynamics (a decline, stagnation or growth). This makes it possible to timely adjust managerial decisions on minimizing effects produced by such factors.

Deep integration interactions between different countries given the globalization and digitalization on the global scale exert their influence on internal policies. Thus, for example, the global trends in sustainable development [16] are presented in this or that form in the programs for national development of the Russian Federation and are being implemented by step-by-step achievement of target indicators outlined in the National projects[4] and state programs[5] taking into account environmental issues[6] and spa-

[1] Daily new confirmed COVID-19 cases and deaths per million people. *Our World in Data*. Available at: https://ourworldindata.org/explorers/coronavirus-data-explorer?zoomToSelection=true&time=2022-01-01..latest&uniformYAxis=0&hideControls=true&Metric=Cases+and+deaths&Interval=Cumulative&Relative+to+Population=false&Color+by+test+positivity=false&country=OWID_WRL~RUS (November 15, 2022).

[2] Kreml' dopustil korrektirovku natsproektov iz-za pandemii [The Kremlin admits necessary adjustment of the National projects due to the pandemic]. *RBK*. Available at: https://www.rbc.ru/politics/03/05/2020/5eaece1e9a7947b157729429 (November 03, 2022) (in Russian); National Projects: Expectations, Results, Outlook. *ROSCONGRESS*. Available at: https://roscongress.org/en/materials/natsionalnye-proekty-ozhidaniya-rezultaty-perspektivy/ (November 03, 2022); A black swan with white feathers. The state of the Russian economy during the pandemic. *ROSCONGRESS*. Available at: https://roscongress.org/en/materials/chernyy-lebed-s-belymi-peryami-ekonomika-rossii-v-epokhu-koronakrizisa/ (November 03, 2022).

[3] IHME is the abbreviation for the Institute for Health Metrics and Evaluation.

[4] Natsional'nye proekty [The National projects]. *The Russian Government*. Available at: http://government.ru/rugovclassifier/section/2641/ (October 16, 2022) (in Russian).

[5] Gosudarstvennye programmy [The State programs]. *The Russian Government*. Available at: http://government.ru/rugovclassifier/section/2649/ (October 16, 2022) (in Russian).

[6] Ob ogranichenii vybrosov parnikovykh gazov: Federal'nyi zakon ot 02.07.2021 № 296-FZ [On limiting emissions of greenhouse gases: The Federal Law issued on July 02, 2021 No. 296-FZ]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_388992/ (November 11, 2022) (in Russian); Ob utverzhdenii strategii sotsial'no-ekonomicheskogo razvitiya Rossiiskoi Federatsii s nizkim urovnem vybrosov parnikovykh gazov do 2050 goda: Rasporyazhenie Pravitel'stva RF ot 29.10.2021 № 3052-r [On Approval of the strategy for socioeconomic development of the Russian Federation with low emissions of greenhouse gases for the period up to 2050: The RF Government Order issued on October 29, 2021 No. 3052-r]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_399657/ (November 11, 2022) (in Russian).

tial differentiation of the RF regions[7]. Successful implementation of these activities largely depends on a regional context in different RF regions since the country territory is relatively extensive and huge. The current officially acknowledged[7] basic issues related to spatial development of the Russian Federation include high intra- and inter-regional socioeconomic inequality, uncomfortable urban environment, poor quality of the environment in large urban agglomerations (where population exceeds 500 thousand people), negative effects produced by the global climate change etc. Complex and differentiated approaches that consider a specific demographic situation, socioeconomic peculiarities and natural and climatic conditions in different RF regions have been selected as the guidelines[7, 8] for finding solutions to the issues outlined above.

According to the forecasts by the RF Ministry of Economic Development, key trends in the economic policy in the middle term are going to include "spatial development that involves reduction in inter-regional differentiation in the quality of life…" and "development of human capital through raising quality and availability of healthcare, education, culture, environment and safety (considering the higher requirements to the quality of life) by using the most advanced technologies" [8].

Given that, it seems only logical that the information space now provides data on region ratings as per the quality of life[9] and management quality[10] made by using calculated indexes and experts estimates as per indicator groups that describe different spheres in people's activities.

Therefore, there are certain predispositions for new emerging risk factors and more precise definition of the existing ones since they modify the medical and demographic situation on the global scale against the background of all the efforts taken to decrease the global burden of diseases, a regional context considered. These predispositions determine the necessity to accomplish studies that address prediction and assessment of available public health potential in a territorial aspect.

The present study develops methodical approaches to predicting changes in life expectancy at birth given the occurring transformations of socio-hygienic determinants that modify it. Previously, the authors created a list of socio-hygienic determinants (SHDs) of life expectancy at birth[11] based on the results obtained by relevant and topical studies on epidemiology of non-communicable diseases. ANNs were applied to create models within 'SHDs – LEB' system followed by estimating a potential growth in LEB in the Russian Federation as a whole [17] and in a given RF region [18] considering the target indicators outlined as goals within the National and Federal projects aimed at improving the quality of life in the country.

The current research in the sphere and the state projects being implemented at the moment gave grounds for the basic scope of the

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[7] Ob utverzhdenii Strategii prostranstvennogo razvitiya Rossiiskoi Federatsii na period do 2025 goda: Rasporyazhenie Pravitel'stva RF ot 13.02.2019 № 207-r (red. ot 30.09.2022) [On Approval of the strategy for spatial development of the Russian Federation for the period up to 2025: The RF Government Order issued on February 13, 2019 No. 207-r (last edited on September 30, 2022)]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_318094/ (November 12, 2022) (in Russian).

[8] Ob utverzhdenii Osnov gosudarstvennoi politiki regional'nogo razvitiya Rossiiskoi Federatsii na period do 2025 goda: Ukaz Prezidenta ot 16.01.2017 № 13 [On Approval of the Fundamentals of the state policy for the regional development in the Russian Federation for the period up to 2025: The RF President Order issued on January 16, 2017 No. 13]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_210967/ (November 12, 2022) (in Russian).

[9] Luchshie regiony dlya zhizni. Reiting RBK [The best regions for living. RBK rating]. *RBK*. Available at: https://www.rbc.ru/economics/26/04/2021/6078136e9a7947d0e9e1b1fb (October 26, 2022) (in Russian).

[10] Turovskii R.F., Orlov D.I. IX reiting effektivnosti upravleniya v sub"ektakh Rossiiskoi Federatsii v 2021 godu [IX rating of management efficiency in the RF regions in 2021]. *APEC: the Agency for Political and Economic Communications*. Available at: http://www.apecom.ru/projects/item.php?SECTION_ID=91&ELEMENT_ID=7691 (October 26, 2022) (in Russian).

[11] MR 2.1.10.0269-21. Opredelenie sotsial'no-gigienicheskikh determinant i prognoz potentsiala rosta ozhidaemoi prodolzhitel'nosti zhizni naseleniya Rossiiskoi Federatsii s uchetom regional'noi differentsiatsii: metodicheskie rekomendatsii [MG 2.1.10.0269-21. Identification of socio-hygienic determinants and prediction of a potential growth in life expectancy at birth for the RF population, regional differentiation taken into account: methodical guidelines]. Moscow, 2021, 113 p. (in Russian).

present study and corroborated its relevance. **The aim** was to establish regularities of influence, both at the country and regional levels, exerted by indicators describing sanitary-epidemiological wellbeing and lifestyle-related ones on LEB and to estimate reserves of managing this public health indicator considering activities performed by Rospotrebnadzor and implementation of the national and regional projects in the middle and long term.

To achieve this aim, several tasks were set and then solved: 1) to identify different types of RF regions and distribute them into specific groups as per these types considering sanitary-epidemiological wellbeing (SEW for short) and lifestyles; 2) to set scenario changes in socio-economic determinants in the middle term (up to 2024) according to the existing trends and targets outlined in the Federal and regional projects; 3) to calculate predicted LEB levels in RF regions (by 2024) with different SEW and lifestyles as per preset scenarios; 4) to establish available reserves for LEB management as regularities and peculiar effects produced on the indicator by regionally differentiated SEW indicators and lifestyle-related ones.

Materials and methods. Life expectancy at birth and factors that can modify it, both environmental and lifestyle-related ones, were selected as our research objects. Official statistical data over the period in 2010–2018 on all the RF regions were taken from the open sources and applied as initial data in socio-hygienic determinants and LEB levels. In conformity with the Methodical guidelines MR 2.1.10.0269-21 'Identification of socio-hygienic determinants and prediction of a potential growth in life expectancy at birth for the RF population, regional differentiation taken into account'[11], the list of the examined indicators included 148 socio-hygienic determinants that were conditionally aggregated into 6 groups: sanitary-epidemiological wellbeing (53 indicators), socio-demographic indicators (34), lifestyle-related indicators (30), economic indicators (14), public healthcare (9 indicators), nature and climate (8 indicators).

We performed k-means clustering to identify what RF regions were comparable as per SEW indicators and lifestyle-related ones; the analysis relied on 2018 data. Each examined group of determinants was divided into 4 clusters. Next, all the RF regions were compared as per their belonging to specific clusters in the analyzed groups of socio-hygienic determinants.

Analysis as per groups of SEW indicators and lifestyle-related ones was performed with these factors viewed as potentially manageable by activities performed by the sanitary-epidemiological service in accordance with its authority and considering the existing trends and additional effects produced by activities accomplished within the projects being implemented in the country.

Clusters were ranked from conditionally 'the best' to 'the worst' ones within the analyzed groups of socio-hygienic determinants; this was done by ranking average cluster values in these groups and the resulting biggest sum of all the ranks in a given group was considered 'the worst'. To perform comparative assessments, we ranked the RF regions as per ultimate effects on LEB produced in clusters of the analyzed indicator groups.

Available reserves for managing LEB through predicting its potential growth were identified in accordance with the algorithm described in the MR 2.1.10.0269-21[11], with using a neural network model (a four-layer perceptron with two internal layers containing 8 and 3 neurons accordingly, the determination coefficient (R^2) of the ultimate model was equal 0.78); analyzing cause-effect relations between environmental factors and lifestyle-related ones (socio-hygienic determinants) and LEB; the next stage involved identifying the ultimate predicted LEB level based on scenario changes in either all the socio-hygienic determinants or some specific ones.

In accordance with the algorithm described in the MR 2.1.10.0269-21[11], scenario changes in socio-hygienic determinants (independent variables) were set within the baseline scenario as equal to their values that were actually registered in 2018 (a pre-COVID period); the data on 2018 were taken from open official statistical sources. To achieve the aim of the study and solve tasks set within it, we parameterized predictive scenarios only as regards SEW indicators and lifestyle-related ones; all the other indicator groups were considered background characteristics of the analyzed territories. Target values set for two analyzed groups of socio-hygienic determinants (sanitary-epidemiological wellbeing and lifestyle) were specified in accordance with the target indicator levels outlined in the National and Federal projects[12] and calculated (predicted) indicator levels as per linear / logarithmic trends by 2024 (the highest value of the determination coefficient R^2 was used as the criterion).

Predicted LEB levels (by 2024) were calculated and reserves for managing this indicator in RF regions with different sanitary-epidemiological wellbeing and lifestyles were identified using an algorithm that included several stages. First, baseline and target scenarios were created to describe changes in indicators characterizing socio-hygienic determinants for different types of the analyzed territories. Next, predicted LEB levels were calculated for different types of territories in accordance with baseline and target scenarios. Last, we calculated a potential growth in LEB for different territories (as difference in model LEB levels as per a baseline and target scenario) followed by identifying reserves for managing this indicator.

We analyzed reserves for managing life expectancy at birth relying on predicted estimations of a growth in this indicator (potential LEB growth). The analysis considered scenario changes in the analyzed socio-hygienic determinants, all-country regularities and regional peculiarities as well as the existing unrealized potential due to targeted management of priority SEW indicators and lifestyle-related ones.

Mathematical modeling and statistical analysis of the initial data with subsequent analysis and visualization of its results were accomplished in software packages for mathematical computations and statistical data analysis (Statistica 10, RStudio, MS Excel 2010).

Results and discussion. K-means clustering gave an opportunity to distribute the RF regions into different clusters within the analyzed groups of socio-hygienic determinants (Figure 1).

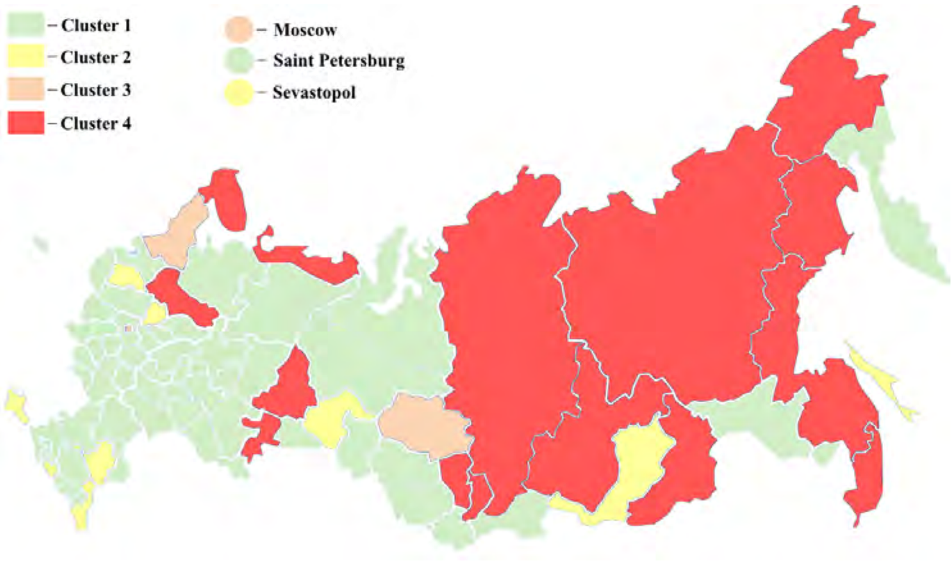
Since there was significant differentiation of the RF regions, we ranked the clusters within each of the analyzed groups of socio-hygienic determinants (Table 1).

Conditionally 'the best' and 'the worst' clusters (Table 1) were identified by ranking average cluster values of indicators in the RF regions as per clusters within the analyzed groups of socio-hygienic determinants.

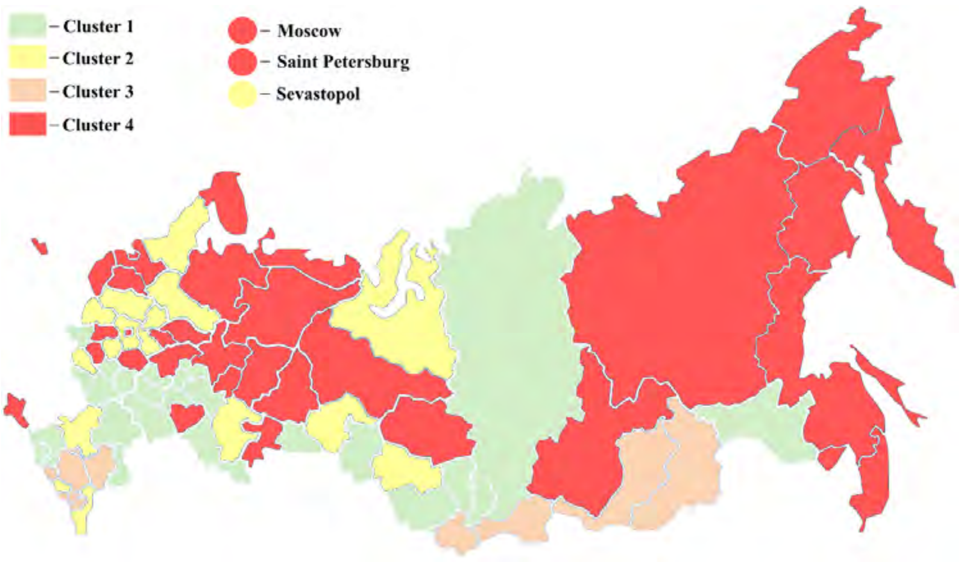
Clusterization of the RF regions within the group comprising **indicators of sanitary-epidemiological wellbeing** (Figure 1a) has revealed that the RF regions that were assigned to the **first cluster** have comparatively higher levels of sanitary-epidemiological wellbeing against other clusters (Table 2).

This cluster has the greatest number of the RF regions, namely 57 (the Leningrad region, Tula region, Belgorod region, etc.). The regions in this cluster typically have the lowest share of drinking water sources that do not conform to the sanitary standards and rules (10.6 % of centralized water supply sources; 11.7 %, non-centralized ones; 14.6 % of surface sources and 10.0 % of underground ones). Also, the share of soil samples deviating from the standards is the lowest in this cluster and equals 3.6 % as regards sanitary-chemical indicators and 2.7 % as regards levels of heavy metals. The cluster has the greatest share of population provided with qualitative drinking water (95.3 % of the urban population and 79.3 % of the rural population). Also, the cluster has the lowest share of food samples that do not conform to the existing sanitary-epidemiological requirements as per sanitary-chemical (0.4 %) and microbiological indicators (3.5 %). The cluster has the lowest share of working population who has to work under conditions deviating from the hygienic standards (34.9 %) including exposure to such

[12] Natsional'nye proekty [The National projects]. *The Russian Government.* Available at: http://government.ru/rugov-classifier/section/2641/ (October 16, 2022) (in Russian).



a



b

Figure 1. Results of clusterization in groups of SEW indicators (a); lifestyle-related indicators (b)

Table 1

Cluster ranking within groups of socio-economic determinants

A group of socio-economic determinants	Number of a cluster*	Rank sum	Number / share of regions in a cluster **
Indicators of sanitary-epidemiological wellbeing	1	120	57 (67.1 %)
	2	147	10 (11.8 %)
	3	149	3 (3.5 %)
	4	174	15 (17.6 %)
Lifestyle-related indicators	1	43	24 (28.2 %)
	2	57	17 (20.0 %)
	3	57	10 (11.8 %)
	4	63	34 (40.0 %)

Note: * means that coloring matches the qualitative attribute of 'the best' (green) and 'the worst' (red) cluster as per the quantitative attribute 'Rank sum'; the higher the sum, the 'worse' (conditionally) the cluster is within the analyzed group of socio-hygienic determinants; ** means that a share of the regions in a cluster out of the total number of RF regions is given in brackets.

Table 2

Cluster ranks within the groups of indicators that describe sanitary-epidemiological wellbeing

Groups of indicators describing sanitary-epidemiological wellbeing	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Ambient air quality	2	3	1	4
Drinking water quality	1	4	3	2
Soil quality	1	3	2	4
Physical factors in urban settlements (noise, EMR)	2	1	4	3
Quality of non-foods	2	4	1	3
Quality of foods	1	2	4	3
Quality of workplaces	2	1	3	1
Typical categories of objects as per their sanitary-epidemiological profiles	2	2	1	2
Economic entities as per risk categories	2	1	1	1
Working conditions deviating from the existing hygienic standards	1	1	2	3
Rank sum	19	22	24	27

Note: different colors of the table cells correspond to average cluster values as per priorities where red means the worst attributes and green means the best ones.

harmful factors as vibration (4.1 %), non-ionizing radiation (0.9 %), work hardness (17.4 %) and intensity (4.3 %). Still, the cluster holds the second rank place as per emissions from stationary sources (157 thousand tons per year) and the first rank place as per the share of soil samples that do not conform to the existing standards as per parasitological indicators (1.5 %). In 2018, LEB levels varied between 66.47 and 82.41 years in the regions in this cluster.

The second cluster as per sanitary-epidemiological wellbeing includes 10 RF regions (the Novgorod region, the Yaroslavl region, Republic of Crimea, etc.). Emissions of pollutants are lower in this cluster, both from stationary and mobile sources (55.6 thousand tons and 98.2 thousand tons per year accordingly). But the share of drinking water sources that do not conform to the sanitary standards and rules is the highest in this cluster (39.5 % of centralized water supply sources and 44.9 % of non-centralized ones; 50.7 % of surface sources and 36.9 % of underground ones). The cluster has a relatively high share of soil samples that deviate from the hygienic standards as per sanitary-chemical indicators and levels of heavy metals (7.0 % and 7.2 % accordingly) but the share of samples deviating from the standards as per microbiological indicators is quite low (3.5 %). The share of working people who have to work under conditions not conforming to the existing hygienic standards is relatively lower than in other clusters (35.1 %); the lowest shares were identified as regards exposure to such occupational factors as noise (15.4 %), biological factors (0.5 %), and adverse microclimate (2.9 %); but the share of working people exposed to chemical factors at their workplaces was rather high (6.9 %). The cluster has the high share of ambient air samples with chemical contents exceeding MPC in rural areas (3.9 %). In 2018, LEB levels varied between 69.92 and 78.69 years in the regions in the second cluster.

The third cluster includes three RF regions (Moscow, Republic of Karelia and the Tomsk region). Just like other clusters, this one has several indicators of sanitary-epidemiological wellbeing with relatively higher average cluster values. In this cluster, there are higher shares of food raw materials and food products deviating from the existing sanitary-epidemiological requirements as per sanitary-chemical and microbiological indicators (0.68 % and 6.5 % accordingly); the cluster also has the highest share of workplaces that do not conform to the existing hygienic requirements as per noise and vibration (29.0 % and 47.3 % accordingly). In 2018, LEB levels varied between 70.56 and 77.84 years in the third cluster.

The fourth cluster includes 15 RF regions (the Chelyabinsk region, the Irkutsk region, Trans-Baikal territory, etc.). The sanitary-epidemiological situation is comparatively unfavorable there. This cluster has the highest average cluster values of emissions that pollute ambient air, both from stationary and mobiles sources (475.8 and 650.6 thousand tons accordingly); the share of ambient air samples with chemical contents exceeding MPC in urban areas is the highest (3.2 %). Drinking water quality is also relatively poor since the share of centralized water supply sources, including underground ones, which do not conform to the sanitary standards and rules, is high (22.8 % and 21.9 % accordingly). There are high shares of soil samples in the regions included into this cluster that do not conform to the hygienic requirements as per sanitary-chemical and microbiological indicators and levels of heavy metals (11.9 %, 11.4 % and 10.9 % accordingly). The cluster has the highest shares of food samples with levels of antibiotics deviating from the hygienic standards, namely 1.2 %, as well as the highest shares of non-foods deviating from the standards as per sanitary-chemical indicators, 10.1 %. The cluster has the highest share of working people who have to work under conditions not conforming to the existing hygienic standards, both as a whole (51.7 %) and as per specific factors (noise, 28.9 %; vibration, 10.3 %; work hardness, 27.7 % etc.). In 2018, LEB levels varied between 63.58 and 72.72 years. It is noteworthy, that most cities included into the 'Clean Air' Federal project (9 cities or 75 %) are located in the regions included into this cluster.

Clusterization of the RF regions within the group comprising **lifestyle-related indicators** (Figure 1b) revealed that the **first cluster** included 24 RF regions (the Orenburg region, the Volgograd region, Republic of Tatarstan, etc.). Just as it was the case with the group of indicators describing sanitary-epidemiological wellbeing, this cluster has a relatively more favorable situation as regards the analyzed group of indicators (Table 3). In this cluster, consumption of basic foods, including vegetables (23.4 %) and fruits (26.3 %) deviates from the recommended standards[13] only slightly; ethanol consumption per capita is comparatively low (7.5 liters per year per an adult person); the share of population who do sports or physical exercises is comparatively high (44.7 %). In 2018, LEB levels in the first cluster as per lifestyle-related indicators varied between 69.11 and 74.35 years.

The second cluster includes 16 RF regions (the Kursk region, the Novosibirsk region, the Yamal-Nenets Autonomous Area etc.). The cluster has the most optimal food consumption (close to the recommended standards with relatively smaller deviations) as regards vegetables (-18.1 %), fruits (-15.8 %), potato (-26.2 %), milk products (-10.2 %), and eggs (-4.2 %); still, some products are consumed in excessive quantities, namely meat and meat products (+37.3 %), sugar (+53.4 %) and butter (+13.8 %). Alcohol consumption is higher in this cluster than in the first or third one but lower than in the fourth one. In this cluster, the total number of sport objects and facilities is lower than in other clusters, in

Table 3

Cluster ranks within the groups of lifestyle-related indicators

A group of lifestyle-related indicators	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Quantitative attributes of food consumption by population	1	2	4	3
Quantitative attributes of retail alcohol sales to population	2	3	1	4
Population's physical activity	1	3	4	2

Note: different colors of the table cells correspond to average cluster values as per priorities where red means the worst attributes and green means the best ones.

[13] Ob utverzhdenii Rekomendatsii po ratsional'nym normam potrebleniya pishchevykh produktov, otvechayushchikh sovremennym trebovaniyam zdorovogo pitaniya: Prikaz Ministerstva zdravookhraneniya RF ot 19 avgusta 2016 g. № 614 [On Approval of the Recommendation on rational food consumption that meet the healthy diet requirements: The Order by the RF Public Healthcare Ministry issued on August 19, 2016 No. 614]. *GARANT: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/71385784/ (November 15, 2022) (in Russian).

particular, there are not enough gymnasiums and stadiums; still, the share of people who do sports or physical exercises is quite high in the cluster (42.24 % or the second rank place). The minimum and maximum LEB levels were 70.47 and 78.69 years accordingly in the regions in this cluster in 2018.

The **third cluster** as per lifestyle-related indicators includes 10 RF regions (Karachay-Cherkess Republic, the Altai Republic, Trans-Baikal territory, etc.). The cluster has the lowest retail alcohol sales (3.8 liters of ethanol per year per an adult person); still there are some deviations in food consumption, which is lower than the recommended standards as regards some foods (vegetables, -35.9 %; fruits, -41.4 %; milk products, -27.4 %; fish and sea foods, -37.1 %). The share of population who do sports or physical exercises is rather low (40.1 %). In 2018, LEB levels in the third cluster as per lifestyle-related indicators varied between 66.47 and 82.41 years.

The fourth cluster includes 34 RF regions (the Kirov region, the Orel region, the Sverdlovsk region, etc.) and the situation there as per lifestyle-related indicators could be described as 'less favorable' in comparison with three other clusters. Retail alcohol sales are the highest in this cluster (10.7 liters per year per an adult person). Some products are consumed in quantities lower than the recommended standards[14] (vegetables, -34.6 %, fruits, -32.8 %, etc.); the share of population who do sports or physical exercises is also low, 39.7 %, despite quite well-developed sport infrastructure (237 sport facilities per 100 thousand people). The minimum and maximum LEB levels were 63.58 and 77.84 years accordingly in the regions in this cluster in 2018.

We relied on the results obtained by clusterization to estimate a potential growth in LEB considering the existing trends and additional influence exerted by the National and Federal projects being implemented now. The estimation revealed significant differentiation of effects produced on LEB by specific sub-groups of SEW indicators and life-style related ones as well as the whole indicator groups in regions that belonged to different types (clusters).

In the RF as a whole, at present we can expect variable effects produced by indicators describing **sanitary-epidemiological wellbeing** by 2024 considering the current trends, from -289 days to +1398 days. Negative effects on LEB as per the current trends are detected in some regions in the 3rd and 4th clusters where deviations from the sanitary standards as per specific SEW indicators are the greatest and / or there are negative short-term trends (by 2024) in determinants included in this group.

The **fourth type** (cluster) is characterized with effects on LEB within the range between (-289) and +220 days due to changes in sanitary-epidemiological determinants by 2024 according to the current trends. Positive effects that predict a LEB growth are produced by improved working conditions, better quality of non-foods, and better drinking water quality (Figure 2b). On average, the smallest effects on LEB according to the current trends are produced in this cluster by soil quality, quality of foods, and risk categories of economic entities as regards possible violations of sanitary-epidemiological requirements. A negative effect on LEB is produced in this cluster, according to the current trends, by soil quality.

Implementation of the 'Clean Air', 'Clean Water' and some other Federal projects in the regions included in this cluster can produce additional positive effects on LEB within the range from 9 to 420 days. These variations occur due to other significant trends in indicators of sanitary-epidemiological wellbeing and internal relations between specific determinants in this group. Still, we have a significant reserve for a LEB growth due to improvement of other sanitary-epidemiological indicators even if changes in them would not lead to significant improvement in the sanitary-epidemiological situation in the regions in this cluster by 2024 (Figures 2a and 2b). Thus, implementation of the National projects and achieving 'better'

[14] Ob utverzhdenii Rekomendatsii po ratsional'nym normam potrebleniya pishchevykh produktov, otvechayushchikh sovremennym trebovaniyam zdorovogo pitaniya: Prikaz Ministerstva zdravookhraneniya RF ot 19 avgusta 2016 g. № 614 [On Approval of the Recommendation on rational food consumption that meet the healthy diet requirements: The Order by the RF Public Healthcare Ministry issued on August 19, 2016 No. 614]. *GARANT: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/71385784/ (November 15, 2022) (in Russian).

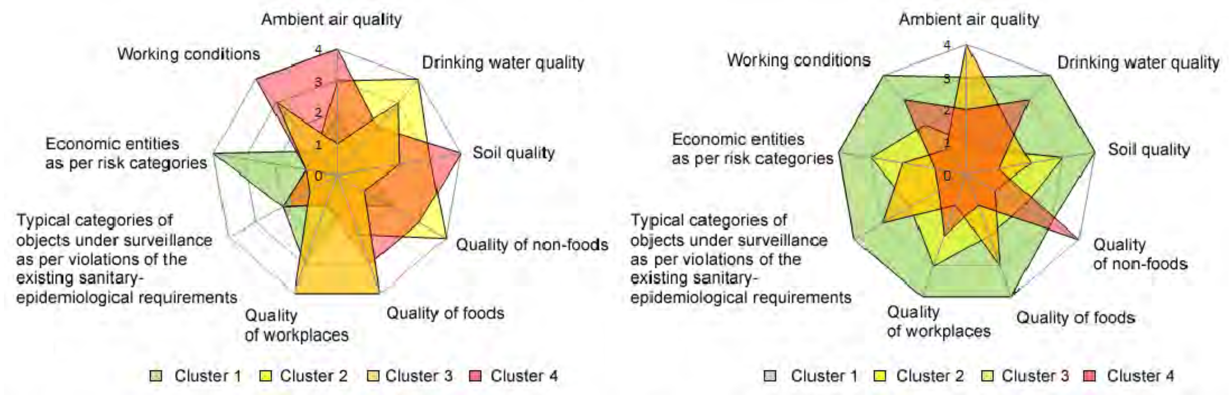


Figure 2. Cluster ranking in the group of indicators that describe sanitary-epidemiological wellbeing: (a) as per rank values of indicators in 2018; (b) as per aggregated effects produced on LEB by indicator sub-groups by 2024 (the 1st rank means the most favorable situation / the smallest effect on LEB; the 4th rank means the least favorable situation / the greatest effect on LEB)

levels of indicators that do not conform to the existing sanitary-epidemiological requirements (achieving levels that are as close to the hygienic standards and requirements as possible) can secure a growth in LEB in this cluster that at best would reach 740 days.

In **the 3rd cluster,** effects produced on LEB by 2024 as per the existing trends vary between -191 days and +1398 days. In the short term, the greatest positive effect on LEB is expected to be produced by better ambient air quality, better quality of foods, and typical categories of objects under surveillance as per violations of the existing sanitary-epidemiological requirements. The smallest effects are expected to be produced by changes in working conditions, quality of workplaces, and quality of non-foods. The current trends in drinking water quality produce negative effects on a potential LEB growth in some regions in the cluster. Implementation of the existing Federal projects can secure an additional growth in LEB up to 42 days. Improved working conditions, quality of workplaces and drinking water quality might be considered additional reserves for a LEB growth by 2024 (Figures 2a and 2b).

The current trends in sanitary-epidemiological determinants in the **2nd cluster** can produce effects on LEB by 2024 that vary between -39 and +853 days. A potential growth in LEB in this cluster is largely expected due to better soil quality, better quality of working places, and risk categories of economic entities. Negative effects on LEB are produced by the current trends in ambient air quality and drinking water quality. Implementation of the National and Federal projects in the regions included in this cluster will secure an additional 10 % growth in effects produced on LEB given the existing trends in sanitary-epidemiological indicators. Besides, there is an additional reserve for a LEB growth due to further improvement of drinking water and ambient air quality.

The regions in the **1st cluster** have a comparatively more favorable sanitary-epidemiological situation against other clusters. According to the current trends, a potential LEB growth by 2024 equals 100–318 days in this cluster. The regions in this cluster have managed to achieve a LEB growth most successfully; this became possible largely due to managing most sanitary-epidemiological indicators that are priority ones in this cluster. They include drinking water quality, soil quality, working conditions etc. Implementation of the Federal projects, in case their targets are achieved as regards relevant indicators, will secure an additional positive effect on LEB by 2024 that will vary between 6 and 57 days. Reserves for an additional LEB growth include minimization of negative effects produced on public health due to further improvement of working conditions, ambient air quality and quality of non-foods.

An expected change **in lifestyle-related indicators** by 2024, considering additional influence exerted by the National and Federal projects being implemented now, can lead to a LEB growth that will vary between 107 and 659 days in the country as a whole. Differences between regions as per effects on LEB occur due to both baseline levels of the analyzed lifestyle-related indicators and registered trends in these determinants (Figure 3a).

The regions in the **1st cluster** have the most favorable levels of lifestyle-related indicators; a potential LLEB growth there varies between 1070 and 280 days. Potentially, the greatest growth in LEB may occur due to achieving targets stipulated by the 'Public Health Improvement' Federal project[15] and 'Sport as a Standard of Life' Federal project[16], namely, a decline in alcohol consumption and greater physical activity in accordance with the regional targets. Potentially, a further LEB growth might be achieved in this cluster as per this indicator group due to even greater decline in alcohol consumption.

In **the second and third clusters,** the current trends by 2024 and implementation of the relevant projects may secure a LEB growth between 229 and 352 days and 266 and 654 days accordingly. This effect on LEB in these clusters can largely be secured due to achieving optimal levels of food consumption (close to rational food consumption recommended by the RF Public Healthcare Ministry[17]). Greater physical activity by population and food consumption close to its optimal levels (vegetables, fruits, meat products, etc.) can secure an additional growth in LEB in these regions.

A potential LEB growth **in the fourth cluster** can be achieved by expected encompassing improvement of all the sub-groups of lifestyle-related indicators. The greatest effect will occur due to alcohol consumption going down to its target levels[17] and optimal food consumption (meat products, milk products, vegetables, etc.). A potential growth in LEB by 2024 in this cluster would vary between 290 and 659 days in case of positive changes in relevant modifying lifestyle-related factors.

Modifying influence exerted by determinants that are being managed by Rospotrebnadzor activities can provide an additional contribution to achieving target LEB levels by 2024 (COVID-related processes excluded): 8–62 % due to an improved sanitary-epidemiological situation on a given territory and 5–45 % due to spreading healthy lifestyle practices.

It is noteworthy that modifying influence exerted on LEB by indicators of sanitary-epidemiological wellbeing and lifestyle-related ones occurs together with background effects produced by concomitant modifying factors (economic, socio-demographic, public healthcare, etc.). Changes in these factors can lead to changing intensity and direction of influence exerted by the analyzed factors that are being managed by Rospotrebnadzor activities.

Figure 3. Cluster ranking in the group of lifestyle-related indicators: (a) as per rank values of indicators in 2018; (b) as per aggregated effects produced on LEB by indicator sub-groups by 2024 (the 1st rank means the most favorable situation / the smallest effect on LEB; the 4th rank means the least favorable situation / the greatest effect on LEB)

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[15] Federal'nyi proekt «Ukreplenie obshchestvennogo zdorov'ya» ['Public Health Improvement' Federal project]. *Ministry of Labor and Social Protection*. Available at: https://mintrud.gov.ru/ministry/programms/demography/4 (November 23, 2022) (in Russian).

[16] Federal'nyi proekt «Sport – norma zhizni» ['Sport is a Standard of Life' Federal project]. *Ministry of Labor and Social Protection*. Available at: https://mintrud.gov.ru/ministry/programms/demography/5 (November 23, 2022) (in Russian).

[17] Ob utverzhdenii Rekomendatsii po ratsional'nym normam potrebleniya pishchevykh produktov, otvechayushchikh sovremennym trebovaniyam zdorovogo pitaniya: Prikaz Ministerstva zdravookhraneniya RF ot 19 avgusta 2016 g. № 614 [On Approval of the Recommendation on rational food consumption that meet the healthy diet requirements: The Order by the RF Public Healthcare Ministry issued on August 19, 2016 No. 614]. *GARANT: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/71385784/ (November 15, 2022) (in Russian).

In all the RF regions, reserves for a LEB growth include further improvement of a sanitary-epidemiological situation as well as individual and population lifestyles together with using the most optimal model for managing relevant determinants, regional differentiation and trends in the current demographic situation taken into account.

Discussion. The results obtained by scenario prediction of changes in socio-hygienic determinants indicate that greater effects might be secured as regards improvement of a medical-demographic situation due to achieving targets stipulated by the Federal and National projects that are being implemented now. Complex activities (National and Federal projects) can provide an effective tool for improving public health, especially on territories where the sanitary-epidemiological situation is unfavorable.

Complex influence exerted by environmental factors on public health is estimated regularly within varied research spheres and in inter-disciplinary studies that rely on using variable methodical approaches relevant to specific research aims and tasks. T.B. Melnikova with colleagues presented the results of regression and factor analysis that concentrated on cause-effect relations between a relatively small number of independent variables and the results obtained by a sampling observation over public health performed by Rosstat (health self-assessment); they established that sanitary-epidemiological factors and lifestyle-related ones made a significant contribution to people's self-assessment of their health [19, 20]. O.V. Kudelina and M.A. Kaneva comparatively analyzed available statistical indicators in their work (LEB, HALE[18], expenses on public healthcare and some others) in order to identify whether it was possible to apply them as indicators (proxy-variables) of health capital. They concluded that there was not any universal indicator and established that LEB and HALE were the optimal Impact[19] indicators; their ultimate conclusion was that an indicator should be chosen based on actual research tasks [21].

I.P. Shibalkov and O.P. Nedospasova applied several tools in their monograph to simulate cause-effect relations between LEB and environmental factors; in particular, the authors used a regression model, factor analysis and cluster analysis of RF regions. They concluded that considerable efforts should be taken to reduce social inequality as regards health along with developing medical services and technologies [22]. The methodical approach accepted in our studies involves analyzing a wider set of indicators and a greater period of their aggregation; it considers the whole set of factors together with their modifying interactions between each other and makes it possible to obtain quantitative estimations of effects produced on LEB, both aggregated ones and as per each factor independently (considering the background influences).

A.I. Piankova and T.A. Fattakhov performed decomposition analysis to estimate reserves for LEB growth in northern regions in Russia owing to declining mortality rates due to circulatory diseases and external causes [23]. In this study, we applied the systemic approach as regards creating scenarios of predicted changes in socio-hygienic determinants for different types of RF regions. This approach involves using both changing trends in a set of independent variables and target values of indicators stipulated in the National and Federal projects with subsequent determination of priorities and management reserves.

A similar approach to comparative assessment of RF regions as per several indicators related to living conditions and sufficient provision of public healthcare with all relevant reserves was applied by S.A. Boitsov with colleagues [24]. The authors noted in their work that quartile division as per YPLL[20] established that RF regions differed significantly as per their economic and geographical properties within quartiles. This indicated that public health depended on multiple factors, relations between the analyzed variables were not lin-

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[18] HALE is healthy life expectancy.

[19] According to LogFrame methodology (the World Bank), the analyzed indicators are distributed as follows: Inputs – Outputs – Outcomes – Impact. The indicators in the Impact category are more efficient for measuring effects produced on certain spheres of human wellbeing.

[20] YPLL means years of potential life lost.

ear, and it was necessary to create multidisciplinary research teams in the field. In our study, an issue related to heterogeneity of the analyzed RF regions with subsequent comparative estimates was solved by identifying different types of regions within the analyzed groups of socio-hygienic indicators and distributing all the regions into different clusters as per these types. Besides, using an ANN model made it possible to consider non-linear interrelations between independent variables.

Extensive data that covered 148 determining indicators over 2010–2018 made it possible to create an optimal neural network model for predicting LEB levels and this is an obvious advantage of our study. But at the same time, the modeling results are limited by these data and it is impossible to make correct predictions of the current LEB levels considering, for example, effects produced by the COVID-19 pandemic, outcomes of economic sanctions and other global processes. Such predictions will require additional training of the ANN model. Indicators were conditionally distributed into groups as per information sources and / or meaning contents of an indicator thereby facilitating data analysis and presentation. However, if the groups are reconfigured, their aggregated effects on LEB might also change. There is one fundamental limitation of this study, namely LEB, the dependable variable we chose. It is considered a hypothetic value that projects the current mortality rates due to all causes onto the next generation. The list of the analyzed independent indicators also can impose certain limitations on the research results concerning effects produced on LEB. More specifically, influence exerted by other modifying factors may be neglected.

There are several promising trends in further development of this research area. They include making more precise lists of independent variables, including other criteria for selecting these independent indicators, standardizing indicator groups (to make them comparable with other studies); adjusting the model or calculating corrections that allow considering effects produced by the COVID-19 pandemic and economic transformations, or creating a new model; examining cause-effect relations between population mortality due to varied causes and socio-hygienic determinants to identify their contributions to final LEB levels and to present more detailed results. Besides, in future we are planning to optimize prediction methods and criteria for determining predictive values of determinants considering their 'essence' and rate of changes; we can also suggest certain solutions to the optimization task and an optimal management model.

Conclusions:

1. Activities performed by Rospotrebnadzor within its authority and aimed at providing sanitary-epidemiological wellbeing and spreading healthy lifestyle practices produce positive modifying effects on the integral health indicator, LEB: their contribution, considering significant differences between the RF regions, to achieving target regional LEB levels by 2024 (COVID-related processes excluded) varies between 8 and 62 % for the group of indicators that describe sanitary-epidemiological wellbeing on a given territory and between 5 and 45 % for the group of lifestyle-related indicators.

2. The RF regions differ as per a level of sanitary-epidemiological wellbeing and lifestyle-related indicators and modifying influence exerted by these factors on a potential growth in LEB in the short term are peculiar in different regions. Priority factors with the maximum positive effects on LEB have been identified for each type of regions. The fourth cluster with the least favorable sanitary-epidemiological situation has the following factors that are priority for management: working conditions, quality of non-foods, drinking water quality and ambient air quality. The fourth cluster with the least favorable lifestyle-related indicators has several priority factors that produce the greatest effects on LEB; they include alcohol consumption, rational food consumption and balanced diets, and physical activity by population.

3. Implementation of the National projects aimed at improving sanitary-epidemiological wellbeing and lifestyle-related indicators can secure an additional LEB growth by 2024 that will vary between 6 and 240 days and 107 and 659 days accordingly provided that the project targets have been achieved against the background of the current trends and regional differentiation.

4. Improved working conditions, better quality of drinking water and ambient air are reserves for a LEB growth for all the types of RF regions. They can secure a regionally differentiated positive effect on LEB both in the short and middle term. All the regions have this reserve for a growth in LEB due to improving the initial indicators of sanitary-epidemiological wellbeing on a given territory and lifestyle-related indicators. Effects produced by changes in them by 2024 will not lead to significant improvement of the sanitary-epidemiological situation in the country and population lifestyles without additional managerial decisions.

5. Indicators that describe a sanitary-epidemiological situation on a given territory and lifestyle-related indicators produce modifying effects on LEB against the background influence exerted by other factors (economic, socio-demographic, public healthcare, etc.); changes in these background factors can lead to changing intensity and direction of influence exerted by factors that are managed by Rospotrebnadzor activities. Differences in effects on LEB between the RF regions occur due to both initial (baseline) values and registered trends in the analyzed determinants.

6. The identified country and regional determinants should be considered when creating an optimization model of LEB management considering all available reserves for its growth.

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Research article

PARAMETERIZATION OF RELATIONSHIPS BETWEEN RISK FACTORS AND PUBLIC HEALTH UNDER CHRONIC EXPOSURE TO COMPLEX AMBIENT AIR POLLUTION

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The relevance of the present study follows from the necessity to establish parameterized cause-effect relationships that describe additional disease cases among population caused by chronic exposure to chemical factors.

In this study, our aim was to explore relationships within the 'environment – public health' system to quantify and predict chronic risks under exposure to chemicals in ambient air.

To achieve this, we collected statistical data on some municipalities located in the Russian Federation with different structures and levels of chemical pollution in ambient air. Data on population incidence and ambient air quality were coordinated at places where calculation points were located; these points were centers of residential buildings and their coordinates were applied in the study. Mathematical modeling of the relationships was conducted by using multiple linear regressions. Pollution indicators (chemical concentrations in ambient air) that met the requirements of biological plausibility and statistical significance of pair correlations were selected as independent variables. The obtained regression models contain 190 factors for 36 chemicals occurring in emission into ambient air from stationary and mobile sources, which allow calculating the frequency of additional disease cases for 29 diseases. The established factors make it possible to perform operative estimations of a number of diseases associated with ambient air quality at a place of residence relying on medical aid applications.

The resulting relationships can be used to predict chronic health risks. Establishing criteria for ranking chemical health risks in zones influenced by hazardous chemical objects can become a next step in development of the suggested approaches.

Keywords: *chronic risk, ambient air pollution, chemicals, mathematical modeling, multiple regression, health risk assessment, incidence, additional cases.*

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At the present stage in the society development, the health risk assessment methodology is in great demand at any level of public administration in Russia. When any activities are being planned, health risk assessment gives grounds for identifying the most effective measures aimed at mitigating threats and hazards. When action plans are being implemented, it gives an opportunity to estimate effectiveness of implemented activities. When it comes down to control and surveillance, health risk assessment makes it possible to identify priorities for concentrating the efforts by relevant authorities on objects that create the highest risks for public health [1, 2].

The necessity to perform health risk assessment is fixed by the RF President Order dated March 11, 2019 No. 97 "On Essentials of the state policy in the Russian Federation on providing chemical and biological safety for the period up to 2025 and beyond". This Order is the fundamental ground for the public administration in the sphere of the RF national security [3, 4]. The tasks set by the 'Clean Air' Federal project of the 'Ecology' National project highlight the importance of assessing health risks caused by exposure to chemicals in ambient air. This project (2019–2024) is aimed at improving the environmental situation and reducing pollutant emissions into ambient air.

Development of the health risk assessment methodology poses some challenges; tackling them involves using advanced scientific and methodical approaches that combine some allied sciences such as medicine, physiology, biology, biomechanics, and mathematics. Use of mathematical modeling is a most promising approach for predicting and estimating contributions made by variable factors into health impairment as well as for establishing cause-effect relations.

At present, the most popular methodical approaches within chemical risk assessment include calculation of hazard indexes and hazard quotients (HI and HQ) of chemicals under various administration ways and classification of risk levels. This approach is easy to implement and relevant data for calculations are also easy to obtain. This makes the method the most available for conducing express-estimations of pollution levels in environmental objects and selecting priority risk factors. The approach is widely used in assessing adverse effects produced by ambient air pollution on health of people in settlements [5, 6]; in assessing health risks created by exposure to pollutants for different population groups [7–9]; in estimating and mitigating influence exerted by an enterprise on environmental objects [10]; in conducting monitoring studies with their focus on pollution in ambient air, water, soils, etc. [11–13]; in urban development and organization of environmental protection; as well as in estimating effectiveness of activities aimed at providing sanitary-epidemiological wellbeing of the population. The method adequacy for health risk assessment is confirmed by its wide prevalence both in Russian and foreign studies [14, 15].

At the same time, our review of available research articles established that calculations and analysis of hazard quotients and indexes did not give an opportunity to quantify such probable negative outcomes for population as additional disease cases [16, 17]. This results in much more narrow range of solvable tasks related to providing chemical and biological safety, public health management and protection, substantiating rehabilitation activities, planning necessary volumes of medical aid rendered to population, predicting expected demographic and economic losses etc.

The methodology for calculating hazard quotients and indexes within risk assessment can be enhanced analytically by introducing certain algorithms. They are based on models describing relationships between environmental factors and negative health outcomes and allow calculating additional likelihood of health disorders associated with exposures to various factors. It is noteworthy, that most published studies with their focus on creating similar relationships address specific cases of effects produced by exposure to a limited number of factors on certain health disorders under specific socioeconomic, weather and climatic and other conditions [18–20].

The methodology for risk evolution modeling involves coordinated use of statistical and analytical models and can be considered one of the most relevant methods for predicting and estimating probable effects produced by environmental factors on public health. This makes it possible to consider effects caused by exposure to heterogeneous factors more comprehensively, including situations when age-related changes should be taken into account. Evolution models give an opportunity to estimate risks that functional disorders would occur in specific organs and systems under present exposure scenarios during the whole lifetime and to analyze contributions made by specific factors and / or their combinations into health risks [21].

By now, multiple studies have established a relation between ambient air pollution and growing incidence of respiratory diseases, diseases of the digestive organs, circulatory system, nervous system, etc., for various population groups, the most sensitive included [22–25]. If we generalize the available materials, we can spot out several limitations of formalized relationships described in research articles. Research results are not repeatable, highly localized and generalized rather poorly for possible use to solve variable tasks. Also, practically all the relationships or their quantitative parameters have not been fixed in regulatory or methodical documents on health risk assessment. Most relationships or their models require further investigations to be applied correctly in risk assessment; a significant share of such relationships were obtained in 1980ties and are not relevant for the current technical and technological development, social sphere, pollution levels in environmental objects, and development of public institutions as regards environmental control and environmental quality and public health management.

In this study, our aim was to examine relationships within the 'environment – public health' system to quantify and predict chronic risks under exposure to chemicals in ambient air.

Materials and methods. The study relied on systemic and statistical analysis. Mathematical models to describe relationships were built by using multiple linear regressions.

We made several basic hypotheses when planning the present study:

– influence exerted by chemical pollution in environmental objects on public health is determined only by levels of the analyzed factors (chemical concentrations or their doses) and combined exposure to any other influencing factors is neglected;

– the additivity property is applied under combined exposure to several chemical factors and their influence on one specific health indicator and any effects able to reinforce or weaken influences exerted by specific chemicals under occurrence of other chemicals are neglected.

To solve the task, we collected statistical data on several large municipalities located in the Russian Federation. Chemical pollution in environmental objects was different in them as per its stricture and levels of chemicals. This condition allowed necessary differentiation in sampling data as regards lists of chemicals and pollution levels for correct statistical modeling.

The modeling procedure involved collecting, preparing and analyzing data on six municipalities over the three-year period from 2019 to 2021 (Perm, Krasnoyarsk, Norilsk, Bratsk, Chita and Shelekhov). We collected the following data on the listed municipalities:

a) data on chemical concentrations in ambient air obtained at the posts belonging to the Centers for Hydrometeorology and Environmental Monitoring (Rosgidromet);

b) data on chemical concentrations in ambient air obtained at the social and hygienic monitoring posts (Rospotrebnadzor);

c) data on pollutant emissions from stationary and mobile sources taken from the aggregated data collections on maximum permissible emissions (Rosprirodnadzor);

d) data on medical aid applications taken from the registers of paid disease cases (regional offices of the Fund for Obligatory Medical Insurance);

e) data on points where residential areas were located within the analyzed municipalities with location 'binding'.

All the data were taken from official sources, coordinated and combined into electronic tables to make them eligible for further relationship modeling procedures.

Data on population incidence and ambient air quality were coordinated at places where calculation points were located; these points were centers of residential buildings and their coordinates were applied in the study. To coordinate and prepare data on ambient air quality, the following operations were accomplished:

– data taken from the location register of electronic maps were actualized and coordinates of the centers of polygons in the 'Buildings and Constructions' layer were identified;

– dispersion of chemicals emitted by stationary and mobile sources was calculated at the selected calculation points that were centers of residential buildings;

– results obtained by calculating dispersion were verified as per data of laboratory control performed at control points;

– verified data for all the analyzed territories were coordinated.

Dispersion of chemicals was calculated according to the methodology MRR 2017 with the 'Ekolog-Gorod' unified program for calculating ambient air pollution, version 4.60.1.

The results obtained by calculating chemical dispersion were verified in accordance with the methodical guidelines MR 2.1.6.0157-19 'Creation of the programs for surveillance over ambient air quality and quantification of population exposure within social-hygienic monitoring', approved by the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on December 02, 2019. According to the aforementioned procedure, the results obtained by calculating ground levels of chemicals at the calculation points on each territory (the points were located within residential areas) were verified by data obtained by field observations at the posts for monitoring of ambient air quality (they were corrected by approximating conformity factors of calculated and field data at the monitoring points). A major condition here was that both calculated and field data were available for a given territory.

When verifying calculated data obtained on various territories, we had to analyze several possible situations regarding specific chemicals:

a) in case there were no field data on some chemicals on all the analyzed territories, they were completely excluded from the modeling;

b) in case there were no calculated data for some chemicals, but their levels were measured by laboratory control at the control points, we approximated their levels in residential areas by applying the backward distance method;

c) in case some chemicals were not measured with laboratory tests but there were calculated data regarding their dispersion, verification was accomplished with using an averaged conformity factor at the control points on other territories.

Coordination of the verified chemical concentrations for all the territories involved creating a joint electronic table. It contained data on levels of all the selected chemicals with binding to territory identifiers and residential areas. As a result, 48 chemicals were selected for constructing relationships. We should note that cause-effect relations can be detected and later applied only at the stage when a model is determined; therefore, it is important to analyze distribution of ambient air pollution indicators. Table 1 provides basic data on distribution of chemical levels on the analyzed territories. These parameters were applied for relationship modeling.

Data on population incidence with binding to the calculation points on the analyzed territories were prepared by using depersonified information on the number of insured people and their medical aid applications. This information was provided by regional offices of the Fund for Obligatory Medical Insurance.

Table 1

Distribution of chemical levels applied in mathematical modeling

Chemical	Concentration, mg/m^3				
	Minimum	25th percentile	Median	75th percentile	Maximum
Nitrogen (II) oxide (Nitrogen oxide)	0	0.009	0.013	0.019	0.167
Nitrogen dioxide (Nitrogen (IV) oxide)	0	0.015	0.024	0.033	0.238
Ammonia	0	0.010	0.016	0.021	1.413
Benz(a)pyrene	0	0	0.109	0.400	5.759
Benzene	7.86E-06	9.06E-04	2.09E-03	0.005	1.636
Particulate matter	0.005	0.055	0.079	0.125	1.767
Particulate matter PM_{10}	0	0	0	0.040	0.267
Particulate matter $PM_{2.5}$	0	0	0	0.018	0.251
Hydroxybenzene (Phenol)	9.87E-06	5.11E-04	9.80E-04	1.72E-03	1.101
Hydrogen chloride	0	3.88E-03	0.011	0.025	0.515
Dialuminum trioxide (recalculated as per aluminum)	0	8.88E-05	1.24E-04	1.95E-04	0.02
Dihydrosulfide (Hydrogen sulphide)	5.5E-05	3.47E-04	5.86E-04	1.73E-03	0.38
Dimethyl benzene (Xylene)	0	1.09E-03	3.86E-03	0.010	0.645
Cadmium oxide (recalculated as per cadmium)	0	0	0.01	0.01	0.01
Cobalt oxide (recalculated as per cobalt)	0	0	0	0	1.59E-04
Manganese and its compounds (recalculated as per manganese (IV) oxide)	2E-06	5.33E-05	0.013	0.033	0.224
Copper (II) oxide (recalculated as per copper)	2.3E-06	4.39E-05	0.012	0.059	9.213
Methylbenzene (Toluene)	3.3E-05	1.10E-03	3.56E-03	9.67E-03	0.243
Methyl mercaptan	0	1.66E-08	7.73E-07	1.94E-06	8.32E-04
Nickel oxide (recalculated as per nickel)	2.25E-06	4.04E-05	1.95E-03	0.018	0.255
Ozone	0	0	0	1.08E-03	0.149
Prop-2-enenitrile	0	3.26E-05	5.14E-05	1.02E-03	0.073
Lead and its non-organic compounds (recalculated as per lead)	5.18E-08	7.70E-06	3.87E-03	0.012	0.17
Sulfur dioxide (Sulfuric anhydride)	2.29E-04	8.90E-04	1.56E-03	0.008	0.696
Sulfuric acid	0	0	0	3.06E-04	0.161
Tetrachloroethylene	0	0	0	6.56E-03	0.46
Trichloroethylene	0	0	0	0.025	46.665
Carbon (Soot)	1.63E-03	0.829	1.205	2.207	34.059
Carbon oxide	0.053	0.422	0.548	0.998	15.520
Formaldehyde	0	3.00E-03	6.91E-03	0.01167	0.104
Poorly soluble non-organic fluorides	0	0	0	3.17E-03	0.04
Gaseous fluoride compounds (recalculated as per fluorine)	3.54E-06	1.27E-03	1.92E-03	3.94E-03	0.043
Chlorobenzene	0	0	0	1.00E-04	4.00E-04
Chromium (hexavalent chromium) (recalculated as per chromium (VI) oxide)	4.48E-07	1.11E-05	4.34E-03	0.021	0.097
Zinc	0	2.05E-03	0.09	0.16433	0.322
Ethylbenzene	0	1.00E-03	2.12E-03	5.65E-03	31.436

The obtained data were 'bound' to location registers of electronic maps of the analyzed territories and differentiated as per age groups (children aged 0–17 years, people of working age, elderly people beyond working age) and classes and groups of diseases as well as specific diseases.

A preliminary biomedical examination made it possible to create a list of 43 priority diseases and their groups; they were considered probable health outcomes as a response to negative effects produced by chemical pollutants in ambient air. It also gave an opportunity to fill the 'factor – response' expert matrix with relevant data. The examination was performed by 11 experts specializing in hygiene and epidemiology as well as Candidates and Doctors of Medical and Biological Sciences with work records in the sphere being longer than 15 years. The examination was conducted in three stages. The first stage involved creating a list of diseases and their groups; it was done by experts resorting to their available experience during a group meeting. At the second stage, each expert filled in the 'factor – response' matrix independently relying on analysis of relevant Russian and foreign literature sources. The score '1' was put in case there was a potential relationship between a chemical pollutant in ambient air and a disease; otherwise, the score '0' was put. The third stage was a group discussion when all the individual expert scores were discussed and the ultimate matrix was filled (by the majority of votes). It is noteworthy that any expert could change his or her opinion during the final voting if persuaded to do so by other experts' arguments or literature sources provided by them.

Two coordinated data arrays were created by preparing all the relevant data for construction of relationships:

1) verified average annual levels of chemicals at the calculation points on the analyzed territories;

2) relative frequency of people's applications for medical aid in residential areas (the calculation points) for three age groups as per selected diseases.

Relationship modeling to quantify chronic health risks was based on all the collected and prepared data and involved accomplishing the following tasks:

– to construct a matrix of possible (probable) relationships between chemical factors and incidence rates (the biological plausibility matrix) according to expert scores and known pathogenetic regularities;

– to create and analyze correlations between chemical factors and health disorders, to identify statistically authentic correlations;

– to formalize and parameterize relationships relying on multiple linear regression analysis.

The correlation analysis relied on calculating Pearson's correlation coefficients and testing statistical hypotheses to identify authenticity of differences with Student's t-test. Modeling was conducted separately for each dependent variable (incidence as per a group of diseases or a specific disease). Pollution indicators (levels of chemicals in ambient air) were taken as independent variables in case they were biologically plausible and met the requirements as per statistical authenticity of pair correlations.

The modeling process itself involved examining models that described cause-effect relations by using multiple linear regression analysis as per the following formula (1):

$$z = a_0 + \sum_i a_i x_i \ , \qquad (1)$$

where z is relative frequency of a disease, cases/100,000;

x_i is a level of exposure to the i-th chemical factor;

a_0, a_i are model parameters.

A step-by-step procedure was developed to build multiple regression models. It entailed exclusion of summands with negative regression coefficients from the complete model for each dependent variable (obtained with multiplicity of selected independent variables). These summands were excluded due to nonconformity with the accepted hypotheses. The applied algorithm made it possible to obtain models with only positive coefficients included in them; this means that these models consider only negative effects produced on health by chemical pollutants in ambient air.

We created a script in the *R-studio* environment to automate the procedure for constructing relationship models since it involved an inspection with using statistical and biological indicators and multiple regression analysis is iterative in its essence.

Results and discussion. In this study, we analyzed approximately 6.3 thousand pair relationships between frequency of medical aid applications by different age groups (children, people of working age, elderly people beyond working age) and levels of chemical in ambient air on the analyzed territories. Based on them, we built 56 multiple regression models that were statistically authentic and biologically plausible. Since standard and measured levels of various chemicals in ambient air can differ considerably (by several orders of magnitude), the model coefficients also have values that differ significantly. Therefore, Table 2 provides parameters of the obtained multiple regression models a_i for each age group that are adjusted as per a unit of the reference concentration (RFC, mg/m^3) for chronic inhalation exposure[10]

The regression models obtained by modeling the relationships within the 'environment (levels of chemical in ambient air) – health (incidence)' system contain 190 coefficients for 36 chemicals occurring in emissions into ambient air from stationary and mobile sources. These coefficients make it possible to calculate frequency of additional cases for 29 various diseases. Coefficients presented in the tables have certain dimensionality that corresponds to relative frequency of additional medical aid applications during one year (cases per 100,000 people) per a change in levels of chemicals in ambient air by one unit of the reference concentration (RFC, mg/m^3) for chronic exposure. When using the obtained formal relationships and model coefficients for health risk assessment and analysis, we should bear in mind several limitations and uncertainties arising in the process. Basic limitations of using the modeling results are as follows: the obtained models have a limited range of definition and exposure parameters for some chemicals in ambient air and health outcomes in population have not been estimated statistically.

When factor values go beyond the model range of definition, we should remember that the process linearity might be disrupted towards its intensification. In this case, when the obtained coefficients are used, calculations of the corresponding risks may yield understated results. The aforementioned facts do not eliminate the possibility to extrapolate the obtained relationships beyond the range of definition (towards its increase); still, when interpreting the results of risk calculations, one should treat them as the lower estimation limit.

There are several uncertainties that should be considered when developing the risk assessment methodology. They include the following:

– the constructed models are liner in their essence;

– errors are accumulated when calculations are performed beyond the model range of definition;

– the territories have not been covered in full when preparing data for the modeling;

– different programs for laboratory control of ambient air quality are used on the analyzed territories.

Despite the considerable number of uncertainties and limitations that occurred during the modeling process, the obtained coefficients give an opportunity to perform operative estimations of a number of diseases associated with ambient air quality in residential areas relying on medical aid applications. The obtained relationships can also be used to predict chronic health risks by substituting relevant predicted levels of exposure to factors into the formula (1). The formal relationships described in this work give grounds for developing and implementing methods for chronic health risk assessment.

[1] Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals (approved and introduced by G.G. Onishchenko, the First Deputy to the RF Public Healthcare Minister and the RF Chief Sanitary Inspector on March 5, 2004). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200037399 (October 17, 2022) (in Russian).

Table 2

Parameters of the models describing ‘environment (levels of chemical in ambient air) – health (incidence)’ relationships, cases per 100,000 people per a unit of the reference concentration (RFC, mg/m^3)

Group of diseases (ICD-10 codes)	Chemical	Children	Working age	Beyond working age
Disorders of conjunctiva (H10, H11)	Trichloroethylene	0.46	1.14	
	Formaldehyde	159.79	73.93	37.70
Certain disorders involving the immune mechanism (D80, D81, D82, D83, D84, D86, D89)	Zinc	2.87	0.44	0.47
Arthrosis (M15–M19)	Fluorides*		2978.39	1288.28
Deforming dorsopathies (M40–M43)	Fluorides *		6242.21	4679.50
Other dorsopathies (M50–M54)	Fluorides *		2589.20	4162.21
	Gaseous fluorides*			1376.33
Diseases of myoneural junction and muscle (G70–G73)	Manganese and its compounds*		0.06	
Demyelinating diseases of the central nervous system (G35–G37)	Hydroxybenzene (Phenol)		51.83	
	Manganese and its compounds*		0.04	0.12
	Methylbenzene (Toluene)		117.33	1235.86
	Lead and its compounds*		2.13	1.31
	Tetrachloroethylene		71.97	
Other degenerative diseases of the nervous system (G30–G32)	Benzene			2.70
	Hydroxybenzene (Phenol)			2.29
	Dimethyl benzene (Xylene)			13.12
	Tetrachloroethylene			4.96
	Trichloroethylene			0.07
Other disorders of the nervous system (G90–G99)	Manganese and its compounds*	2.46		1.49
	Tetrachloroethylene	885.92		
Polyneuropathies and other disorders of the peripheral nervous system (G60–G64)	Manganese and its compounds*	0.06		
Cerebral palsy and other paralytic syndromes (G80–G83)	Tetrachloroethylene	527.71		
Episodic and paroxysmal disorders (G40–G47)	Tetrachloroethylene	648.11	372.81	
Other diseases of upper respiratory tract (J30, J31, J32, J34, J35, J37)	Nitrogen (II) oxide		793.42	709.29
	Nitrogen dioxide	1667.65	692.41	281.97
	Particulate matter	25.09	7.29	
	Particulate matter $PM_{2,5}$			2.10
	Hydroxybenzene (Phenol)	1514.42	404.60	
	Dihydrosulfide	13.97		
	Dimethyl benzene (Xylene)	457.03		
	Manganese and its compounds*		0.55	
	Methyl mercaptan	6809.72		
	Nickel oxide *		0.14	
	Ozone	6363.46	1295.37	406.06
	Carbon (Soot)	1.83	4.52	7.82
	Fluorides *	462.31	809.60	964.30
Acute upper respiratory infections (J00, J01, J02, J03, J04, J05)	Nitrogen (II) oxide		1015.42	1426.06
	Nitrogen dioxide	4446.44	110.04	
	Particulate matter	5036.79	307.21	329.62
	Particulate matter $PM_{2.5}$	1511.14		
	Hydroxybenzene (Phenol)	9252.80	1083.52	47.07

End of the table 2

Group of diseases (ICD-10 codes)	Chemical	Children	Working age	Beyond working age
	Hydrochloride		142.53	722.86
	Cadmium oxide*		2.65	
	Cobalt oxide *		781.06	936.20
	Methylbenzene (Toluene)		3320.82	
	Ozone			1957.86
	Prop-2-enenitrile	2355.55		
	Sulfur dioxide	17510.12		72.92
	Carbon (Soot)	296.52	9.23	5.82
	Formaldehyde	1770.93		
	Fluorides *			305.56
	Zinc		1.99	
Pneumonia, organism unspecified (J18)	Nitrogen (II) oxide	1260.76		
	Particulate matter		3.86	3.87
	Particulate matter PM_{10}		93.85	120.92
	Particulate matter $PM_{2.5}$		110.87	71.41
	Hydroxybenzene (Phenol)	240.28		
	Hydrochloride	22.73		27.20
	Cobalt oxide *		812.75	612.30
	Copper (II) oxide *		0.02	0.01
	Nickel oxide *	0.40		0.14
	Prop-2-enenitrile	98.62	30.82	38.74
	Sulfur dioxide			29.44
	Formaldehyde			13.37
	Gaseous fluorides *		344.32	384.01
	Zinc	1.25		
Chronic lower respiratory diseases (J40, J41, J42, J44.1, J44.8, J44.9, J45, J46)	Nitrogen (II) oxide	703.75		
	Nitrogen dioxide	12.93		
	Ammonia	127.48		
	Particulate matter	128.09		
	Hydroxybenzene (Phenol)	188.99	2248.89	
	Hydrochloride	51.03		
	Dialuminum trioxide *	73.34		
	Dimethyl benzene (Xylene)	201.68		
	Methylbenzene (Toluene)	991.40		
	Nickel oxide *		0.21	
	Sulfuric acid	16.86		
	Formaldehyde	87.20		
	Fluorides *	168.23		
	Chromium *		1.05	
	Zinc		12.41	
Glomerular diseases (N00–N08)	Hydroxybenzene (Phenol)		155.81	
	Cadmium oxide *	0.30	0.21	
	Trichloroethylene		0.25	
	Ethylbenzene		45.90	
Other disorders of kidney and ureter (N25, N28)	Dimethyl benzene (Xylene)			2.17
	Cadmium oxide *	0.01		
	Chlorobenzene	339.13		234.45
	Ethylbenzene	7.72		
Renal failure (N17, N18, N19)	Hydroxybenzene (Phenol)	0.50	10.17	
	Dimethyl benzene (Xylene)		8.68	
	Trichloroethylene	0.00		

End of the table 2

Group of diseases (ICD-10 codes)	Chemical	Children	Working age	Beyond working age
Renal tubulo-interstitial diseases (N10, N11, N12, N13, N14, N15)	Hydroxybenzene (Phenol)	143.68		
	Dimethyl benzene (Xylene)	158.03		
	Cadmium oxide *	0.47		
	Lead and its compounds*	1.37		
	Chlorobenzene		9043.25	
	Ethylbenzene		8.10	
Congenital malformations, deformations and chromosomal abnormalities	Benz(a)pyrene	0.004		
	Lead and its compounds*	69.38		
	Trichloroethylene	9.52		
Hypertensive diseases (I10, I11, I12, I13)	Particulate matter PM_{10}		5150.12	60.71
	Particulate matter $PM_{2.5}$		868.02	88.42
	Hydroxybenzene (Phenol)		5437.70	104.95
	Carbon oxide		4531.99	863.09
Other forms of heart disease (I30.0, I30.8, I30.9,I31, I33, I34, I35, I36, I37, I38, I40.1, I40.8, I40.9, I42, I45, I49, I50)	Hydroxybenzene (Phenol)			0.02
	Carbon oxide			2.07
Other and unspecified disorders of the circulatory system (I95.0, I95.8, I95.9, I99)	Benzene			0.18
	Particulate matter PM_{10}	14.41		0.21
	Particulate matter $PM_{2.5}$	5.08		0.04
	Carbon oxide	1.97		
Ischaemic heart diseases (I20, I21, I22, I24.0, I24.8, I24.9, I25)	Benzene			43.61
	Particulate matter $PM_{2.5}$			40.24
	Hydroxybenzene (Phenol)			30.00
	Carbon oxide			443.08
Aplastic and other anaemias (D60–D64)	Nitrogen dioxide	37.97		
	Nickel oxide *		0.05	
	Lead and its compounds*	0.07	0.46	
	Zinc	0.75		
Other diseases of blood and blood-forming organs (D70, D71, D72.1, D72.8, D72.9, D74.8, D74.9, D75.8, D75.9)	Nitrogen (II) oxide		45.78	0.26
	Nitrogen dioxide			43.39
	Benzene		0.22	
	Nickel oxide *	0.02		
	Lead and its compounds*	0.70		
	Carbon oxide		73.25	60.61
	Chlorobenzene	7486.82	3279.83	
Purpura and other haemorrhagic conditions (D69.0; D69.1, D69.2, D69.4, D69.6, D69.8, D69.9)	Lead and its compounds*	1.33		
Endocrine, nutritional and metabolic disorders	Cadmium oxide *		9.36	
	Lead and its compounds*	11.45	20.63	
	Trichloroethylene	6.46		

N o t e : *some chemicals were given shortened denominations in the table; the full ones are: Poorly soluble non-organic fluorides, Gaseous fluoride compounds (recalculated as per fluorine), Manganese and its compounds (recalculated as per manganese (IV) oxide), Lead and its non-organic compounds (recalculated as per lead), Cadmium oxide (recalculated as per cadmium), Cobalt oxide (recalculated as per cobalt), Copper (II) oxide (recalculated as per copper), Nickel oxide (recalculated as per nickel), Dialuminum trioxide (recalculated as per aluminum), Chromium (hexavalent chromium) (recalculated as per chromium (VI) oxide).

Conclusion. The present study has enhanced our knowledge on quantitative characteristics of influence exerted by chemical pollution in ambient air on public health. It has also given a certain insight into how they can be applied in future when assessing and predicting health risks without abandoning the existing methods that are being actively used at present. To obtain the maximum objective models of the relationships, we collected relevant data on ambient air quality in residential areas on several territories in our country. These territories differ considerably both in their geography and frequency of detected diseases. The required relationships have been modeled based on the collected and systematized data; as a result, we build 56 multiple regression models for chronic exposure.

The table with model coefficients for the obtained relationships is the major result of this study. These coefficients describe how intensively health disorders develop under exposure to chemical pollution in ambient air; they have been obtained within the hypothesis that influence on the model ranges of definition is linear. It is noteworthy that it seems hardly possible to consider all the uncertainties within the present study; still, this can become possible in future. The results of this study can provide a basis for further research with its focus on modeling cause-effect relations between ambient air pollution and public health. This research may rely on more complicated and enhanced system of initial data as well as on more complicated and enhanced relationship models. Establishing criteria for ranking chemical health risks in zones influenced by hazardous chemical objects can become a next step in development of the suggested approaches.

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Research article

ASSESSMENT OF AEROGENIC RISKS FOR PEOPLE LIVING IN CLOSE PROXIMITY TO ULBA METALLURGICAL PLANT

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In this study, our aim was to assess public health risks caused by ambient air pollution in close proximity to production facilities of "Ulba Metallurgical Plant" JSC (JSC "UMP") located in Ust-Kamenogorsk, Kazakhstan.

The study relied on the health risk assessment methodology under exposure to chemical environmental factors.

Our research objects were chemical pollution in ambient air in close proximity to production facilities of "Ulba Metallurgical Plant" JSC and its effects on public health.

We assessed public health risks caused by chemical pollution in ambient air in close proximity to production facilities of JSC "UMP". The assessment relied on data obtained at monitoring posts of RSE "Kazhydromet" between 2018 and 2021. We identified six carcinogens in the analyzed area that created unacceptable public health risks. High non-carcinogenic risks were typically caused by exposure to particulate matter (dust), $PM_{2.5}$ and PM_{10}, sulfur dioxide and sulfuric acid.

Our assessment of non-accidental mortality risks caused by exposure to PM2.5 in ambient air established unacceptable relative risks (RR: 1.27–1.78), individual risks of non-accidental ($1.5–2.1·10^{-3}$) and cardiopulmonary mortality (between $8.3·10^{-4}$ and $1.0·10^{-3}$).

Unfavorable meteorological factors and geographic location can promote high levels of ambient air pollution and created aerogenic health risks for people living in the analyzed industrial area.

The established risks require developing and implementing scheduled health-improving activities aimed at raising quality of the environment.

Keywords: *metallurgical plant, risk assessment, public health, ambient air, pollutants, hazard quotient, hazard index, Ust-Kamenogorsk.*

At present, environmental protection and urban public health are given much attention all other the world due to the intensified urbanization and growing levels of pollution and emissions into ambient air [1]. There have been multiple studies that address effects produced by ambient air pollution on the human body [2].

Exposure to industrial emissions is known to induce varied adverse health outcomes [3, 4].

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Elevated levels of solid particles (dusts, $PM_{2.5}$, PM_{10},), ozone and nitrogen oxide (dioxide) in ambient air result in high mortality rates among people living on a given territory [5–7].

Also, some research works have proven that levels and dynamics of pollutant concentrations in ambient air in big cities largely depend on meteorological conditions and a season [8, 9].

Similar studies have been accomplished in the Republic of Kazakhstan (RK) as well since intensified production and urbanization create elevated pollution levels in ambient air in various regions in the country. This, together with some local geographical, climatic and socio-economic peculiarities, causes negative health outcomes in population. Given that, it is extremely important to assess public health risks and health harm [10–14].

Experts have long established direct links between contents of adverse chemicals in ambient air (solid particles (dusts), $PM_{2.5}$, PM_{10}, nitrogen dioxide, sulfur dioxide, phenol, formaldehyde, arsenic, benz(a)pyrene and others) and incidence of respiratory and cardiovascular diseases, cancer incidence and mortality [15, 16].

Preliminary studies that examined public health depending on ambient air pollution were accomplished in the country in 2019 [17]. Following their results, it was recommended to perform more comprehensive examinations of public health depending on air pollution levels in some cities in the RK. Given that, the research works in the sphere continued, namely, health risk rates caused by exposure to ambient air pollution were determined in seven most significant industrial regions in Kazakhstan. The research works were accomplished within the project "The National program for implementing patient-specific and preventive medicine in the Republic of Kazakhstan" IRN OR12165486.

We have assessed aerogenic health risks for people living in close proximity to the production facilities of "Ulba Metallurgical Plant" JSC (hereinafter JSC "UMP") located in Ust-Kamenogorsk, the East Kazakhstan region, Kazakhstan. This article presents the assessment results.

The environmental situation in Ust-Kamenogorsk is influenced not only by industrial emissions but also by some other factors. The city is located in a secluded hollow and this prevents pollutants from dispersing. Meteorological conditions are often unfavorable there due to weak winds or their absence (it is total calm on approximately 98–100 days a year) and high air humidity due to frequent radiation and river fogs. Elevated levels of ambient air pollution occur in 80–90 % of days with fogs. According to the Republican State Enterprise "Kazhydromet" (RSE "Kazhydromet" for short), there have been persistently high levels of some pollutants in ambient air in Ust-Kamenogorsk including PM_{10}, sulfur dioxide, carbon oxide, nitrogen dioxide, hydrogen sulfide, and phenol. Sulfuric anhydride concentrations reach 4–5 daily average MPC[1].

The authors of the work [16] established that cancer incidence was by two times higher in Ust-Kamenogorsk than on average in the country and by 1.4 times higher than on average in the region. Deaths from cancer were by 1.9 times higher than on average in the republic. Cancer incidence was examined in dynamics; as a result, it was revealed that its growth amounted to 6.5 % in Ust-Kamenogorsk whereas it was 13.5 % in the republic. A relationship was suggested between cancer incidence and ambient air being polluted with adverse chemicals.

A separate study revealed that long-term living in Ust-Kamenogorsk led to developing skin pathologies and weakened immunity [18].

Therefore, according to data available in research works, high levels of chemical pollution in ambient air in Ust-Kamenogorsk occur due to its peculiar physical and geographical position and the synoptic situation. They cause elevated cancer incidence and weakened immunity among people living there.

All the above indicates the issue is topical for the examined area.

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[1] Monthly newsletter on the state of the environment. *RSE "Kazhydromet"*. Available at: https://www.kazhydromet.kz/en/ecology/ezhemesyachnyy-informacionnyy-byulleten-o-sostoyanii-okruzhayuschey-sredy/2018 (June 19, 2022).

In this study, our aim was to assess public health risks caused by ambient air pollution in close proximity to production facilities of "Ulba Metallurgical Plant" JSC (JSC "UMP") located in Ust-Kamenogorsk, Kazakhstan.

To achieve this, the following tasks were set:

1) To provide quantitative description of carcinogenic and non-carcinogenic public health risks depending on levels of ambient air pollution in the examined area relying on data collected at the monitoring posts of the RSE "Kazhydromet" regional office in Ust-Kamenogorsk;

2) To assess risks of non-accidental and cardiopulmonary mortality depending on $PM_{2.5}$ concentrations in ambient air;

Materials and methods. Our research objects were chemical pollution in ambient air in close proximity to the "Ulba Metallurgic Plant" production facilities and their impacts on public health in Ust-Kamenogorsk. Pollutant concentrations in ambient air were estimated as per data taken from the Information bulletins about the environmental situation issued by the RSE "Kazhydromet". We analyzed data collected over the last four years (2018–2021).

Ust-Kamenogorsk is one of the largest industrial centers in Kazakhstan. There are many working enterprises in the city and this leads to substantial chemical emissions into ambient air. "UMP" JSC is an established manufacturer of uranium, beryllium, tantalum and niobium products; it is among world's leading enterprises in the sphere. The enterprise is a part of the National Atomic Company "Kazatomprom" JSC. The production facilities are located in Ust-Kamenogorsk and border the production facilities of another company in the south-west. This company is Ust-Kamenogorsk lead and zinc plant that belongs to "Kazzinc", a large Kazakhstan manufacturer of zinc, lead, copper and precious metals. The closest residential areas near the production facilities of "UMP" JSC are those of Ust-Kamenogorsk. The distances from the residential areas to the boundaries of these production facilities are 890 meters in the west, 570 meters in the east and 290 meters in the south-east.

Emissions from the production facilities of "UMP" JSC, together with emissions from other enterprises in the city, make a certain contribution to the overall background pollution there. This background pollution is measured at seven monitoring posts that belong to the RSE "Kazhydromet" and are located at different points within the city. Three are in the closest proximity to the production facilities of "UMP" JSC.

Several chemicals are included into monitoring programs accomplished at these posts. They are solid particles (dusts); $PM_{2.5}$; PM_{10}; sulfur dioxide; carbon oxide; nitrogen dioxide; nitrogen oxide; phenol; hydrogen sulfide; hydrogen fluoride; hydrogen chloride; formaldehyde; chlorine; sulfuric acid and sulfates; lead; zinc; cadmium; copper; beryllium; ozone; ammonia. We took actual single maximum and average annual concentrations of the above-listed chemicals in ambient air to calculate aerogenic health risks. The data we used in calculations were collected in 2018–2021 and taken from the Information bulletins about the environmental situation issued by the RSE "Kazhydromet"[1].

Carcinogenic and non-carcinogenic health risks were assessed in accordance with the Guide R 2.1.10.1920-04 "Human Health Risk Assessment from Environmental Chemicals" (hereinafter the Guide R 2.1.10.1920-04)[2].

We did not calculate population carcinogenic risk levels since it was impossible to define a precise number of people exposed to emissions from the "UMP" JSC production facilities.

In this study, hazard quotients (HQ) and hazard indexes (HI) were applied to assess non-carcinogenic risk levels. They were calcu-

[2] The Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals (approved and validated by G.G. Onishchenko, the First Deputy to the RF Public Healthcare Minister and the RF Chief Sanitary Inspector on March 5, 2004). *KODEKS: electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/1200037399 (June 18, 2022) (in Russian).

lated as per the formulas provided in the Guide R 2.1.10.1920-04[2].

We applied a more complicated scheme to calculate risks of mortality caused by exposure to average annual $PM_{2.5}$ concentrations in ambient air in Ust-Kamenogorsk, namely, a log-linear model as per the following formulas [19]:

$$R = \frac{RR-1}{RR} M\rho; \quad (1)$$

$$RR = \left(\frac{C+1}{C_0+1}\right)^{\beta}, \quad (2)$$

where RR is relative risk;

R is population risk;

$M\rho$ are background levels of non-accidental and cardiovascular mortality (we used official statistical data provided by the Republican State Enterprise “The Republican Center for Electronic Public Healthcare” of Ministry of Health of the Republic of Kazakhstan);

β is concentration – response factor (the average value is 0.15515);

C is $PM_{2.5}$ concentration;

C_0 is threshold concentration (it usually equals $7.5\frac{\mu g}{m^3}$);

M is background mortality (cardiopulmonary);

ρ is exposed population (the whole city population was considered in these calculations).

The analysis was based on available information including identified (current and established) concentration and background mortality. Typically, only PM_{10} concentrations have been determined in ambient air in the city; $PM_{2.5}$ concentrations were first registered in 2021 and have been identified ever since (Table 1).

Average annual $PM_{2.5}$ concentrations were calculated as per the method described in the work [19] relying on average annual concentrations of solid particles (dusts) provided in the Informational bulletins by the RSE “Kazhydromet”[1]. The following formula was used to calculate them:

$$PM_{2.5} = (0.33 - 0.36) \cdot TSP, \quad (3)$$

where TSP is total solid particles; (0.33–0.36) are recalculation quotients [19]. The quotient value 0.36 was used for the analyzed region given its climatic conditions.

The data on population numbers and population mortality were taken from the materials provided by the National Statistics Bureau of the Agency for Strategic Planning and Development of the Republic of Kazakhstan[3] and the Republican State Enterprise “The Republican Center for Electronic Public Healthcare” of Ministry of Health of the Republic of Kazakhstan[4].

Results and discussion. According to the research aim and tasks, we assessed public health risks depending on levels of ambient air pollution in close proximity to the production facilities of “UMP” JSC.

Table 1

Cancer incidence among population in Ust-Kamenogorsk estimated as per data on medical aid appealability (per 100 thousand people)

Regions	2018	2019	2020
The Republic of Kazakhstan (urban population)	952.1	899.6	876.5
The East Kazakhstan region (urban population)	1777.8	1713.5	997.8
Ust-Kamenogorsk	2576.4	2522.7	1710.3

Note: the data were provided by the Republican State Enterprise “The Republican Center for Electronic Public Healthcare” of Ministry of Health of the Republic of Kazakhstan[4].

[3] Demographic statistics. *Agency for Strategic planning and reforms of the Republic of Kazakhstan*. Available at: https://stat.gov.kz/region/264990/statistical_information/industry/6361 (June 18, 2022).

[4] Respublikanskii tsentr elektronnogo zdravookhraneniya [The Republican Center for Electronic Public Healthcare]. Available at: https://rcez.kz (June 18, 2022) (in Russian).

Carcinogenic and non-carcinogenic public health risks were assessed as per data taken from the Information bulletins issued by the RSE "Kazhydromet" [5].

Carcinogenic risks. The cancer incidence is high in the analyzed area (Ust-Kamenogorsk) with its rates being by 1.4 and 2.7 times higher accordingly than in the region and the republic as a whole (Table 1). Given that, it is obvious that carcinogenic health risks existing in the area should be assessed.

The quantitative description of carcinogenic risks was based on values of individual carcinogenic risks identified for six chemicals from the analyzed list since these chemicals have carcinogenic properties according to Table 2.4 in the Appendix 1 of the Guide R 2.1.10.1920-04[6]. Table 2 provides these values of individual carcinogenic risks.

Obviously, values of individual carcinogenic risks identified for cadmium, benz(a)-pyrene and arsenic fell within the range between $1.1 \cdot 10^{-4}$ and $8.6 \cdot 10^{-4}$, that is, they were acceptable for occupational groups and unacceptable for population at large. Such a risk requires developing and implementing scheduled sanitary activities in settlements.

Non-carcinogenic risks. In this study, we applied a hazard quotient (HQ) to assess non-carcinogenic health risks. This quotient is a ratio of a certain exposure level (concentration *C*) to a reference level of a certain chemical under acute (ARfC) and chronic (RfC) exposure (the formulas to calculate it are provided in the Guide R 2.1.10.1920-04)[6].

In case there is combined exposure to several chemicals, the total non-carcinogenic risk is described with a hazard index (HI).

Average daily and single maximum concentrations of pollutants that have been controlled in ambient air over the analyzed years are given as per data provided by the Information bulletin of the RSE "Kazhydromet"[5] (Table 3).

Figures 1 and 2 show the results obtained by calculating HQ and HI (respiratory organs being the target ones) under acute and chronic exposure.

HQ values identified for some chemicals under acute exposure were higher than a permissible (acceptable) risk level, which is equal to 1.0 or lower. Unacceptable acute inhalation risks were identified for exposure to sold particles (dusts), PM_{10}, $PM_{2.5}$ (2021); sulfur dioxide, hydrogen sulfide (2018); sulfuric acid and arsenic. The total hazard indexes (HI) identified for ambient air pollutants were high as regards respiratory organs being the target ones (Figure 1).

A similar picture was observed for chronic non-carcinogenic risk values. The total hazard index identified for major ambient air pollutants in Ust-Kamenogorsk was also high for respiratory organs as target ones (Figure 2).

Table 2

Individual carcinogenic risks in Ust-Kamenogorsk

Chemical	2018	2019	2020	2021
Formaldehyde	6.0E-05	6.6E-05	4.5E-05	3.9E-05
Cadmium	1.7E-04	1.4E-04	1.1E-04	4.5E-05
Lead	3.8E-06	3.7E-06	4.1E-06	1.7E-06
Beryllium	2.7E-07	2.7E-07	2.4E-07	1.7E-07
Benz(a)pyrene	6.2E-04	7.5E-04	7.5E-04	7.5E-04
Arsenic	8.6E-04	8.6E-04	4.3E-04	*

Note: * means that no data on a concentration were provided in the Information bulletin[5].

[5] Monthly newsletter on the state of the environment. *RSE "Kazhydromet"*. Available at: https://www.kazhydromet.kz/en/ecology/ezhemesyachnyy-informacionnyy-byulleten-o-sostoyanii-okruzhayuschey-sredy/2018 (June 19, 2022).

[6] The Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals (approved and validated by G.G. Onishchenko, the First Deputy to the RF Public Healthcare Minister and the RF Chief Sanitary Inspector on March 5, 2004). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200037399 (June 18, 2022) (in Russian).

Table 3

Average daily and single maximum concentrations of pollutants in ambient air in Ust-Kamenogorsk

Chemical	Average daily concentrations (mg/m^3)					Single maximum concentrations (mg/m^3)				
	RFC, mg/m^3	2018	2019	2020	2021	ARFC, mg/m^3	2018	2019	2020	2021
Solid particles (dusts)	0.075	0.1262	0.109	0.0955	0.084	0.3	2.2	2	1	0.9
$PM_{2.5}$	0.015	*	*	*	0.025	0.065	*	*	*	0.88
PM_{10}	0.05	0.0475	0.05	0.0476	0.028	0.15	0.99	0.99	0.99	0.96
Sulfur dioxide	0.05	0.1183	0.09	0.1085	0.076	0.66	5.70	4.93	5.47	4.95
Carbon oxide	3	0.7403	0.68	0.6027	0.653	23	28.70	14.47	20.71	11.3
Nitrogen dioxide	0.04	0.0729	0.06	0.0438	0.036	0.47	0.56	0.77	0.42	0.29
Nitrogen oxide	0.06	0.0132	0.003	0.002	0.005	0.72	1.08	0.68	1.57	1.48
Ozone	0.03	0.0379	0.029	0.0411	0.06	0.18	1.37	0.16	0.15	0.14
Phenol	0.006	0.0016	0.001	0.0017	0.002	6	0.02	0.01	0.02	0.04
Hydrogen sulfide		*	*	*	*	0.1	1.05	0.19	0.16	0.063
Hydrogen fluoride	0.014	0.0069	0.007	0.0032	0.003	*	*	*	*	*
Chlorine	$2\cdot10^{-4}$	$3.9\cdot10^{-3}$	0.004	0.0052	0.007	0.2	0.07	0.09	0.07	0.09
Hydrogen chloride	0.02	0.0309	0.03	0.0665	0.057	2.1	0.15	0.15	0.22	2.1
Ammonia	0.1	0.0045	0.004	0.0029	0.002	0.35	0.054	0.19	0.06	0.06
Sulfuric acid	0.001	0.0143	0.014	0.0106		0.1	0.5	0.18	0.35	
Formaldehyde	0.003	0.0046	0.005	0.0034	0.002	0.048	0.07	0.06	0.03	0.01
Arsenic	$3\cdot10^{-5}$	$2\cdot10^{-4}$	$2\cdot10^{-4}$	$1\cdot10^{-4}$	*	*	*	*	*	*
Lead	$5\cdot10^{-4}$	$3.1\cdot10^{-4}$	$3.1\cdot10^{-4}$	$3.4\cdot10^{-4}$	$1.8\cdot10^{-4}$	*	*	*	*	*
Copper	$2\cdot10^{-5}$	$5.4\cdot10^{-5}$	$5.6\cdot10^{-5}$	$4.3\cdot10^{-5}$	$1.8\cdot10^{-5}$	*	*	*	*	*
Beryllium	$2\cdot10^{-5}$	$1\cdot10^{-7}$	$1.13\cdot10^{-7}$	$1\cdot10^{-7}$	$6.5\cdot10^{-8}$	*	*	*	*	*
Cadmium	$2\cdot10^{-5}$	$9.5\cdot10^{-5}$	$7.5\cdot10^{-5}$	$6.1\cdot10^{-5}$	$2.4\cdot10^{-5}$	*	*	*	*	*
Zinc	$9\cdot10^{-4}$	$1.3\cdot10^{-3}$	$1.2\cdot10^{-3}$	$1.1\cdot10^{-3}$	$4.3\cdot10^{-4}$	*	*	*	*	*

Note: * means that no data on a concentration were provided in the Information bulletin[5].

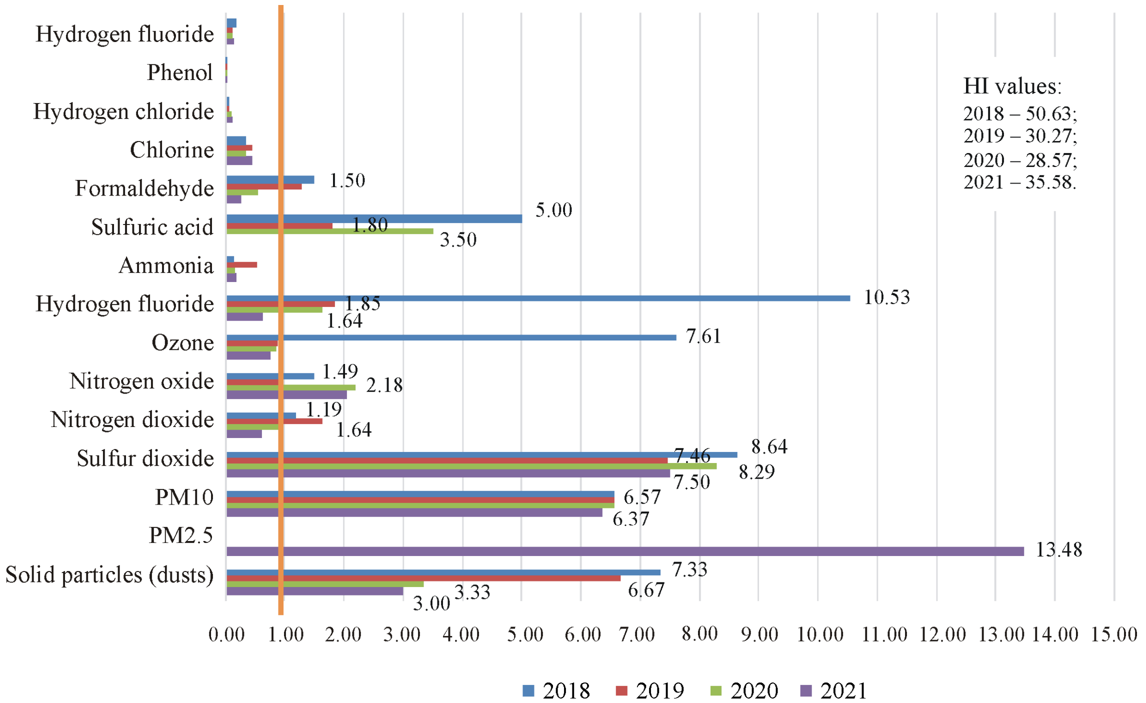

Figure 1. Hazard quotients (HQ) and hazard indexes (HI) under acute exposure (respiratory organs as target ones)

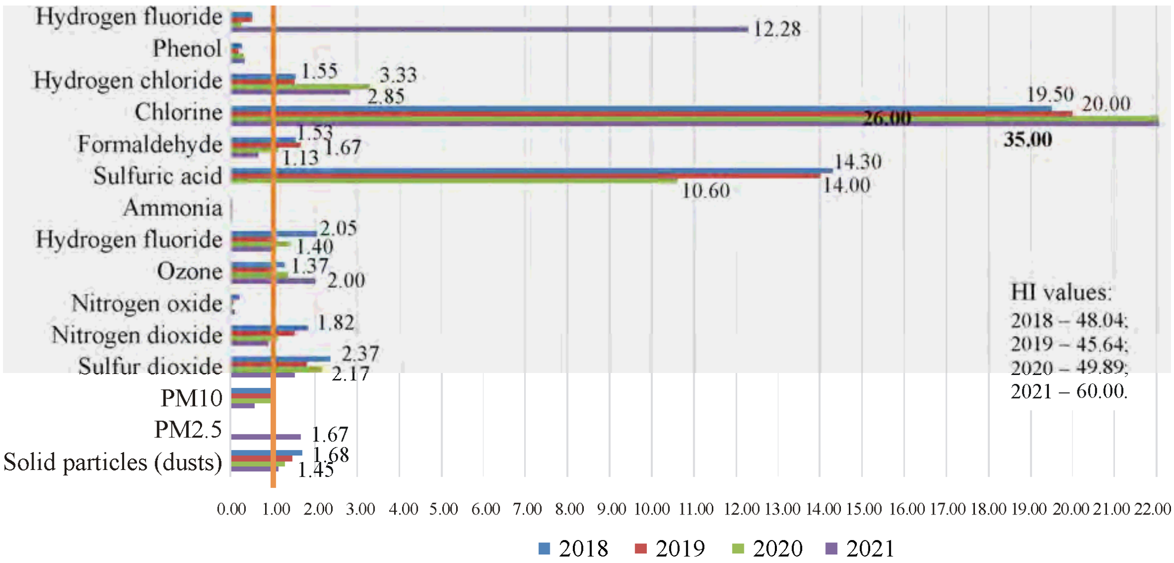

Figure 2. Hazard quotients (HQ) and hazard indexes (HI) under chronic exposure (respiratory organs as target ones)

The highest values of hazard quotients under chronic exposure were identified for chlorine and sulfuric acid and for such heavy metals as cadmium, arsenic and copper (Table 4).

HQ values for chlorine under chronic exposure were identified as high due to its low reference concentrations.

Hazard quotients for heavy metals identified in ambient air in concentrations equal to and / or higher than permissible risk levels indicate possible effects produced by emissions from the lead and zinc plant of the "Kazzinc" company.

The total hazard index identified for ambient air pollutants in Ust-Kamenogorsk was high for respiratory organs as target ones. Given the summation of these targeted effects on the body organs and systems, the identified heavy metals produce them on the kidneys, blood and the hormonal systems.

Therefore, the identified levels of acute and chronic non-carcinogenic health risks occur due to exposure to sulfur dioxide and sulfuric acid in ambient air in Ust-Kamenogorsk.

At the same time, when air humidity is high, sulfur dioxide increases a concentration of sulfuric acid as it is indicated by its abnormally high hazard quotients under chronic exposure. These chemicals are emitted from smoke plumes of enterprises powered by coal fuel and natural gas or are contained in exhausts from motor transport.

Table 4

Non-carcinogenic risks under chronic epxosure to heavy metals

Metal	HQ values			
	2018	2019	2020	2021
Arsenic	6.67	6.67	3.33	*
Lead	0.63	0.622	0.676	0.35
Copper	2.7	2.8	2.15	0.90
Beryllium	0.005	0.006	0.005	0.0033
Cadmium	4.75	3.75	3.05	1.20
Zinc	1.491	1.34	1.27	0.48
Hygienic standard	HQ ≤ 1.0			

N o t e : * means that no data on a concentration were provided in the Information bulletin[5].

Assessing risks of non-accidental and cardiopulmonary mortality depending on levels of ambient air pollution with $PM_{2.5}$. Our quantitative description of non-carcinogenic public health risks identified their high levels due to exposure to solid particles (dusts) and PM_{10} in ambient air in the analyzed city. PM_{10} produce certain effects on mortality, incidence of respiratory and cardiovascular diseases as well as on some other health indicators [19].

The WHO experts believe approximately 3.0 % of deaths from cardiopulmonary diseases and 5.0 % of deaths from lung cancer to be caused by exposure to particulate matter (PM). In different European regions, this share varies between 1 and 3 % and between 2 and 5 % accordingly. At the same time, some studies established that adverse effects produced by exposure to particulate matter $PM_{2.5}$ caused up to 3.1 million deaths and up to 3.1 % of lost years of healthy life all over the world [20].

Given all the above-stated, we assessed risks of non-accidental mortality in Ust-Kamenogorsk caused by exposure to ambient air pollution with $PM_{2.5}$ with their concentrations being calculated as per the formula (3).

Table 5 summarizes the results obtained by calculating risks of non-accidental mortality caused by ambient air pollution with $PM_{2.5}$ in Ust-Kamenogorsk over 2018–2020. Non-accidental mortality in 2021 was not calculated since we did not have any official background data on non-accidental and cardiovascular mortality in that year.

Non-accidental and cardiopulmonary mortality was rather high in 2018–2020 (Tables 5 and 6).

The data in Table 5 clearly show that a relative risk of the overall non-accidental mortality (*RR*) varied between 1.27 and 1.78 over the analyzed period whereas the actual pollution threshold was usually 7.5 $\frac{\mu g}{m^3}$, and a risk was determined at the above-mentioned levels. When calculating a population risk caused by exposure to $PM_{2.5}$ concentrations in ambient air in Ust-Kamenogorsk, we identified from 521 to 740 non-accidental deaths per year. This number was rather high and made a considerable contribution (up to 20 %) into the overall mortality in the analyzed city (Table 5).

Table 5

Risks of non-accidental mortality due to ambient air pollution with $PM_{2.5}$ in Ust-Kamenogorsk

Years	Calculated average $PM_{2.5}$ concentration (mg/m^3)	Average population number	Non-accidental mortality (number of deaths)	Relative risk, RR	Population risk, R	Additional deaths (AD)	Individual risk, IR $PM_{2.5}$
2018	0.045	342,422	3173	1.30	577	127	$1.7\cdot10^{-3}$
2019	0.039	344,953	3319	1.27	521	133	$1.5\cdot10^{-3}$
2020	0.344	347,480	4069	1.78	740	163	$2.1\cdot10^{-3}$

Table 6

Risk of cardiopulmonary mortality under exposure to ambient air pollution with $PM_{2.5}$ in Ust-Kamenogorsk

Year	Calculated average $PM_{2.5}$ concentration (mg/m^3)	Average population number	Cardiopulmonary mortality (number of deaths)	Relative risk, RR	Population risk, R	Additional deaths (AD)	Individual risk, IR $PM_{2.5}$
2018	0.045	342,422	1281	1.3	349	51	$1.0\cdot10^{-3}$
2019	0.039	344,953	1225	1.27	288	49	$8.3\cdot10^{-4}$
2020	0.344	347,480	1636	1.78	446	65	$1.3\cdot10^{-3}$

Individual risks of non-accidental mortality were above $1.0 \cdot 10^{-3}$ (Table 5); this meant they were unacceptable both for occupational groups and population at large. In case such a risk occurs, urgent sanitary activities are to be developed and implemented to mitigate it.

According to our calculations, a population risk of mortality from respiratory and cardiovascular diseases varies between 288 and 446 deaths per year. The number of additional deaths per each 10 $\mu g/m^3$ of $PM_{2.5}$ varies between 49 and 65 (Table 6).

To sum up, we assessed risks of mortality due to inhalation exposure to $PM_{2.5}$. The calculated individual risks of non-accidental and cardiopulmonary mortality were within the third (higher than $1 \cdot 10^{-4}$) and fourth ranges (higher than $1 \cdot 10^{-3}$). This requires developing and implementing sanitary activities.

However, as we assessed public health risks as per the data provided by the RSE "Kazhydromet", we established high health risks caused by exposure to pollutants in ambient air. High health risks were identified under exposure to particulate matter, sulfur dioxide, sulfuric acid and heavy metals.

Calculated risks of non-accidental and cardiopulmonary mortality caused by exposure to $PM_{2.5}$ turned out to be unacceptable.

These established high public health risks can be also associated with unfavorable meteorological conditions (calms, high air humidity etc.) and geographical peculiarities of the examined area.

Given all the above stated, our study has enabled us to make the following conclusions:

1. Six carcinogens out of the ambient air pollutants in the analyzed area were established to create unacceptable health risks for people living there (identified risks are between $8.6 \cdot 10^{-4}$ and $1.1 \cdot 10^{-4}$). These carcinogens are cadmium, lead, arsenic, formaldehyde, benz(a)pyrene, and beryllium.

2. Hazard quotients (HQ) calculated for solid particles, particulate matter (PM_{10} and $PM_{2.5}$), sulfur dioxide, sulfuric acid and heavy metals (under chronic exposure) were higher than permissible (acceptable) risk levels. The total hazard index (HI) calculated for ambient air pollutants in the region was high for respiratory oirgans as target ones.

3. Individual risks of non-accidental and cardiopulmonary mortality caused by exposure to $PM_{2.5}$ were unacceptable for population at large (IR is higher than $1 \cdot 10^{-4}$).

4. High levels of health risks caused by exposure to ambient air pollution in the analyzed region can be also associated with its geographical location and unfavorable meteorological conditions (frequent calms and high air humidity).

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Research article

MATHEMATICAL MODELING OF AMMONIA EMISSION RATE IN NEWLY CONSTRUCTED BUILDINGS

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A rapid growth in monolithic residential construction over recent decades has created a problem associated with ammonia contamination inside newly constructed buildings. Absence of substantiated preventive actions aimed at minimizing ammonia emissions hinders commissioning of new residential buildings and may create an unfavorable sanitary-epidemiological situation with obvious olfactory-reflex and irritating effects on public health.

The aim of this study was to develop a scientifically grounded method to predict when ammonia concentrations emitted from concrete constructions would reach their permissible levels in air inside contaminated premises in newly constructed buildings.

Ammonia emissions were estimated based on data of laboratory tests that involved analyzing indoor air samples taken in Saint Petersburg and the Leningrad region. Indoor air was analyzed in 4 newly constructed residential buildings (165 premises, 57 test protocols, 893 air samples tested to identify ammonia in them). Relationships between changes in ammonia concentrations and ventilation time were obtained by using regression analysis (regression equation, least square method). To establish reproducibility of the results and a possibility to compare them, we tested variances for homogeneity by using Fisher criterion. Sampled populations were compared with Student's t-test in case the data fitted to a normal distribution (Kolmogorov – Smirnov test, Shapiro – Wilks test). Critical significance was taken at 0.05 in all the statistical comparisons.

We have developed a method for predicting when ammonia concentrations that occurred in indoor air inside newly constructed buildings due to multi-day emissions from building materials would reach their permissible levels. The method involves multi-day measurements (y, mg/m^3) of ammonia concentrations sequentially in each premise inside a newly constructed building on any day of measurements during the time period t; building up relationships between averaged ammonia concentrations (y_{av}, mg/m^3) and a time moment t; mathematical analysis of the obtained relationships by parameterization and statistical analysis of the obtained kinetic parameters.

Keywords: *monolithic residential buildings, indoor air, ammonia, ammonia emission, mathematical modeling, building materials, concrete.*

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A rapid growth in residential construction based on using monolithic reinforced concrete as a building material has created a problem associated with ammonia contamination in indoor air. This contamination is largely caused by ammonia compounds that are contained in raw components of concrete mixes. These compounds are introduced with chemical modifiers that are usually added to concrete and concrete mixes (hardeners, antifreeze admixtures etc.) or with auxiliary components in concrete production (grinding intensifiers). Industrial wastes used as mineral fillers (slag or ash) can be another source of ammonia in indoor air [1–12].

Ammonia is a result of hydrolysis and is generated from amides, amines and ammonia compounds.

Studies that addressed ammonia emission from concrete into indoor air have been accomplished in many countries; their results indicate the chemical occurs in indoor air in considerably high concentrations. Thus, Z. Bai with colleagues (2006) established ammonia concentrations varying between 2.30 and 5.85 mg/m^3 in indoor air inside newly constructed buildings; these concentrations occurred due to use of urea-based antifreeze admixtures in concrete [8]. T. Lindgren (2010) described a case when an elevated ammonia concentration was detected in a newly built office in Beijing and this was also due to antifreeze admixtures in concrete blocks [10].

Russian studies report similar results. D. Fokin (2011) showed in his work that ammonia migration from walls in a newly built residential building made of monolithic reinforced concrete created indoor air contamination at a level equal to 2 mg/m^3 (50 MPCav.d.). The author pointed out that ammonia concentrations in indoor air were not influenced by furniture or decoration [13].

The work [4] describes the results obtained by instrumental measurements of indoor air inside residential and public buildings that were accomplished in Saint Petersburg and the Leningrad region over the period between 2012 and 2018. These tests were both scheduled or performed as per developers' and / or citizens' claims. Ammonia was detected in concentrations higher than MPCav.d. in 1147 samples out of 2839 (40.4 %). The established levels were by 163 times higher than the existing hygienic standards (MPCav.d.) in some cases. Ammonia was fixed in a concentration lower than sensitivity of the applied chemical-analytic method only in 1.1 % of the analyzed samples.

Intensity of ammonia migration from concrete into indoor air, just as any other chemical, depends on air temperature and humidity and on volumes of construction materials present in a given premise [1, 3]. Z. Bai with colleagues (2006) showed in their studies that an increase in air temperature leads to more intense ammonia emission and to higher ammonia emission rates. Besides, an ammonia concentration directly depends on ventilation in a premise. According to the work [8], it takes more than ten years to reach complete ammonia emission from concrete that contains urea-based admixtures. However, there have been no studies so far with their focus on determining a period over which ammonia concentrations in indoor air drop down to safe levels regulated by hygienic standards.

Ammonia in air has been established to produce olfactory-reflex and irritating effects even in small and average concentrations. High concentrations can induce acute poisoning. An issue related to providing safe environment in residential buildings given ammonia emissions from building materials is quite urgent; this is confirmed by multiple complaints about indoor air in residential and public buildings made by citizens and workers to federal and regional executive authorities [4, 7–19].

Although the issue is truly vital, there are still no available scientifically substantiated methods to predict how long it will take ammonia concentrations emitted from concrete construction in indoor air to fall down to their permissible levels. This hinders commissioning of new residential buildings [4, 12–14, 16, 20–24].

In this study, our aim was to develop a scientifically grounded method to predict when ammonia concentrations emitted from concrete constructions would reach their permissible levels in air inside contaminated premises in newly constructed buildings made of monolithic reinforced concrete.

Materials and methods. Relationships between ammonia concentrations in indoor air and time were built and analyzed by using 57 test protocols that described the results of laboratory tests of indoor air in buildings in Saint Petersburg and the Leningrad region. The tests were performed by the Hygiene and Epidemiology Center of Saint-Petersburg and Leningrad region.

Air tests were performed following citizens' complaints about unpleasant smells as well as within production control of buildings that were to be commissioned. Overall, 893 air samples were taken in 165 premises to identify ammonia in them and 285 tests were performed.

Sampling was accomplished with electrical aspirators OP-824 TZ in accordance with the State Standard GOST R 57256-2016[1]. The process involved three sequential measurements of ammonia concentrations in air 8 hours after 15 minutes of cross ventilation with fully opened windows done once a day for 20 minutes.

Ammonia mass concentrations were measured as per a procedure based on "catching ammonia in air with an acid solution and its photometric detection as per indophenol…"[2].

The research objects were four apartment houses (hereinafter called objects) with their load-bearing walls made of monolithic concrete. All four objects had natural ventilation that provided intense airing inside them. Air was tested to identify ammonia in it in all four objects. The tests in objects No. 1 and 2 were performed prior to their commissioning; in objects No. 3 and 4, after the commissioning. The objects were at different stages regarding completion of decoration works inside them.

There was no interior decoration inside the objects No. 1 and 2. Floors and ceilings were made of concrete slabs; walls were made of concrete and foam concrete blocks. Premises inside the objects No. 3 and 4 had finished interior decoration and 6 premises already had some furniture.

Air sampling was made considering preliminary identification of microclimate indicators that conformed to the established sanitary-epidemiological requirements. It was done to eliminate any influences exerted by environmental factors that would facilitate ammonia emission from concrete.

Mathematical analysis and parameterization were accomplished by using the least square method (non-linear regression) as per the equation (1); Statistica 10 software package was used in the process[3]. That is, we determined average values of the basic kinetic parameters A_{av}, B_{av} and C_{av} for all the relationships established for all the objects:

$$y_{av}(t) = A_{av} \cdot \exp(-B_{av} \cdot t) + C_{av}, \qquad (1)$$

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where $y_{av}(t)$ is the exponential relationship between a decline in ammonia concentration y_{av} in indoor air in residential premises and time t;

A_{av} is the average value of the total change in ammonia concentration y_{av} over the whole analyzed time t, mg/m^3, the parameter identified by the graph analysis;

B_{av} is the average value of the decline rate constant for ammonia concentration y_{av} in indoor air in residential premises, the parameter identified by the graph analysis and associated with the essence of the migration process, ammonia emission from concrete materials and a material itself, day^{-1};

C_{av} is the average value of the minimal residual ammonia concentration in indoor air at the end of the experimental period, mg/m^3, the parameter identified by the graph analysis.

The formula (1) establishes the exponential relationship between a decline in ammonia concentration y_{av} in indoor air in residential premises and a time t; that is, it describes ammonia emission from building materials over time. The equation (1) with average values of the basic parameters A_{av}, B_{av} and C_{av} calculated with the least square method describes a parameterized approximated curve. This curve goes through all the experimental points in the graph in the best way.

We calculated expanded uncertainty (inaccuracy) $U(T)$ of the value T (in days) for a given object as per the formula (2) at $p < 0.05$:

$$U(T) = \frac{\sigma_T}{\sqrt{3 \cdot n}} \cdot t_{P=0.95;\, f=n-1}, \qquad (2)$$

where the standard deviation σ_T was calculated by summing all the standard uncertainties considering three contributions made by the standard deviations σ_A, σ_B and σ_C of all three kinetic parameters A_{av}, B_{av} and C_{av}, calculated within the analysis, the very value T depending on them:

$$\sigma_T = \frac{1}{B_{av}} \cdot \sqrt{\left(\frac{\sigma_B}{B_{av}}\right)^2 \cdot \left(\ln\left|\frac{C^* - C_{av}}{A_{av}}\right|\right)^2 + \left(\frac{\sigma_{C_{av}}}{C^* - C_{av}}\right)^2 + \left(\frac{\sigma_A}{A_{av}}\right)^2}, \qquad (3)$$

where C^* is the selected standard concentration, for example, equal to MPCav.d.; the declining extrapolated curve crosses with it at the time moment T.

Results and discussion. Average initial ammonia concentrations C_0 (mg/m^3) in indoor air were considerably higher than its MPCav.d. in premises inside all the analyzed objects; that is, C_0 / MPCav.d. ratio was within a range between 3.55 to 30.4 in different premises.

Ammonia concentrations were measured in indoor air in all four objects within a multiday study; as a result, we observed a monotonically decreasing relationship between an ammonia concentration and time, which is shown as a descending curve on the graph.

The Figure provides an example of a typical curve describing a relationship between an ammonia concentration y_{av} in indoor air and a time t for the object No. 1 (each point represent an average value out of three or six ammonia concentrations y in air inside each premise).

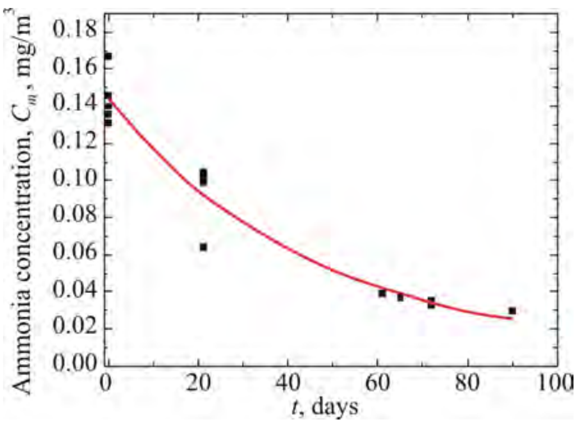

Figure. A relationship between an ammonia concentration in indoor air in residential premises inside the object No. 1 and time (all the measures were performed under identical conditions)

For the object No. 1, as well as for all the other analyzed objects, the created graphic relationships between average values y_{av} of ammonia concentrations (in mg/m^3) and an emission time t are exponential curves that asymptotically tend to the average minimal concentration C_{av}. All the relationships satisfy the equation (1). Therefore, the analysis made it possible to take the exponential relationship between a decline in an ammonia concentration y_{av} in

Table

Time moments when ammonia concentrations reached their permissible levels at the objects No. 1–4

The analyzed object	Average initial concentration, $(A+C)^*$, mg/m^3	Average initial concentration to MPCav.d. ratio	Total change in concentration A^*, mg/m^3	Observed change rate constant B^*, days^{-1}	Residual concentration $C_{av} \pm \Delta C^*$, mg/m^3	A time moment when MPCav.d. was reached T^*, days
1	0.144 ± 0.023	3.6	0.138 ± 0.02	0.02209 ± 0.00742	0.0065 ± 0.0121	64 ± 11.2
2	0.145 ± 0.01	3.55	0.122 ± 0.008	0.02419 ± 0.00483	0.0228 ± 0.00513	81.2 ± 8.7
3	0.163 ± 0.01	3.98	0.145 ± 0.005	0.01947 ± 0.00891	0.01784 ± 0.0084	96.4 ± 28.6
4	1.24 ± 0.141	30.4	1.215 ± 0.134	0.01736 ± 0.00463	0.02475 ± 0.0451	252.2 ± 88.3

indoor air inside premises and a time t as a physical law that describes ammonia emission from building materials and is mathematically given with the equation (1). In future this law can be applied to analyze and parameterize all the experimentally obtained points in graphic relationships.

The Table above provides all the calculated average values of the basic kinetic parameters A_{av}, B_{av} and C_{av} for all the relationships established for all the analyzed objects and their uncertainties (inaccuracies).

As an ammonia concentration y_{av} in indoor air changes, at a certain time moment t, which is equal to T, it can reach any target permissible ammonia concentration C^* in indoor air, including the established maximum permissible level (MPL) C^* for ammonia equal to 0.04 mg/m^3. We can assume a time moment T when the kinetic curves reach any permissible ammonia concentration C^* relying on values of the kinetic parameters A_{av}, B_{av} and C_{av} in the equation (1). In particular, this level can be equal to MPC established for ammonia, which is 0.04 mg/m^3.

We determined a time moment T when the kinetic curves reach MPC for ammonia equal to 0.04 mg/m^3 for each object as per the equation (4) using numerical values of the parameters A_{av}, B_{av} and C_{av} in the equation (1) of the experimental relationships established for each analyzed object:

$$T = -\frac{1}{B_{av}} \cdot \ln\left|\frac{C^* - C_{av}}{A_{av}}\right|, \qquad (4)$$

where T is a predicted time moment when a permissible concentration C^* of ammonia emitted from building materials into indoor air inside a new building is reached, starting from the first day of measuring ammonia concentrations, days;

B_{av} is the average value of the decline rate constant for ammonia concentration y_{av} in indoor air in residential premises, the parameter identified by the graph analysis and associated with the essence of the migration process, ammonia emission form concrete materials and the material itself, days^{-1};

C^* is the established permissible ammonia concentration, mg/m^3, for example, MPC;

C_{av} is the average value of the minimal residual ammonia concentration in indoor air at the end of the experimental period, mg/m^3, the parameter identified by the graph analysis.

A_{av} is the average value of the total change in ammonia concentration y_{av} over the whole analyzed time t, mg/m^3, the parameter identified by the graph analysis.

We determined the time T for each object as per the equation (4) as follows:

1) The object No. 1:

$$T = -\frac{1}{0.02209}\ln\left|\frac{0.04 - 0.00653}{0.13759}\right| = 64 \text{ days;}$$

2) The object No. 2:

$$T = -\frac{1}{0.02419}\ln\left|\frac{0.04 - 0.02281}{0.12251}\right| = 81.2 \text{ days;}$$

3) The object No. 3:

$$T = -\frac{1}{0.01947}\ln\left|\frac{0.04 - 0.01784}{0.14479}\right| = 96.4 \text{ days;}$$

4) The object No. 4:

$$T = -\frac{1}{0.01736}\ln\left|\frac{0.04 - 0.02475}{1.215}\right| = 252.2 \text{ days.}$$

The values *T* calculated with the equation (4) and their inaccuracies calculated as per the formula (2) for all the analyzed objects are given in the Table.

We established that in case the initial conditions are the same (average initial ammonia concentrations in indoor air are almost identical and do not exceed 4 MPCav.d.) a time period up to 125 days (according to the upper confidence limit) ensures that an ammonia concentration falls below MPCav.d. in premises without interior decoration (the objects No. 1 and 2) as well as in variable premises with decoration and furniture (the object No. 3).

High values of the time period *T* that is necessary for ammonia concentrations to reach MPCav.d (8–11 months) at the object No. 4 are due to the initial ammonia concentrations (*A* + *C*) that were substantially higher than at three other objects (more than 30 MPCav.d.).

Following the research, the invention No. 2760762 was patented in the RF as "The method for predicting a time moment when concentrations of ammonia that occur in indoor air inside newly constructed buildings due to multi-day emissions from emitted from building materials will reach their permissible level"[4].

Conclusions. Therefore, we established that an exponential equation should be used to describe ammonia emission from building materials into indoor air inside newly constructed buildings associated with time of ventilation. The suggested method for predicting how ammonia concentrations will decline in new premises has been developed using mathematical procedures and provided with statistical substantiation. This makes it possible to use it to analyze any experimentally established relationship to estimate ammonia emission from building materials into indoor air.

The identified quantitative kinetic parameters of the equation for approximated curves give an opportunity to calculate ammonia emission rate and a time period over which ammonia concentrations will fall down to their permissible levels (MPC).

The suggested method for predicting a time moment when ammonia concentrations fall down to their permissible levels makes it possible to effectively control safety of indoor air and to establish when residential and public buildings should be commissioned provided that they do not pose any health harms for people.

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Research article

ROUTINE USE OF MOBILE ELECTRONIC DEVISES BY SCHOOLCHILDREN AND STUDENTS AND ITS CORRECTION BY HYGIENIC EDUCATION

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Many research works have described negative effects produced by use of electronic devices, mobile ones (smartphone, tablets) included, on children, adolescents and youth. However, the problem has many aspects and not all of them have been explored profoundly.

In this study, our aim was to conduct hygienic assessment of routine use of mobile electronic devices by schoolchildren and students and to give grounds for its correction by hygienic education.

By conducting a survey, we obtained data on use of mobile electronic devices by 1218 schoolchildren and students both in their educational and spare time activities and created a profile of a work and rest routine when working with mobile electronic devices. 943 students and schoolchildren were examined by an ophthalmologist. Next, issues related to hygienic education were integrated into the training program for medical students at the Hygiene Department of the Pirogov Russian National Research Medical University. The emphasis was on creating a safe routine of using mobile electronic devices.

Schoolchildren and students who adhered to a healthy work and rest routine when working with mobile electronic devices complained about health disorders authentically less frequently ($p \leq 0.05$).We established a statistically significant relative risk for visual acuity if schoolchildren and students did not pursue a safe routine of using mobile electronic devices. Its level was RR = 3.07 (95 %, CI =1.88–5.03).

By the end of hygienic studies with their focus on creating a safe routine of using mobile electronic devices, medical students had an authentic ($p \leq 0.05$) increase in visual acuity due to decline in such states as routine accommodative excess and pre-myopia.

Work and rest routines accepted by children, adolescents and youth when they use mobile electronic devices are a manageable risk factor of health disorders in these population groups. This study shows that hygienic education may be quite effective for correcting a routine of using mobile electronic devices by schoolchildren and students.

Keywords: *schoolchildren, students, visual acuity, accommodation, mobile electronic devices, work and rest routine, hygienic education.*

Protection and strengthening of children's health is a priority trend in the development of the public healthcare and education in the Russian Federation. On July 26, 2017, the RF Government approved the Profile of the priority project 'Creation of a healthy lifestyle'. Its major goal is to raise a share of citizens who pursue a healthy lifestyle up to 60.0 % by the

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end of 2025; the project involves providing hygienic education to various population groups and spreading the best educational practices in the sphere.

Rapid development of the digital environment marked the beginning of the 21st century. The report by the UNICEF entitled The State of the World's Children 2017: Children in a Digital World was published 10 years after the first iPhone was introduced on the market; it stated that children tended to start using the Internet at a younger age and the trend was persistent. In Europe, more and more children aged 3–5 years become active Internet users and this involves using mobile electronic devices (MEDs) [1].

In 2015, the RF Government approved The Concept of Information Safety for Children (The RF Government Order issued on December 02, 2015 No. 2471-r). The document established a major strategic goal of the state policy as regards providing information safety for children. This goal is to secure harmonious development of rising generations due to minimizing effects produced by all the adverse factors associated with the digital environment[1].

The modern digital environment has substantial influence on educational activities of children, adolescents and youth, their spare time, socialization and lifestyle [2].

Experts believe that in primary school children should spend no more than 10 minutes working on a PC. In middle school, this time should not exceed 15 minutes in 5–7th grades and 20 minutes in 7–9th grades. In senior school, adolescents should spend no more than 30 minutes working on a PC during the first class and no more than 20 minutes during the second one[2].

Multiple studies have addressed effects produced by MEDs use on children, adolescents, and young people. It is noteworthy that students who use MEDs often have asthenopic complaints, functional state of their eyes (organs of sight) and musculoskeletal system is impaired, and they tend to suffer from developing psychological dependence etc. [3–8].

Previously, any potential hazard posed by MEDs use was associated only with exposure to physical factors and keeping mobile devices in the closest proximity to the head. Today, a smartphone is a compact but still very powerful PC, which keeps receiving audio- and video-data uninterruptedly; therefore, a potential hazard associated with using it is more and more frequently associated with timing of MEDs use [9–14].

All the above stated requires analyzing effects produced by different routines of MEDs use on health and, in particular, eyes of students; another necessity is to search for effective ways to compensate them.

In this study, we aimed to conduct hygienic assessment of routine use of mobile electronic devices by schoolchildren and students and to give grounds for its correction by hygienic education.

Materials and methods. In 2017–2021 academic years, we conducted a one-time examination and questioning of 1218 schoolchildren and students in Moscow and the Moscow region (150 of them attended primary school; 225, middle school; 200, senior school; 643, freshmen and sophomores). Data on routine MEDs use by schoolchildren and students were obtained by using a standardized questionnaire recommended by the Research Institute of Hygiene and Health of Children and Adolescents for multi-centered surveys. All the participants had been using a MED for at least one year prior to the survey. We examined a period and variants of combined MEDs use by schoolchildren and students of different age, how often and for how long they used MEDs during classes, weekends and holidays.

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All the obtained data were supplemented with those obtained by using our own questionnaires developed by experts of the Hygiene Department, Pediatrics Faculty at Pirogov's Russian National Research Medical University; all these experts were certified in such fields as Common Hygiene, Hygienic Education, Children and Adolescents Hygiene, and Epidemiology. The questionnaires had questions about conditions of MEDs use (an organized workplace, a possibility to keep a comfortable working posture, sufficient lighting of a working surface); routines of their use (any breaks during work, their frequency and duration); any accomplished prevention activities and their description (a number of breaks and their timeliness, prevention activities occurring during them); 'screen time' of MEDs use. Schoolchildren in senior school and students were offered to fill in the questionnaires by using Google Forms. Two hundred schoolchildren in senior school and 518 freshmen and sophomores took part in the survey.

We copied some results of examinations performed by an ophthalmologist; overall, 943 schoolchildren and students were examined to establish their visual acuity. The examination results were written down as follows: $Vis^{without/}_{correction}$ (OD = …, OS = …).

We applied several criteria to include participants into our sampling. First, a participant should attend either a school or a higher educational institution (HEI); second, a written informed consent was submitted; a participant was examined by an ophthalmologist and the examination results were available; a questionnaire was filled in correctly by a respondent or his or her legal representative (for children in primary school); duration of MEDs use was one year and longer. Several criteria were applied to exclude a participant: other age group; an informed consent was not submitted; results of an examination by an ophthalmologist were not available; a participant suffered from chronic diseases of the eye that allowed ranking him or her as having the 4^{th} or 5^{th} health group; a correctly filled-in questionnaire was not submitted; duration of MEDs use was shorter than one year.

In 2018–2019, hygienic education was integrated into the training program for medical students at the Hygiene Department, Pediatrics Faculty at Pirogov's Russian National Research Medical University; its focus was on priority trends in developing skills of safe use of electronic devices (EDs) and on hygienic principles of eyesight protection. Students who were getting basic medical education or specialized in pediatrics were being taught by using active learning (case studies, active trainings, etc.). Effectiveness of the accomplished activities was checked based on certain objective indicators (visual acuity) at the beginning of the training at the Hygiene Department and after it was completed. One hundred and twenty-eight medical students in the test group and 128 in the reference one were observed in dynamics. Both groups were comparable as per age and sex, duration of EDs use, health, functional abnormalities and chronic diseases of the eye.

The conducted study did not pose any danger for its participants and fully conformed to biomedical ethics and provisions stipulated in the Declaration of Helsinki (1983 revision); it was approved by the Ethics Committee of Pirogov's Russian National Research Medical University (The meeting proceedings No. 159 dated November 21, 2016 and No. 203 dated December 20, 2020).

All the data were statistically analyzed with Statistica 13 PL software package.

Results. The study made it possible to identify the leading risk factors that could cause negative health outcomes for the eye. They included non-compliance with the rules for safe MEDs use as well as absence of healthy lifestyle components in daily routines of schoolchildren and students as regards MEDs use.

A smartphone is the most frequently used MED in educational and spare time activities of children, adolescents and youth. Duration of its use is not regulated by any valid sanitary standard and this device is not enlisted among those recommended for use in the educational process. In primary school, a smartphone accounts for about 50.0 % of the total daily time spent on MEDs use in educational and spare time activities during an academic year; in middle school, 55.0 %; in senior school and HEI, 65.0 %. During holidays, schoolchildren in primary and secondary school tend to use a smartphone for a longer period, on average, by 15–40 minutes longer.

When using a smartphone, schoolchildren and students made their first asthenopic complaints after 30 minutes in 44.00 ± 3.5 % of the cases whereas it was in 12.0 ± 2.3 % of the cases when a stationary ED was used.

We established that schoolchildren and students who adhered to safe routines of MEDs use complained about their health authentically rarer ($p \leq 0.05$); only 7.5 % of the respondents with routine MEDs use conforming to the safe standards complained about head aching and heaviness whereas 92.5 % of those who did not use MEDs safely had such complaints. Computer vision syndrome was detected in 17.8 % and 82.2 % of the respondents accordingly (Pearson's contingency coefficient was 0.511 ± 0.034, $p \leq 0.05$).

Functional impairments and chronic diseases of the eye in schoolchildren and students were associated ($p \leq 0.05$) with their failure to comply with rules for safe MEDs use: insufficient lighting (Pearson's contingency coefficient was 0.713 ± 0.037), an irrational working posture (Pearson's contingency coefficient was 0.822 ± 0.030), absence of proper breaks in work (Pearson's contingency coefficient was 0.836 ± 0.031), absence of a 'smartphone-free day' a week (Pearson's contingency coefficient was 0.827 ± 0.031), failure to do eye gymnastics (Pearson's contingency coefficient was 0.709 ± 0.039), using MEDs in transport (Pearson's contingency coefficient was 0.813 ± 0.032).

To explore a correlation between routine MEDs use by children, adolescents, and youth and the functional state of their eyes, we applied Spearman's correlation analysis. Statistically significant inverse correlations ($p \leq 0.05$) were established between visual acuity of the right eye (OD) and the total time of MEDs use ($r = -0.308$) on a weekday. We also established statistically significant inverse correlations ($p \leq 0.05$) between visual acuity of the left eye (OS) and the total time of MEDs use ($r = -0.32$) on a weekday. The obtained correlations were moderate according to the Chaddock scale.

Statistically significant inverse correlations ($p \leq 0.05$) were also established between visual acuity of the right eye (OD) and uninterrupted MEDs use (that is, a period of work with MEDs that was followed by a break) ($r = -0.392$) on a weekday. We established statistically significant inverse correlations ($p \leq 0.05$) between visual acuity of the left eye (OS) and uninterrupted MEDs use ($r = -0.335$) on a weekday. The obtained correlations were mostly moderate as per the Chaddock scale.

We established a statistically significant relative risk of impaired visual acuity due to unsafe MEDs use by schoolchildren and students; its value was $RR = 3.07$ (95 %, $CI = 1.88$–5.03).

The next stage in the study involved substantiating a possibility to correct a routine of MEDs use by providing hygienic education for medical students.

In accordance with the transition to the Federal State Educational Standard for Higher Education 2020, the Hygienic Education module was included into the training program for medical students. Among the topics it covered, it is noteworthy to mention 'Developing skills of safe use of electronic devices' with its aim to create general cultural and occupational competences in safe EDs use including those necessary to perform occupational activity as a physician with basic medical education or specializing in pediatrics. This concerns accomplishing the occupational function 3.1.4 'Accomplishment of prevention activities including educational ones among children and their parents' by a pediatrist and the occupational function 3.1.5 'Accomplishment of activities aimed at prevention and creating a healthy lifestyle as well as sanitary-hygienic education of the population with control of their effectiveness' by a physician with basic medical education.

Training provided for medical students within the Hygienic Education module involved using active learning methods (case studies, role plays, active trainings, etc.). They created necessary conditions to effectively motivate students to master the learning materials on their own using initiative and a creative approach and developed students' skills in preparing and implementing hygienic educational programs among patients and population in general with their aim to promote hygienic knowledge.

Examining basic requirements to a rest and work routine in EDs use became the major trend in training within the Hygienic Education module for medical students as future doctors and healthy lifestyle promoters. These requirements covered the total daily time of use; duration of uninterrupted use; regulation of breaks in work with MEDs and recommended activities during them; having a 'MED-free day' a week; ending MEDs use at least 1 hour prior to going to bed; hygienic requirements to lighting at a workplace; hygienic requirements to workplace ergonomics; doing eye gymnastics; etc.

Practical classes enabled medical students to acquire and master skills and competences as regards the following:

– assessing whether a patient is well informed about how to use MEDs safely, developing and implementing hygienic education schemes on the matter;

– searching, analyzing, systematizing and providing reliable information about how to prevent adverse effects produced by unsafe MEDs use on health, the eye included; to achieve this, students were provided with relevant regulatory and methodical documents;

– working with open access sources including those available in the Internet. Medical students got acquainted with official web-sites of medical organizations dealing with prevention, for example, the National Health and Research Center of Preventive Healthcare, Rospotrebnadzor's Center of Hygienic Education for Population, the Yamal Center for Public Health and Medical Prevention as well as web-sites of social movements working in the sphere. The aim was to get acquainted with information provided on these web-sites and to use it when accomplishing tasks within a practical class.

At the beginning of the module, visual acuity of the students from the test and reference groups did not have any authentic differences and was equal to 0.60 ± 0.04 (OD), 0.60 ± 0.04 (OS). At the end of the module, we established that visual acuity improved authentically ($p \leq 0.05$) in the medical students from the test group and equaled 0.85 ± 0.03. It was achieved due to a decrease in such states as usual accommodative excess and pre-myopia and this was an objective indicator. We did not detect any changes in visual acuity of the students from the reference group (Figure).

The Hygienic Education module included into the training programs for future physicians makes it possible to develop both general cultural and occupational competences as regards essentials of health protection. This has a positive effect on the functional state of students' eyes.

Discussion. MEDs use by children, adolescents and youth is accompanied with an increase in the static component, elevated visual loads, nervous and emotional involvement thereby creating a wide range of risk factors able to cause health disorders including diseases of the eye.

MEDs have certain well-known construction peculiarities, which can have drastic influence on schoolchildren's and students' health. There are several unresolved issues that determine health risks associated with their use: irrational keyboard (QWERTY) ergonomics; uncomfortable interface that makes user's hands keep a forced position, difficulties in using a device when walking (it influences one's walk and raises a possibility of getting injured) [15–17].

Big-sized MED's sensory screens were established to have an obvious advantage over smaller sensor screens as regards available space that could be used to accommodate and transfer graphical information. Some studies revealed that users thought a tablet to be the most useful in a situation when precision of graphical interpretation is important and there are no time limits [18].

A text size and wider interlineage also make a text much easier and more comfortable to read on a tablet but screen oversaturation requires much more time for reading and processing data [19].

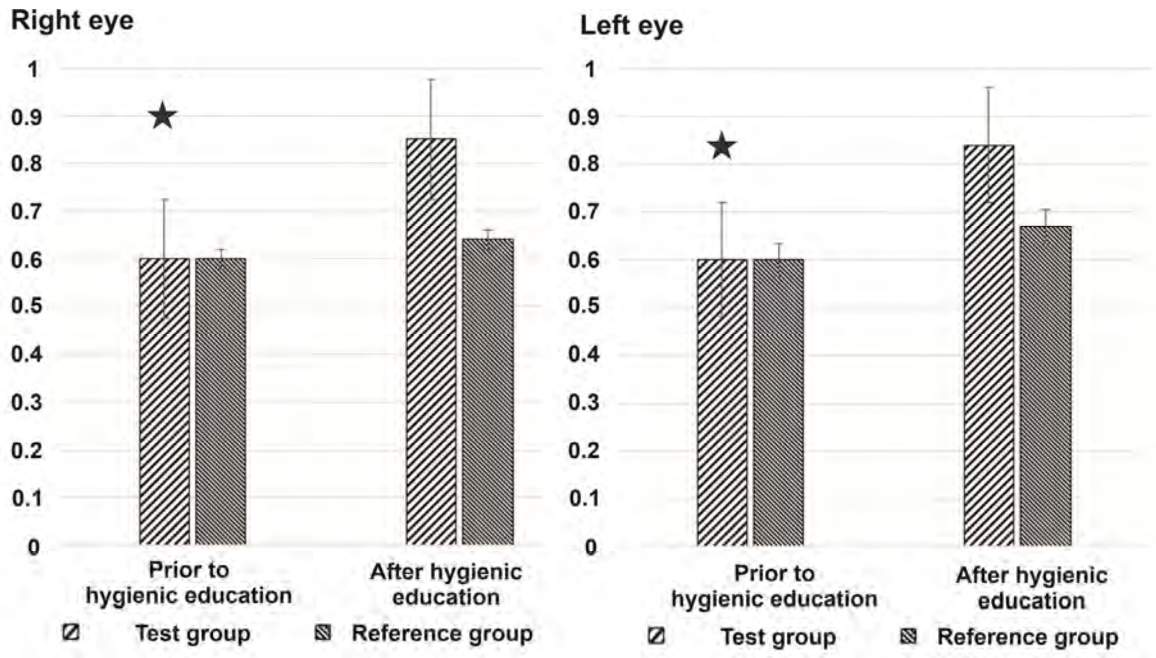

Figure. Visual acuity of the medical students from the test and reference groups prior to and after hygienic educations addressing major aspects in eyesight protection, M ('asterisk' corresponds to $p \leq 0.05$)

There are also several studies addressing influence exerted by routine MED (smartphones, tablets) use in spare time activities [20].

Still, available research data on influence exerted by different routines of MEDs on young people's health cannot be considered comprehensive and reflect only certain aspects of the issue.

Several studies revealed that imposing a limitation on time spent by schoolchildren and students on MED's use both produces favorable effects on the functional state of the body as a whole by preventing fatigue and makes for prevention of functional abnormalities and chronic diseases of the eye.

Therefore, it is still vital to explore harmful effects produced on health by constructive peculiarities of various MEDs. The issue requires further investigation. But another significant issue is absence of any healthy lifestyle components as regards safe MEDs use in schoolchildren's and students' daily routines. This study has established that it is possible to correct MEDs use by hygienic education. Effectiveness of hygienic education, which was confirmed by such objective indicator as visual acuity, was exemplified by its inclusion into training programs for medical students.

Conclusions:

1. Use of mobile electronic devices (tablets and smartphones) is widely spread among schoolchildren in primary, middle and senior school as well as among HEI students.

2. Work and rest regime of MED use by children, adolescents and youth is a manageable health risk factor for the rising generation.

3. The study has shown effectiveness of using hygienic education for correcting routine MEDs use by schoolchildren and students.

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Research article

ASSESSMENT OF POTENTIAL HAZARDS POSED BY INFLUENCE OF RISK-INDUCING ENVIRONMENTAL FACTORS AND FACTORS RELATED TO THE EDUCATIONAL PROCESS ON SOMATIC HEALTH OF SCHOOLCHILDREN IN DIFFERENT SCHOOLS

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Contemporary school should create favorable conditions for schoolchildren. The aim of the study was to comparatively analyze risk factors related to the educational process in different schools and to develop a new approach to objective assessment of combined exposure to environmental factors and factors related to the educational process and its influence on schoolchildren's health.

The study focused on the following research objects: 1) five different secondary schools, the test ones providing profound educational programs and the reference ones being ordinary secondary schools with the same or lower levels of pollution in environmental objects; 2) quality of components in the school environment (organization of the educational process, quality of meals provided by school, quality of ambient air on a school territory and inside a school, quality of drinking water, socioeconomic conditions); 3) health of 756 schoolchildren. The study was conducted by using sanitary-epidemiological, sanitary-hygienic and sociological methods; clinical and laboratory examinations; chemical analytical tests. Fuzzy logic was applied to estimate combined influence exerted by factors related to the educational process and environmental factors.

We established several determinants of negative effects produced by the educational process on schoolchildren's health. They included elevated intensity and monotony of educational and intellectual loads, shorter breaks between classes and recovery index deficiency. Diet-related factors included excess consumption of fats and carbohydrates, overall caloric contents being too high, protein and micronutrient deficiency. Chemical factors were elevated levels of metals, aromatic hydrocarbons, aldehydes, and chlorinated organic compounds in biological media. Risk-inducing factors of schoolchildren, regardless of a school and age, include organization of the educational process (Ipj = 0.45–0.58) and school meals (Ipj = 0.41–0.54); the group potential hazard index for these factors reached its peak values in primary school (Ipj = 0.49–0.58 and Ipj = 0.46–0.54). The maximum value of the integral potential hazard index (I_{pdk} = 0.41–0.46) caused by combined exposure to factors related to the educational process and environmental factors, regardless of a school type, was detected in senior schoolchildren in school with profound studies of natural sciences (I_{pdk} = 0.41); the minimum value was detected in a military school (I_{pdk} = 0.33).

Keywords: *schoolchildren, health risk factors, educational process, diet, environment, fuzzy sets.*

Children's health protection is one of the key trends in the state social policy and sanitary-epidemiological wellbeing of the RF population is a way to provide it [1]. The years 2018–2027 have been declared the Childhood Decade by the RF President Order No. 240 issued on May 29, 2017. The priority activities that are to be performed during this period include providing a comfortable and safe educational environment for the rising generation. School should create favorable conditions for schoolchildren to study there and combine both educational activities and those beyond the educational process relying on health-protecting teaching technologies [2, 3]. The contemporary educational process covers not only issues concerning how education activities are organized at school but also tasks related to providing

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schoolchildren with a safe in-school environment and adequate meals. It should include teaching them useful skills as regards how to correctly organize their activities beyond school and how to pursue a healthy lifestyle [4]. The reforms of the contemporary secondary education involve, among other things, creating specialized schools (gymnasiums, schools with advanced studies of various subjects, military schools etc.). These specialized schools have the right to develop a wide range of their own educational programs and apply variable teaching technologies [5, 6]. However, these newly developed teaching technologies do not often undergo any sanitary-hygienic examination to establish their safety for schoolchildren's health whereas data obtained by multiple studies reveal unfavorable trends in basic health indicators of children when they are examined in dynamics over school years [7, 8]. Educational loads are growing and the educational process itself is being intensified and involving wider use of IT; given that, educational programs can only be mastered by significant exertion of schoolchildren's functional capabilities [9, 10].

At present, many studies focus on examining influence exerted by chemical pollutants in ambient air, air inside school buildings and drinking water on schoolchildren's health [5]. Air inside schools and on near-school territories is often polluted with formaldehyde, phenol, styrene and ethylbenzene in concentrations being higher than hygienic standards; drinking water is often polluted with chlorinated organic compounds in unsafe concentrations. All this leads to these chemicals occurring in children's biological media in concentrations being higher than permissible ones with subsequent adverse general somatic effects and those affecting specific organs or systems [11, 12].

Nutrition is a most significant factor influencing health of a growing body [13]. Diseases that result from improper nutrition can become apparent not only in childhood but also at later stages in ontogenesis [14]. Children's unhealthy diets are of growing concern not only in the RF but also abroad since they result in alimentary-dependent diseases and elevated risks of cardiovascular and other systemic diseases at an older age [15]. Children spend a significant amount of their time at school; despite that, we can't fail to consider a substantial role that belongs to socioeconomic factors in children's family homes as well as their activities beyond school. Some studies have established that half of schoolchildren have physical activity below levels recommended by the WHO whereas the rapidly developing information-interactive space involves more and more of them in becoming its active users [16].

Over the last decade, many studies have concentrated on examining effects produced by various factors related to the educational process on biological constants of development and schoolchildren's somatic health. However, issues related to combined exposure to such factors have not been given enough attention [16, 17]. Targeted management of health risks for schoolchildren associated with the educational process requires developing a new approach to objective assessment of combined exposure to intra-school factors and those beyond it. Such an approach makes it possible to identify priority risk factors and differentiate relevant sanitary-hygienic and medical-preventive activities.

In this study, our aim was to analyze priority risk factors associated with the educational process in different schools and to develop a new approach to objective assessment of combined exposure to environmental factors and factors related to the educational process and its influence on schoolchildren's health.

Materials and methods. Our research objects included the following:

– five different types of secondary schools; the test schools implemented educational programs with advanced studies of some subjects: type I school provided more profound studies of natural sciences; type II school focused on general development (more profound studies of humanitarian sciences); type III school had an additional educational program focusing on sports and physical training (more profound military training). The reference schools were ordinary secondary ones with the same (type IV school) and lower (type V school) levels of pollution in environmental objects;

– quality of components in the school environment (organization of the educational process, quality of meals provided at school, quality of ambient air on a school territory and inside a school, quality of drinking water, socioeconomic conditions)

– health of 756 schoolchildren.

The study design involved using sanitary-epidemiological, sanitary-hygienic and sociological procedures; performing clinical-laboratory tests and chemical analytical tests; using risk assessment, probabilistic analysis and fuzzy sets.

Organization of the educational process in primary, middle and senior school was estimated by comparatively analyzing them. The analysis aimed to identify their conformity with the requirements stipulated by the Sanitary Rules and Standards SanPiN 2.4.3648-20[1] and SanPiN 1.2.3685-21[2]. Intensity of the educational process was examined in accordance with the Federal Recommendations FR ROSHUMZ-16-2015[3].

Hygienic assessment of ambient air quality on near-school territories, air inside school premises and drinking water from centralized water supply systems was conducted by using field observation results. Outdoor and indoor air samples were taken according to the State Standards GOST 17.2.3.01 and GOST R ISO 16017-1. Air quality was estimated as per contents of phenol, benzene, toluene, ethylbenzene, manganese, lead, nickel, and chromium. Phenol was identified in ambient air by using spectrophotometry; formaldehyde, high performance liquid chromatography (HPLC); metals, mass spectrometry; aromatic hydrocarbons (benzene, toluene and ethylbenzene), gas chromatography; all identifications were performed as per conventional procedures[4]. Drinking water was analyzed to identify chloroform, tetrachloromethane, and 1,2-dichloroethane by using gas chromatography according to the State Standard GOST 31951-2012; formaldehyde, by using HPLC according to the State Standard GOST 55227-2012; metals, by using mass spectrometry with inductively coupled plasma. Water samples were taken from a source of centralized water supply (tap). We calculated simple mean concentrations of controlled chemicals in water. Quality of indoor and outdoor air as well as water quality was estimated by comparing single and average daily concentrations of the analyzed chemicals with the existing hygienic standards (single maximum MPC, average daily MPC, RfC, MPC) as well as with the same indicators on reference territories. Chemical analysis of phenol, formaldehyde, aromatic hydrocarbons, chloroform, manganese, nickel, chromium, and lead in blood was performed as per conventional

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[1] SanPiN 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 № 28) [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 dated September 28, 2020 No. 28)]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 54 p. (in Russian).

[2] SanPiN 1.2.3685-21. Gigienicheskie normativy i trebovaniya k obespecheniyu bezopasnosti i (ili) bezvrednosti dlya cheloveka faktorov sredy obitaniya [Sanitary Rules and Standards SanPiN 1.2.3685-21. Hygienic standards and requirements to providing safety and (or) harmlessness of environmental factors for people]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573500115 (October 19, 2022) (in Russian).

[3] Gigienicheskaya otsenka napryazhennosti uchebnoi deyatel'nosti obuchayushchikhsya: federal'nye rekomendatsii po okazaniyu meditsinskoi pomoshchi obuchayushchimsya [Hygienic assessment of the intensity of educational activities by students: federal recommendations on providing medical care for schoolchildren]. Moscow, 2015, 18 p. (in Russian).

[4] RD 52.04.186-89. Rukovodstvo po kontrolyu zagryazneniya atmosfery [RD 52.04.186-89. Guide on control over ambient air pollution]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200036406 (October 19, 2022) (in Russian); MUK 4.1.1045-01. VEZhKh opredelenie formal'degida i predel'nykh al'degidov (C_2–C_{10}) v vozdukhe [MUK 4.1.1045-01. Control procedures. Chemical factors. HPLC applied to determine formaldehyde and saturated aldehydess (C_2–C_{10}) in ambient air]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200029341 (October 19, 2022) (in Russian); MUK 4.1.3481-17. Izmerenie massovykh kontsentratsii khimicheskikh elementov v atmosfernom vozdukhe metodom mass-spektrometrii s induktivno svyazannoi plazmoi [MUK 4.1.3481-17. Measuring mass concentrations of chemicals in ambient air by mass spectrometry with inductively coupled plasma]. *The library for regulatory documentation*. Available at: https://files.stroyinf.ru/Index2/1/4293735/4293735234.htm (October 19, 2022) (in Russian); MUK 4.1.3167-14. Gazokhromatograficheskoe opredelenie geksana, geptana, benzola, toluola, etilbenzola, m-, o-, p-ksilolov, izopropilbenzola, n-propilbenzola, stirola, a-metilstirola, benzal'degida v atmosfernom vozdukhe, vozdukhe ispytatel'noi kamery i zamknutykh pomeshchenii [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]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200119249 (October 19, 2022) (in Russian).

procedures[5]. Reference regional background levels as well as levels identified in the reference groups were used as criteria for estimating chemical pollution in biological media.

Meals provided at school were comparatively estimated by analyzing daily menus and identifying the chemical structure and energy values of these meals; ratios between basic nutrients were estimated to determine their conformity with the requirements stipulated in the SanPiN 2.3/2.4.3590-20[6]. We analyzed daily meals to comparatively estimate whether an average daily food set corresponded to the established standards. An average weight of a helping and actual food consumption were identified by individual weighing that took place during four weekdays, 10 weight measurements taken each time [18]. We calculated nutrient and energy value of meals taken for breakfast and lunch (and additionally for a mid-afternoon snack in the type III school). Data on daily consumption of basic foods were obtained by questioning; they made it possible to calculate individual contents of macro- and micronutrients in a daily diet. All these calculations relied on data taken from the reference book on the chemical structure of foods in Russia[7].

Socioeconomic risk factors and factors related to lifestyle were analyzed relying on data of a social survey accomplished by handing out questionnaires to be filled in by participants. The questionnaire included five sets of questions to describe parents' education, family incomes, living conditions, schoolchildren's spare time, intensity of learning activities beyond school, and physical activity (65 questions overall). The results were estimated as per an integral indicator with its score varying between 1 and 3 (the higher the score, the better qualitative indicators).

Clinical and functional examination of children was performed as per conventional procedures and included anthropometric measurements (SD score was used as a reference value); bioimpedance analysis (BIA) to estimate BMI (kg/m^2), fat mass (FM, %), phase angle (PA, grades), bone and muscle mass (BMM, kg), body cell mass (BCM, kg) and fat-free mass (FFM, kg); electroencephalography (EEG); psychological testing (RT-test to assess a reaction time, motor function velocity, and attention level; STROOP-test to estimate cognitive functions; CORSI-test to estimate visual-spatial short-term working memory; and Luscher test to estimate psychological stress level); heart rate variability examination; analysis of children's outpatient medical records (the medical Form No. 112/u). Children's health

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[5] MUK 4.1.2108-06. Opredelenie massovoi kontsentratsii fenola v biosredakh (krov') gazokhromatograficheskim metodom [MUK 4.1.2108-06. Determination of mass concentration of phenol in biological media (blood) with gas chromatography]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200065240 (October 21, 2022) (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 [MUK 4.1.2111-06. Determination of mass concentration of formaldehyde, acetaldehyde, propionic aldehyde, fatty aldehyde and acetone in blood samples by high performance liquid chromatography]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200065243 (October 21, 2022) (in Russian); MUK 4.1.765-99. Gazokhromatograficheskii metod kolichestvennogo opredeleniya aromaticheskikh (benzol, toluol, etilbenzol, o-, m-, p-ksilol) uglevodorodov v biosredakh (krov') [MUK 4.1.765-99. Gas chromatography applied to quantify aromatic (benzene, toluene, ethylbenzene, o-, m-, p-xylene) hydrocarbons in biological media (blood)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200039012 (October 21, 2022) (in Russian); MUK 4.1.2115-06. Opredelenie massovoi kontsentratsii khloroforma, 1,2-dikhloretana, tetrakhlormetana v biosredakh (krov') metodom gazokhromatograficheskogo analiza ravnovesnogo para [MUK 4.1.2115-06. Determination of mass concentration of chloroform, 1,2-dichloroethane, tetrachloromethane in biological media (blood) by gas chromatography analysis of equilibrium vapor]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200065247 (October 21, 2022) (in Russian); MUK 4.1.3230-14. Izmerenie massovykh kontsentratsii khimicheskikh elementov v biosredakh (krov', mocha) metodom mass-spektrometrii s induktivno svyazannoi plazmoi [MUK 4.1.3230-14. Determination of chemical mass concentrations in biological media (blood, urine) with mass spectrometry with inductively coupled plasma]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/495856222 (October 21, 2022) (in Russian); MUK 4.1.3161-14. Izmerenie massovykh kontsentratsii svintsa, kadmiya, mysh'yaka v krovi metodom mass-spektrometrii s induktivno svyazannoi plazmoi [MUK 4.1.3161-14. Determination of mass concentrations of lead, cadmium and arsenic in blood with mass spectrometry with inductively coupled plasma]. *The library for regulatory documents*. Available at: https://files.stroyinf.ru/Index2/1/4293766/4293766470.htm (October 21, 2022) (in Russian).

[6] SanPiN 2.3/2.4.3590-20. Sanitarno-epidemiologicheskie trebovaniya k organizatsii obshchestvennogo pitaniya naseleniya [Sanitary Rules and Standards 2.3/2.4.3590-20. Sanitary-epidemiological requirements to organizing catering provided for population]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/566276706 (October 19, 2022) (in Russian).

[7] Khimicheskii sostav rossiiskikh pishchevykh produktov: spravochnik [Chemical structure of foods in Russia: the reference book]. In: Professor I.M. Skurikhin and RAMS Academician, Professor V.A. Tutelyan eds. Moscow, DeLi print, 2002, 236 p. (in Russian).

was assessed by analyzing the results of clinical examinations performed by a pediatrician, allergologist, neurologist, gastroenterologist, ENT doctor, ophthalmologists, an expert in exercise therapy and a cardiologist[8]. Results of the laboratory tests were examined as per protocols of general clinical, biochemical, immunologic and immune-enzyme analysis[9].

The obtained data were analyzed with Statistica 6.0 software package and embedded statistical functions in Microsoft Excel 2010. The study results were mathematically treated with parametric statistic methods with preliminary assessment of their conformity with the normal distribution. Student's t-test and Fisher's test were applied to estimate authenticity of differences. The social survey results were statistically analyzed by calculating and comparing simple means, identifying frequency and structural properties. Authenticity of differences between social indicators was estimated by using Kruskal – Wallis and Mann – Whitney criteria considering children's age and a school they attended. We estimated influence exerted by the educational process, meals at school, chemical pollutants and socioeconomic factors on anthropometric indicators, children's somatic and mental health by using one-factor logistic regression modeling of 'dose – likelihood of response (effect)' relationships. The model significance was estimated as per Fisher's test (F) with giving a constant value (b_0), regression coefficient (b_1), and Nagelkerke determination coefficient (R^2). Any differences were considered statistically significant at $p \le 0.05$. Overall, the logistic regression model was formulated as follows:

$$p = \frac{1}{1 + e^{-(b_0 + b_1 x)}}, \quad (1)$$

where p is a probability that a response will deviate from the standard; x is exposure level; b_0, b_1 are parameters of the mathematical model.

Combined effects produced by organization of the educational process, meals at school and anthropogenic contaminants were estimated by a method based on fuzzy logic (fuzzy sets). Each factor was estimated with integral values to identify whether it belonged to a set of a scaled indicator. To estimate influence exerted by each of three indicator groups on schoolchildren's health, we used a scale with score values between 0 and 1. It graded a potential hazard index considering weight contributions by specific indicators (I_{pdi}) and a whole group of indicators (I_{pdj}) to an ultimate result. Weight contributions made by meals at school, educational process and chemical environmental factors to severity of potential impairments of schoolchildren's biological status were taken as equal to 0.4; 0.5; 0.1, and primary, middle and senior school were taken as equal to 0.3; 0.2; 0.5 accordingly. The potential hazard index (I_{pd}) was considered as a basic quantitative value of contributions made by specific indicators. This value was used to estimate an integral index (I_{pdk}) and was calculated as a sum of products obtained by multiplying potential hazard indexes of each indicator group (I_{pdj}) and their weight contributions to severity of potential impairments of schoolchildren's biological status (V_g). When calculating a membership degree for a potential hazard index to identify whether it belonged to a certain set of the scale, the fuzzy set method implied the sets had fuzzy boundaries (± 20 % fuzzy). As a result, values within the neighboring sets of the scale could intersect. Each range of indicator values was a trapezoid fuzzy number with the membership function showing whether it belonged or did not belong to a certain set of the scale. The method was implemented by using the membership function $\mu(x)$ to a trapezoid fuzzy number, which was given by four numbers (a_1, a_2, a_3, a_4) as the basic instrument. In its general view, the membership function for an indicator x to a specific trapezoid number was given with the following formula:

$$\mu(x) = \begin{cases} 0, & \text{if } x < a_1, \\ \frac{x - a_1}{a_2 - a_1}, & \text{if } a_1 \le x < a_2, \\ 1, & \text{if } a_2 \le x \le a_3, \\ \frac{x - a_4}{a_3 - a_4}, & \text{if } a_3 < x \le a_4, \\ 0, & \text{if } x > a_4. \end{cases} \quad (2)$$

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[8] All the examinations were performed by medical experts of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

[9] All the examinations were performed by experts from the Departments for clinical, cytogenetic and immune biological diagnostic methods of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

The value $\mu(x)$ reflected that a set of an indicator value belonged to a set of a scale showing potential hazard index values.

When estimating a potential hazard index for chemical factors, we identified a negative health outcome for each indicator in accordance with the Guide R 2.1.10.1920-04 and Methodical Guidelines 2.1.10.3014-12 as well as other relevant research data. A negative health outcome (a group of diseases in accordance with the ICD-10 from A00 to R99) was given a rank l considering its severity ranked within a range between 0 and 1. We determined a weight (frequency) of each group of diseases ranked as per its severity in the total negative health outcome (W_l) as per Fishburne's Rule:

$$W_l = \frac{2(K-l+1)}{(K+1)K}, \quad (3)$$

where W_l is a weight of a ranked group of diseases in the total negative health outcome; K is the total number of groups of diseases identified in the total negative health outcome; l is a rank of a negative health outcome (a group of diseases) as per its severity.

The calculated weight of each group of diseases (W_l) was applied to a weight of each indicator in this group participating in the total negative health outcome (G_i). To estimate the potential hazard index as per meals provided at school and factors related to the educational process, we established a weight of each indicator (G_i) as per the following formula:

$$G_i = \frac{1}{m}, \quad (4)$$

where m is the number of all the analyzed indicators in the group.

$$w_j = \sum\nolimits_i G_i \cdot \mu_{ji},\ j = 1, 2, 3, 4, 5, \quad (5)$$

where w_j is a share of indicators belonging to a specific potential hazard index (or to the k-th scaled set of the potential hazard index and its rank) of the total number of indicators in the group;

G_i is a weight of each indicator contributing to the total negative health outcome;

μ_{ji} is the membership function for each indicator showing its belonging to the j-the scaled set of the potential hazard index and its rank.

We calculated an index for each group of indicators (I_{pdj}) allowing for their share contributions to all kinds of negative health outcomes as per the following formula:

$$I_{pdj} = \sum_{j=1}^{5} \overline{I_j} \cdot w_j, \quad (6)$$

where I_{pdj} is a value of an index for each indicator group allowing for their share contributions to all kinds of negative health outcomes;

$\overline{I_j}$ is the middle of each set on the scale with values of the potential hazard index determined by impacts exerted by each group of indicators;

w_j is a share of indicators belonging to a specific potential hazard index (or to the k-th scaled set of the potential hazard index and its rank) of the total number of indicators in a group.

The integral potential hazard index (I_{pdk}) as per all the indicator groups was calculated as per the following formula:

$$I_{pdk} = \frac{1}{\overline{V_g}} \sum_{p=1}^{5} I_{pdj} \cdot V_g, \quad (7)$$

where I_{pdj} is a value of an index for each indicator group allowing for their share contributions to all kinds of negative health outcomes;

V_g is a weight contribution made by a group of indicators to the integral potential hazard index;

$\overline{V_g}$ is an average weight contribution made by all groups into the integral index.

The indexes were estimated separately for primary, middle and senior school; as per each factor and a group of factors; for each separate school. The obtained values allowed assigning a rank to each school as per potential hazards posed by its environment for schoolchildren's health.

Results and discussion. We analyzed how the educational process was organized in all the examined schools. The analysis revealed that the Sanitary Rules and Norms SanPiNH 2.4.3648-20[10]

[10] SanPiN 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 № 28) [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 dated September 28, 2020 No. 28)]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 54 p. (in Russian).

were violated in all of them. However, the educational process in the schools with advanced studies of some subjects involved by 1.4 times more intensive educational loads and irrational use of electronic teaching aids as opposed to the ordinary secondary schools. The educational process itself was by 1.4–1.9 times more intensive in such schools.

Our assessment of outdoor and indoor air quality established that toluene and ethylbenzene occurred in air in all the analyzed schools; in addition, we identified elevated (against average daily MPC equal to 0.01 mg/m^3) phenol and formaldehyde levels. They were by 1.6–1.7 times higher than average daily MPC in ambient air (0.0162 ± 0.0022–0.0168 ± 0.0034 mg/m^3) and by 1.2-2.0 times higher in air inside school premises (0.0124 ± 0.0025–0.02136 ± 0.0044 mg/m^3), $p < 0.001$.

Quality of drinking water was established to deviate from the requirements stipulated in the SanPiN СанПиН 1.2.3685-21[11] in both groups of the analyzed schools (test and reference ones). Chloroform (0.138 ± 0.01 mg/m^3 and 0.186 ± 0.007 mg/m^3) and formaldehyde (0.123 ± 0.004 mg/m^3 and 0.094 ± 0.002 mg/m^3) levels in drinking water were higher than MPC (0.06 and 0.05 mg/m^3 accordingly) ($p < 0.001$). The chloroform level (0.138 ± 0.01 mg/m^3) was by 1.4 times lower and formaldehyde (0.123 ± 0.004 mg/m^3) and manganese (0.0093 ± 0.0015 mg/m^3) levels were by 1.3 and 28.2 times higher accordingly in the test schools against the same indicators in the reference ones (0.186 ± 0.007 mg/m^3; 0.094 ± 0.002 mg/m^3; 0.0033 ± 0.0004 mg/m^3 accordingly, $p < 0.001$).

We examined chemical contents in children's biological media and established elevated levels of certain chemicals that were higher than the regional background ones in all the analyzed schools. Thus, phenol levels were by 1.5–5.0 times higher (0.0100 ± 0.0036 μg/ml) and formaldehyde levels were by 2.2–16.8 times higher (0.0050 ± 0.0014 μg/ml) in primary, middle and senior school (0.0150 ± 0.0050–0.0502 ± 0.0073 μg/ml and 0.0110 ± 0.0007–0.0840 ± 0.0310 μg/ml accordingly; $p < 0.001$). Benzene and toluene were identified in biological media of children in the type I school; chromium contents were by 1.4–1.8 times higher than their reference values (0.0027 ± 0.00199 μg/ml) in biological media of children from the type II school: 0.0037 ± 0.0005–0.0049 ± 0.0010 μg/ml ($p < 0.001$), and we also identified benzene, toluene and chloroform in them; chromium levels were by 1.9–2.7 times higher than the reference ones in biological media of children in the type III school: 0.0052 ± 0.0005–0.0072 ± 0.0008 μg/ml ($p < 0.001$) and we also identified benzene, toluene and chloroform in them; benzene and ethylbenzene were identified in biological media of children in the type IV school; benzene, toluene and chloroform, the type V school.

Comparative hygienic assessment of meals provided at the analyzed schools revealed that the standard macronutrients ratios (1:1:4) were violated in menus in all of them. Meals provided at the type IV school were the closest to the recommended standards (1 : 1.1 : 3.8); meals provided at the type I school had total nutrient deficiency (0.8 : 0.7 : 2.6); the type II (1.6 : 1.6 : 5.0), III[12] (1.4 : 1.5 : 4.3) and V (1.4 : 1.3 : 4.8) schools had nutrients in excessive quantities. The lowest micronutrient deficiency was established in the type I school (calcium, vitamin A); the highest micronutrient deficiency was identified in the type III school (B_1, B_2, C, A, iron, calcium, magnesium, and phosphor). It is noteworthy that calcium deficiency was identified in meals provided at all the analyzed schools.

We established that, given the maximum contribution made to a daily diet by home meals (76.0–92.2 %), schoolchildren attending most of the analyzed schools consumed some products in excessive quantities (sausages, by 1.7–2.0 times higher than recommended; macaroni, by 1.4–1.5 times; confectionary, by 7.8–8.0 times) whereas others were consumed in insufficient ones (vegetables, fruits, fish and cereals were consumed in quantities by 1.6–3.8 times lower than recommended). Three meals provided at the type

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[11] SanPiN 1.2.3685-21. Gigienicheskie normativy i trebovaniya k obespecheniyu bezopasnosti i (ili) bezvrednosti dlya cheloveka faktorov sredy obitaniya [Sanitary Rules and Standards SanPiN 1.2.3685-21. Hygienic standards and requirements to providing safety and (or) harmlessness of environmental factors for people]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573500115 (October 19, 2022) (in Russian).

[12] Diets provided at the type III school were compared in accordance with the requirements fixed for military schools.

III school were the closest to the recommended daily diet for schoolchildren.

Our study of socioeconomic factors and children's lifestyles revealed that family incomes were substantially higher in families of children who attended innovative schools and their parents had higher education more frequently than parents of children who attended ordinary secondary schools. Schoolchildren in innovative schools tended to spend by 3.5 times more time on additional educational activities and sports.

Comparative assessment of schoolchildren's physical development established that schoolchildren who attended the type I school tended to develop harmoniously in primary and middle school and this harmonious development was by 1.8–2.4 times more frequent among them than in the reference schools. Still, a risk of disharmonious development grew in senior school and was by 4.0 times higher than in an ordinary school. Schoolchildren in the type II school typically had height, weight, and hand dynamometry corresponding to age-specific norms; still, a relative risk of excessive height was by more than 6.0 times higher for them than in the reference schools by the end of school studies. Schoolchildren attending the type II school tended to have average weight, a well-developed chest and there was a trend established for them that the chest excursion would grow by 1.6 times by senior school; a risk of overweight was by 3.0 times higher for school leavers of the type II school than in the reference schools. Senior schoolchildren in the analyzed ordinary secondary schools tended to have average height; however, each third adolescent in these schools had overweight ($p = 0.02$).

Our assessment of the nutritive status established that a number of children with BMI and FM being by 1.4–1.8 times higher than the standard grew by 2.0–2.3 times in senior school in the analyzed ordinary secondary schools. Simultaneously, low BMM and BCM were identified in 14.0–27.6 % and 25.6–31.0 % of the senior schoolchildren in these schools accordingly whereas there were only isolated cases of them in primary and middle schools. The schoolchildren in the type I school typically had by 2.5 times lower BMI and FM in senior school together with growing PA, BCM and BMM, which were by 1.3–2.6 times higher than in primary and middle school. The schoolchildren in the type II school tended to be physically active during the whole educational period; the fact is confirmed by higher BMM which is by 1.6–2.3 times more frequent in this school and a number of children with proper BMM being by 3.2 times higher in senior school. BIA results in the type III school revealed better physical form and activity than in the analyzed ordinary schools evidenced by BCM, BMM and PA being by 1.2–1.8 times higher in the schoolchildren who attended it.

Having analyzed EEG results, we established that frequency of impaired cerebral rhythms went down with age in all the analyzed schools. In the ordinary secondary schools (types IV and V), dysfunctions were identified in 10.0 % and 7.6 % of the senior schoolchildren accordingly whereas we detected a discontinuous trend in the type I and II schools: bioelectrical cerebral activity stabilized in most middle schoolchildren (86.4–100.0 %) and then destabilization was identified in 23.8–26.0 % of the senior schoolchildren. A relative risk that a number of children with deviations from the physiological standards would grow in senior school was by 3.8 times higher in the type I school than in the reference ones. In the type III school, a share of schoolchildren with their EEG indicators corresponding and not corresponding to the age-specific standard equaled 50.0 %. By senior school, a delay in cortical rhythm development was identified only in 13.3 % of the schoolchildren in the type II school; however, a relative risk that dysfunctional bioelectrical cerebral activity would persist was by 1.8 times higher for the schoolchildren in the type II school than in the reference ones.

Psychological tests identified by 1.2–2.9 times lower stress and anxiety levels and higher cognitive flexibility in the schoolchildren from the type I, II, and III school than in their counterparts from the ordinary schools (types IV and V). We compared test results obtained at the beginning and end of a school year and revealed a decrease in children's effectiveness in all the analyzed schools. This difference in test results was more typical for the schoolchildren in the type I and III schools (by 2.8 times). The schoolchildren who attended schools with advanced studies of some subjects had by 1.2–2.9 times more stable working memory but also higher stress, anxiety, fatigue and excessive excitation by senior school. A relative risk that a number of children with some mental stress would grow by senior school was by 12.0 times higher in the type I school and by more than 4.0 times higher in the type II school than in the reference ones.

We estimated functional state of children's autonomic nervous system and identified that the eutonic type of the initial vegetative tone (IVT) prevailed in the schoolchildren in all the analyzed schools in primary, middle, and senior school. In the type I school, a number of children with the eutonic IVT went down by 1.6 times (from 67.6 to 41.7 %); a relative risk that a number of children with the vagotonic IVT would grow in senior school was by 2.0 times higher than in the reference schools. A share of children with sympathicotonic vegetative reactivity (VR) remained stable in primary, middle and senior school in the reference schools and the type III school whereas it tended to decline by 1.3–1.8 times with age in the type I and II schools. The schoolchildren who attended the analyzed schools with profound studies of some subjects tended to have asympathicotonic VR by 4.0 times more frequently in senior school than in primary and middle school (from 0.0–5,9 % to 15.4–23.8 % cases).

Our analysis of incidence established that eye diseases occurred by 3.1–5.2 times more frequently in senior school in all the analyzed schools (the incidence grew from 7.7–18.2 % to 23.5–71.6 %); this was the most typical for the type I school (71.6 %) and the least typical for the type III school (23.0 %). Incidence of nervous diseases tended to grow by 1.7 times among the schoolchildren in the type I school (from 54.2 % to 89.5 %). Incidence of endocrine diseases was established to grow in all the other schools (from 35.4–77.1 % to 45.1–89.5 %). Somatic incidence of most groups of diseases tended to be by 1.2–3.1 times higher among the schoolchildren in the ordinary secondary schools.

We analyzed how the schoolchildren were distributed as per the health groups. The analysis revealed that the number of schoolchildren in the 1st and 2nd health group was by 1.2–1.8 times lower and the number of schoolchildren in the 3rd health group was by 1.8–2.5 times higher in primary and secondary school in the test schools than in the reference ones. In middle school, a relative risk that a number of schoolchildren in the 3rd health group would grow was by more than 3.0 times higher in the analyzed schools with advanced studies of some subjects than in the reference schools; in senior school, the same indicator was by 1.9 times higher in them.

We estimated regularities of developing negative health outcomes relying on the results obtained by laboratory tests. It revealed that a situation when factors related to the educational process did not conform to the standards, chemical contents in ambient air, air inside school premises, drinking water and biological media were higher than maximum permissible and background regional / reference levels, macro- and micronutrients balance and caloric contents of school meals did not conform fully to the age-specific standards, and social factors did not satisfy schoolchildren's physiological needs properly resulted in developing imbalance of the neuroendocrine and neuro-vegetative regulation (R^2 = 0.26–0.31), impaired oxidative-antioxidant processes (R^2 = 0.10–0.22), imbalance of basic metabolism types (R^2 = 0.13–0.89) and developing secondary transitory immune deficiency against background sensitization (R^2 = 0.10–0.68). Imbalance of the neuroendocrine and neuro-vegetative regulation in the children from the test group was evidenced with by 1.4 times lower serotonin and hydrocortisone levels, by 1.2 times lower free T4, by 1.7 times lower acetylcholine and by 2.0 times higher TSH. Impaired oxidative-antioxidant homeostasis was confirmed by 1.9 times more frequent lower AOA in the test group than in the reference one, by 2.9 times higher MDA levels and by 4.4 times higher Δ-ALA. Basic metabolic disorders became apparent through bilirubinemia, hyperglycemia and less active bone remodeling; they were by 1.3–1.6 times more frequent in the test group. Secondary immune suppression was evidenced with by 1.2–1.4 times higher sensitization indicators together with by 1.2–1.6 items lower levels of leukocytes, monocytes, CD19, IL-4 and phagocytic activity.

Our assessment of impacts exerted by the set of the analyzed factors on schoolchildren's body composition revealed that elevated phenol and chromium levels decreased a possibility that BCM would reach its physiological norm whereas iron, vitamin C and B_1 consumed with food in age-specific standard quantities increased it. BMM was more likely to reach its physiological standard in case calcium, magnesium, phosphor and proteins were consumed with food in balanced quantities; this likelihood went down due to elevated nickel and formaldehyde levels in blood. BCM and BMM were less likely to develop properly in case educational loads grew. FFM development was authentically affected in primary school by monotonous and intensive educational loads; in secondary school, by shorter breaks between classes and reduced recovery indexes as well as elevated chromium and formaldehyde levels in blood. In senior school, FFM development was affected considerably by magnesium and protein deficiency in school meals. Basic metabolic indicators were largely impaired by elevated educational loads, shorter breaks and reduced recovery index as well as growing chromium and formaldehyde levels in blood. Lower educational loads and reduced intellectual components in them as well as shorter breaks made FM accumulation and BMI growth more probable whereas elevated chromium levels in blood made these indicators decline. In primary school, elevated benzene levels in children's blood made excessive FM accumulation more likely. Adequate consumption of proteins, fats and carbohydrates became especially vital in middle and senior school. Elevated nickel and lead levels in blood reduced growth indicators in primary school, whereas likelihood that height would conform to its physiological standard grew in middle and senior school provided that meals corresponded to the recommended standards as per calcium, phosphor, iron and vitamins B_1, B_2, and C contents.

Next, we estimated effects produced by the set of the analyzed factors on incidence among the examined schoolchildren. The estimation revealed that cardiovascular diseases developed mostly due to influence exerted by such factors as elevated benzene, nickel and lead levels in blood, growing educational loads and recovery index deficiency, as well as shorter breaks. Diseases of the nervous system were more likely to occur due to excessive caloric contents in school meals; magnesium and vitamin B_1 deficiency; growing monotony of learning, daily educational and emotional loads; elevated lead, formaldehyde and phenol levels in blood. Chloroform in blood as well as levels of nickel, formaldehyde, chromium, lead and phenol in it being higher than maximum permissible ones made diseases of the genitourinary, digestive and respiratory systems more probable. Diseases of the digestive system might be induced by monotonous learning; growing daily educational loads and greater deficiency of recovery index; shorter breaks; magnesium, protein and vitamin C deficiency; excessive fat consumption. Respiratory diseases were more likely to develop due to monotony and intensity of learning, growing number of classes a day, as well as toluene in biological media. Prevalence of diseases of the eye grew due to elevated formaldehyde levels in blood, vitamins A and C deficiency in school meals as well as due to growing emotional and intellectual loads and more classes a day than recommended by the hygienic standards. Diseases of skin and subcutaneous tissue were induced by chromium and nickel levels in blood being higher than reference ones as well as growing monotony and intensity of learning and daily educational loads. We established a relationship between diseases of the musculoskeletal system and excessive caloric contents of school meals, excessive quantities of carbohydrates in them, vitamin C deficiency, a growing number of classes a day, increasing intensity of learning and its intellectual component as well as lead and manganese contents in blood. Diseases of the endocrine systems developed due to excessive quantities of carbohydrates, protein and vitamin C and B_1 deficiency in school meals as well as due to shorter breaks.

The next estimation focused on influence exerted by the set of the analyzed factors on the results obtained by psychological testing. It established that greater educational loads, longer classes, growing monotony of learning, increasing intellectual and emotional loads impaired visual-spatial short-term working memory; at the same time, a lower index of recovery deficiency and longer breaks improved it. Cognitive processes became less effective due to longer classes, shorter breaks, growing intellectual and daily educational loads. Our assessment of effects produced by anthropogenic contaminants revealed lower cognitive functions among schoolchildren of all ages in case they had elevated manganese, lead, nickel, phenol and chloroform levels in blood. Spatial-visual short-term working memory was established to be impaired by elevated nickel level in primary school; phenol and chloroform, in middle school; and manganese and nickel, in senior school. Elevated magnesium, nickel, lead, and phenol levels as well as chloroform contamination lead to more probable increase in reaction time in schoolchildren of all ages. Estimated effects produced by meals on the results of psychological testing included lower flexibility of cognitive processes due to protein, calcium, iron and vitamin C and B_1 deficiency and excessive carbohydrates and total caloric contents. Visual-spatial short-term working memory was impaired by calcium, vitamin C, A, and B_2 and protein deficiency and excessive fat consumption. Reaction time in psychological testing was authentically prolonged by calcium, iron, magnesium, vitamin C and protein deficiency and excessive carbohydrate contents. We established a relationship between impaired cognitive functions and growing intensity of learning in primary, middle and secondary school whereas an increase in physical activity made cognitive processes more flexible. Effectiveness of working memory directly depended on socioeconomic conditions in a family; however, a contribution made by this factor did not exceed 6.0–10.0 % in primary, middle and senior school.

Therefore, we identified several basic determinants of negative effects produced by the educational process on schoolchildren's development, somatic and mental health. They included growing intensity and monotony of learning; increased intellectual loads; shorter breaks and recovery deficiency index; as for meals provided at school, these factors were excessive fat and carbohydrate contents, excessive caloric contents. protein and micronutrient deficiency; chemical factors included elevated manganese, lead, chromium, nickel, phenol and formaldehyde levels in blood as well as occurrence of aromatic hydrocarbons in biological media.

The fuzzy logic was applied to identify values and a structure of a potential hazard index and to rank it; this index fully describes effects produced by exposure to factors related to the educational process and environmental factors on schoolchildren's morphofucntional state, their somatic and mental health. For the type I school the potential hazard index associated with improper school meals equaled I_{pdj} = 0.065 in primary school; I_{pdj} = 0.033–0.082 in middle and senior school; its overall value for all age groups equaled I_{pdj} = 0.180 (hazard was low, negligible). The potential hazard index due to improper organization of the educational equaled I_{pdj} = 0.087 in primary school, I_{pdj} = 0.052 in middle school and I_{pdj} = 0.123; the overall value for all age groups was I_{pdj} = 0.262 (low hazard). The potential hazard index associated with anthropogenic contaminants equaled I_{pdj} = 0.005–0.003 in primary and middle school but there was an ascending trend in it in senior school up to I_{pdj} = 0.008; the overall value for all age groups was I_{pdj} = 0.016 (negligible hazard) (Table).

The integral potential hazard index associated with multi-component influence exerted by school meals, the educational process and anthropogenic contaminants equaled I_{pdk} = 0.39 (average-low) in primary and middle school and I_{pdk} = 0.46 (average) in senior school. In the type I school, the integral potential hazard index was by 2.6–3.1 times higher than its safe level (I_{pd} < 0.15) for all age groups and grew by 1.2 times from primary to senior school. The overall integral potential hazard index for impaired biological status equaled I_{pdk} = 0.47 (average) for the schoolchildren of all ages who attended the type I school. This value was by 3.0 times higher than the upper limit of its safe level (Figure).

In the type II school, the potential hazard index associated with improper meals equaled I_{pdj} = 0.052 in primary school, I_{pdj} = 0.033 in middle school and I_{pdj} = 0.082 in senior school; its total value for all age groups reached I_{pdj} = 0.166 (negligible or low hazard). The potential hazard index associated with the factors of the educational process equaled I_{pdj} = 0.074 in primary school, I_{pd} = 0.045 in middle school and I_{pd} = 0.123 in senior school; its total value for all age groups was I_{pdj} = 0.242 (negligible or low hazard). The potential hazard index associated with anthropogenic contaminants equaled I_{pdj} = 0.005 in primary school, I_{pdj} = 0.003 in middle school and I_{pdj} = 0.008 in senior school; its total value for all age groups was I_{pdj} = 0.016 (negligible hazard). The integral potential hazard index for impaired schoolchildren's biological status under exposure to all the analyzed factors equaled I_{pdk} = 0.34 in primary school, I_{pdk} = 0.32 in middle school (low hazard); its value grew by 1.2–1.32 times in senior school and reached I_{pdk} = 0.42 (average-low hazard). The integral potential hazard index for all schoolchildren who attended the type II school equaled

The potential hazard indexes for schoolchildren's impaired biological status in different schools

Risk factors	Type I school			Type II school			Type III school			Type IV school			Type V school		
	Primary (I), middle (II) or senior (III) school														
School meals	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Proteins, g	0.06	0.05	0	0	0	0	0.04	0.03	0.05	0	0.04	0	0	0.03	0
Fats, g	0	0.04	0.07	0.07	0.07	0.07	0.03	0.03	0.04	0.1	0.03	0.07	0.10	0.05	0,07
Carbohydrates, g	0	0.04	0.06	0.05	0.06	0.06	0.04	0.04	0.05	0.1	0.04	0.06	0.10	0.05	0,06
Caloric contents, kcal	0	0.04	0.05	0.07	0.05	0.04	0.04	0.03	0.04	0.1	0.04	0.05	0.10	0	0,05
B_1, mg	0.07	0.04	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0	0.03	0.05	0	0.05	0,05
B_2, mg	0.06	0.03	0.05	0.07	0.06	0.06	0.04	0.03	0.05	0	0.05	0.05	0	0.05	0,05
C, mg	0.06	0.04	0	0	0	0	0.06	0.05	0.06	0.09	0.04	0	0.09	0.05	0
A, mg	0.08	0.05	0	0.06	0	0	0.05	0.05	0.05	0	0.07	0	0	0.03	0
Ca, mg	0.06	0.07	0.08	0	0.08	0.08	0.05	0.05	0.05	0	0.06	0.08	0	0.03	0,08
P, mg	0.06	0.03	0.05	0.07	0.05	0.05	0	0	0	0.08	0.06	0.05	0.08	0.05	0,05
Mg, mg	0.06	0	0	0	0	0	0.04	0.03	0.05	0	0.04	0	0	0.04	0
Fe, mg	0.05	0	0	0	0	0	0.05	0.04	0.03	0	0.04	0	0	0	0
Total (I_{pd})	0.54	0.42	0.41	0.43	0.41	0.41	0.46	0.42	0.49	0.46	0.53	0.41	0.46	0.43	0,41
I_{pdj} per age groups	0.065	0.033	0.082	0.052	0.033	0.082	0.054	0.033	0.098	0.055	0.042	0.082	0.055	0.034	0,082
I_{pdj} for a school as a whole	0.180			0.166			0.185			0.180			0.171		
Educational process															
A break between classes and additional studies	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.09	0.14	0.17	0.17	0.14	0.14	0.15
The longest break	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0	0	0.08	0.08	0.08	0.08	0.08	0.07
Breaks between classes	0.1	0.1	0.1	0.07	0.07	0.07	0.08	0	0	0.1	0	0	0.1	0.1	0.07
A number of students in a class, m^2	0.08	0.08	0.08	0.06	0.06	0.06	0.06	0.1	0.1	0.06	0.08	0.08	0.06	0.06	0.06
Weekly educational loads	0.08	0.06	0.07	0.08	0.06	0.07	0.06	0.08	0.08	0.07	0.09	0.08	0.07	0.07	0.06
Daily educational loads, classes	0.08	0.05	0.07	0.08	0.05	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07
Total (I_{pd})	0.58	0.52	0.55	0.49	0.45	0.49	0.50	0.38	0.34	0.53	0.49	0.48	0.53	0.51	0.47
I_{pdj} per age groups	0.087	0.052	0.123	0.074	0.045	0.123	0.068	0.038	0.085	0.080	0.049	0.120	0.080	0.051	0.012
I_{pdj} for a school as a whole	0.262			0.242			0.191			0.249			0.142		
Anthropogenic contaminants															
Phenol, mg/dm^3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01
Toluene, mg/dm^3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Benzene, mg/dm^3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Formaldehyde, mg/dm^3	0.01	0.01	0.02	0.03	0.02	0.02	0.00	0.00	0.00	0.02	0.02	0.02	0.01	0.01	0.02
Manganese, mg/dm^3	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Chromium, mg/dm^3	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Nickel, mg/dm^3	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroform, mg/dm^3	0.03	0.03	0.03	0.03	0.03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
1,2-dichloroethane, mg/dm^3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethylbenzene	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Carbon tetrachloride, mg/dm^3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lead, mg/dm^3	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total (I_{pd})	0.15	0.15	0.16	0.17	0.16	0.16	0.15	0.15	0.15	0.16	0.16	0.16	0.15	0.15	0.16
I_{pdj} as per age group	0.005	0.003	0.008	0.005	0.003	0.008	0.004	0.003	0.007	0.005	0.003	0.008	0.005	0.003	0.008
I_{pdj} for a school as a whole	0.016			0.016			0.014			0.016			0.016		
The integral I_{pdk} as per age group	0.39	0.39	0.46	0.34	0.32	0.42	0.34	0.30	0.32	0.36	0.37	0.42	0.35	0.37	0.41
Total as per age group, %	100.0	4.60 95.30	100.0	16.53 83.47	42.89 57.11	23.85 76.15	2.36 97.64	87.1 21.9	67.43 32.57	100.0	100.0	29.49 70.51	100.0	6.58 93.42	36.17 63.83
The integral (I_{pdk}) for a school as a whole	0.472			0.425			0.400			0.444			0.435		
Total as per school, %	100.0			25.46 74.54			50.05 49.95			5.63 94.37			15.2 84.8		

Note:

- negligible hazard (blue)
- low hazard (green)
- average hazard (yellow)
- high hazard (orange)
- extremely high hazard (red)

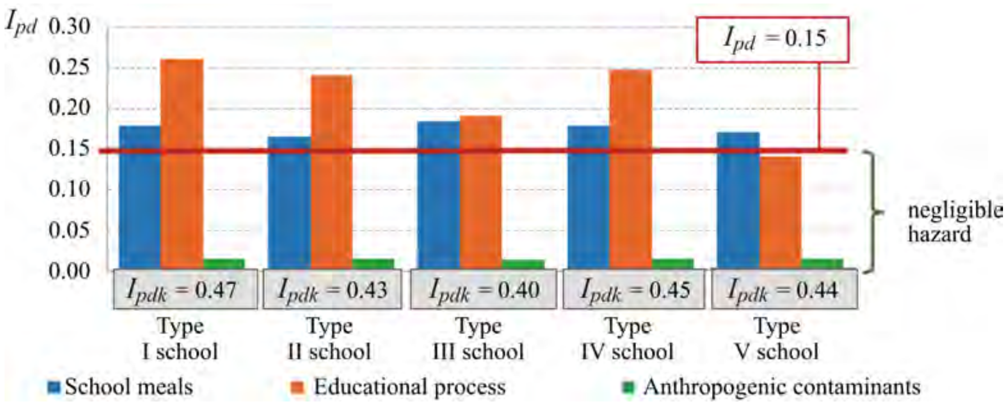

Figure. Profiles of potential hazard indexes for impaired schoolchildren's biological status under combined exposure to the set of the analyzed environmental factors ad factors related to the educational process

I_{pdk} = 0.43 (average-low hazard) and this value was by 2.9 times higher than its permissible level. In the type III school, the potential hazard index associated with improper school meals equaled I_{pdj} = 0.054 in primary school, I_{pd} = 0.033 in middle school and I_{pd} = 0.098 in senior school. Its total value for all age groups reached I_{pdj} = 0.185 (negligible – low hazard). Improper organization of the educational processes created the potential hazard index being equal to I_{pdj} = 0.068 in primary school, I_{pdj} = 0.038 in middle school and I_{pdj} = 0.085 in senior school; its total value for all age groups equaled I_{pdj} = 0.191 (negligible – low hazard). The potential hazard index associated with anthropogenic contaminants equaled I_{pdj} = 0.004 in primary school, I_{pdj} = 0.003 in middle school and I_{pdj} = 0.007 in senior school; its total value for all age groups equaled I_{pdj} = 0.014 (negligible hazard). The integral potential hazard index for impaired schoolchildren's biological status under exposure to all the analyzed factors equaled I_{pdk} = 0.34 in primary school; I_{pdk} = 0.30 in middle school and I_{pdk} = 0.32 in senior school (low hazard). The integral potential hazard index for all schoolchildren who attended the type III school equaled I_{pdk} = 0.40 (average-low hazard) and this value was by 2.7 times higher than its permissible level (I_{pd} < 0.15). In the type IV school, the potential hazard index associated with improper school meals equaled I_{pdj} = 0.055 in primary school, I_{pd} = 0.042 in middle school and I_{pdj} = 0.082 in senior school; its total value for all age groups reached I_{pdj} = 0.180 (negligible – low hazard). The potential hazard index associated with improper organization of the educational process equaled I_{pdj} = 0.080 in primary school, I_{pdj} = 0.049 in middle school and I_{pdj} = 0.120 in senior school; its total value for all age groups equaled I_{pdj} = 0.249 (negligible – low hazard). The potential hazard index associated with anthropogenic contaminants equaled I_{pdj} = 0.005 in primary school, I_{pdj} = 0.003 in middle school and I_{pdj} = 0.008 in senior school; its total value for all age groups reached I_{pdj} = 0.016 (negligible hazard). The integral potential hazard index for impaired schoolchildren's biological status under exposure to all the analyzed environmental factors and factors related to the educational processes actors equaled I_{pdk} = 0.36 and I_{pdk} = 0.37 in primary and middle school accordingly and I_{pdk} = 0.42 in senior school (average-low hazard) and therefore was unacceptable (I_{pdk} > 0.15) for all age groups growing by 1.2 times from primary to senior school. The integral potential hazard index for all schoolchildren who attended the type IV school equaled I_{pdk} = 0.45 (average hazard) and this value was by 3.0 times higher than its permissible level. In the type V school, the potential hazard index associated with improper school meals equaled I_{pdj} = 0.055 in primary school, I_{pdj} = 0.034 in middle school and I_{pdj} = 0.082 in senior school; its total value for all age groups reached I_{pdj} = 0.171 (negligible – low hazard). The potential hazard index associated with improper organization of the educational process equaled I_{pdj} = 0.080 in primary school, I_{pdj} = 0.051 in middle school and I_{pdj} = 0.012 in senior school; its total value for all age groups was I_{pdj} = 0.142 (negligible hazard). The potential hazard index associated with anthropogenic contaminants equaled I_{pdj} = 0005 in primary school, I_{pdj} = 0.003 in middle school and I_{pdj} = 0.008 in senior school; its total value for all age groups reached I_{pdj} = 0.016 (negligible hazard). The integral potential hazard index under exposure to all the analyzed environmental factors and factors related to the educational processes equaled I_{pdk} = 0.35 in primary school, I_{pdk} = 0.37 in middle school and I_{pdk} = 0.41 in senior school (average – low hazard) growing by 1.2 times from primary to senior school. The integral potential hazard index for all schoolchildren who attended the type V school equaled I_{pdk} = 0.44 (average – low hazard) and this value is by 2.9 times higher than its safe level (I_{pd} < 0.15).

Overall, the primary meal-related factors with the greatest contributions to the potential hazard index for schoolchildren of all ages include deficiency of micronutrients, especially vitamins C, B_2, A, calcium, phosphor and magnesium and excessive quantities of macronutrients (fats and carbohydrates). Among factors related to the educational process, the greatest contributions to the integral potential hazard index are made by excessive daily and weekly educational loads, shorter breaks between classes and additional education, and excessive numbers of schoolchildren in a class. Benzene, toluene, ethylbenzene and chloroform are the most significant anthropogenic contaminants. Although their effects do not exceed permissible levels (I_{pdj} = 0.15–0.16, negligible – low hazard), they make their contribution to the integral potential hazard index for schoolchildren's somatic and mental health given the combined exposure to them together with other risk-inducing factors.

We compared the obtained values of the integral potential hazard index as regards probable health disorders in schoolchildren of all age groups in all the analyzed schools with the scale sets. The comparison revealed that these values (I_{pdk} = 0.40–0.47) were by 2.7–3.1 times higher than the permissible level ($I_{pd} \leq$ 0.15). The first rank place identified as per simultaneous exposure to all the analyzed adverse risk-inducing factors belonged to the type I school with profound studies of natural sciences; the second one, ordinary secondary schools (type IV and V); the third one, type II school; the fourth one, type III school.

Conclusions:

1. Basic determinants of negative effects produced by the educational process on schoolchildren's health include elevated intensity and monotony of learning, elevated intellectual loads, shorter breaks and the recovery deficiency index; meal-related factors include excessive fats, carbohydrates and total caloric contents of meals provided at school, protein and micronutrient deficiency; primary chemical factors include elevated levels of metals, aromatic hydrocarbons, aldehydes, and chlorinated organic compounds in biological media.

2. Regardless of schoolchildren's age and a type of a school, the leading risk-inducing factors are organization of the educational process (I_{pj} = 0.45–0.58) and meals provided at school (I_{pj} = 0.41–0.54). The potential hazard index for this indicator groups reaches its peak values (I_{pj} = 0.49–0.58 and I_{pj} = 0.46–0.54 accordingly) in primary school.

3. The highest value of the integral potential hazard index (I_{pdk} = 0.41–0.46) caused by combined exposure to factors of the educational process, meal-related ones and anthropogenic contaminants is detected in senior school regardless of a type of a school.

4. Under combined exposure to environmental factors and factors related to the educational process, the highest values of the integral potential hazard index as regards schoolchildren's somatic and mental health was established for the school with profound studies of natural sciences (I_{pdk} = 0.41); the lowest value was established for the military school (I_{pdk} = 0,33).

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Research article

MYCOTOXIN CONTAMINATION OF FRESH BERRIES AND FRUITS MARKETED IN THE CENTRAL REGION OF RUSSIA

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New emerging strains of toxigenic molds in agricultural areas and insufficient data on levels of their toxic metabolites occurring in domestic horticultural fruits and berries require risk assessment of MT contamination for this plant group of mass consumer products.

This study concentrated on samples of fresh fruits and berries sold on the consumer market (185 samples, including 127 intact and 58 with signs of deformation and molding). We applied our own developed technique for quantification of mycotoxins based on HPLC-MS/MS.

In this study, we were the first in the RF to examine contamination of garden strawberries, raspberries, currants, huckleberries, blueberries, gooseberries, dogwood, plums, blackthorn, apples, pears) with 27 MT including poorly studied emergent MT (EMT), produced by Aspergillus, Penicillium, Fusarium and Alternaria.

Strawberries, gooseberries, black currants and raspberries turned out to be the most contaminated with MT; red currants, apples and pears were less contaminated. The greatest variety of MT and EMT species was found in strawberries (20 MT), gooseberries (8 MT), black currants (7 MT) and raspberries (6 MT).

Among the regulated MT, fumonisins B1 and B2, deoxynivalenol, zearalenone, T-2 toxin, ochratoxin A and aflatoxin B1 were detected in intact strawberries; patulin, in raspberries; deoxynivalenol and zearalenone, in black currant. As for damaged and moldy berries and fruits, the list of detectable toxins was expanded, primarily due to the detection of several types of unregulated EMTs. EMT tenuazonic acid was mainly detected in moldy berries; its levels increased manifold in almost all species, except for strawberries in which penicillic acid prevailed.

These new data on MT contamination in fruits and berries indicate the necessity to perform in-depth hygienic assessment of such products sold on the Russian market to identify MT, EMT and their producers. The obtained results will be used to identify hazards at the first stage in risk assessment with its focus on MT and EMT contamination of fresh fruits and berries.

Keywords: *mycotoxins, emergent mycotoxins, strawberry, raspberry, contamination, HPLC-MS/MS.*

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The Russian market for fresh fruits and berries has been growing quite steadily. According to the Russian Federal State Statistics Service (Rosstat), this year harvests of fruits and berries are higher by 8.1 % than last year ones and have reached 2.6 million tons[1]. Development of intensive gardening and implementation of technologies that allow long-term storage of harvests have enabled Russian producers to reduce dependence on imports on the Russian market. Increasing the availability of fruits and berries cultivated in our country has led to a substantial growth in their consumption.

Fruits and berries are a significant component in human diets. They are an important source of vitamins, microelements, and biologically active substances, including antioxidants; the latter protect the human body from oxidative stresses and prevent ageing. Based on the principles of healthy nutrition and the need to increase the consumption of fresh fruits and berries, the Order of the Russian Ministry of Healthcare approved the norms for the consumption of fresh fruits per person in the amount of 100 kg per year[2].

Cultivated fruits and berries, like other agricultural plants, are known to be susceptible to microbial infection and spoilage during vegetation, harvesting and storage. The major role in these processes belongs to fungi from *Fusarium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Geotrichum*, *Rhizopus*, *Botrytis*, *Cladosporium*, *Sclerotinia*, *Colletotrichum*, and *Phytophthon* genus; many of them produce dangerous mycotoxins (MTs) [1–4]. Identified fungi species can vary depending on farming or storing conditions and this involves changes in an MT spectrum [5]. In addition to well-known and controlled MTs, it is quite possible to identify toxic fungi metabolites that are currently unregulated. These emergent MTs [6–8] should be investigated and hazards posed by their occurrence in foods should be assessed. Occurrence of new and poorly explored mold strains in agricultural areas [9–11] and insufficient data on MT contamination in domestically grown fruits and berries justify the necessity to explore the nature and levels of MT and EMT contamination in this plant group of mass consumer products.

The greatest health risk caused by MTs is associated with their chronic intake with consumed foods. MT occurrence in a food chain is a key concern due to their ability to produce adverse toxic effects even when contamination levels are rather low. The sanitary legislation in the EAEU set contents of only one MT, patulin (PAT); its levels in fruits and berries (apples, tomatoes, sea-buckthorn, viburnum berries and products made of them) are regulated in the Customs Union Technical Regulation 'On food safety'[3] (CU TR 021/2011).

Growing consumption of fresh fruits and berries by the RF population can be directly linked to a growing MT burden that has not been estimated before due to, among other things, absence of available highly sensitive methods for their identification. The authors of the present study have developed methods for quantitative analysis of a wide MT range [12]; these methods made it possible to estimate MT occurrence in food grains and in some non-grain plant products including dried fruits, coffee, cocoa, tea, spices, etc., given different threshold values at levels below the limit of detection.

People are exposed to various MTs consumed with foods. It is known that MTs could

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[1] Valovoi sbor plodov, yagod, vinograda, chainogo lista i khmelya po Rossiiskoi Federatsii (po kategoriyam khozyaistv) [The gross harvest of berries, grapes, tealeaves and hops in the Russian Federation (as per categories of farms)]. *Rosstat*, 2022. Available at: https://rosstat.gov.ru/search (June 06, 2022) (in Russian).

[2] Ob utverzhdenii Rekomendatsii po ratsional'nym normam potrebleniya pishchevykh produktov, otvechayushchikh sovremennym trebovaniyam zdorovogo pitaniya: prikaz Ministerstva zdravookhraneniya Rossiiskoi Federatsii ot 19 avgusta 2016 g. № 614 (s izmeneniyami na 1 dekabrya 2020 goda) [On Approval of the Recommendations on rational standards of food products consumption that conform to the up-to-date requirements of healthy diet: the Order by the RF Public Healthcare Ministry issued on August 19, 2016 No. 614 (last edited on December 1, 2020)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/420374878 (June 06, 2022) (in Russian).

[3] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: Tekhnicheskii reglament Tamozhennogo soyuza [CU TR 021/2011. On food safety: the Customs Union Technical Regulation]. *The Eurasian Economic Commission*. Available at: http://www.eurasiancommission.org/ru/act/texnreg/deptexreg/tr/Pages/PischevayaProd.aspx (October 12, 2022) (in Russian).

produce a variety of adverse combine health effects [13–15]. Thus it is essential to carry out mycotoxins survey in fresh fruits, berries and vegetables considering all data, including low detectable levels.

In this study, we aimed to examine the nature and levels of contamination with regulated MTs, their derivatives and emergent MTs in domestically grown fruits and berries that are sold on the consumer market in the RF.

Materials and methods. Samples of fresh fruits and berries were brought from the Tambov region (Michurinskii and Morshanskii districts); some samples were bought in retail outlets in Moscow and the Moscow region. A total of 185 samples were examined (apples, pears, strawberries, raspberries, black and red currant, dogwood, gooseberries, plums, blackthorn, huckleberries and blueberries). The research objects were the sorts of fruits and berries that were the most resistant to non-quarantine diseases typical for plants cultivated in the Tambov region.

MT levels were identified with high performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS). MTs identified in fruits and berries included those regulated in plant foods such as ochratoxin A (OTA), patulin (PAT), deoxynivalenol (DON), fumonisins B1 and B2 (FB1, FB2), T-2 toxin, aflatoxin B1 (AFL B1), zearalenone (ZEN); their derivatives and structural analogues (aflatoxins B2, G1, G2, HT-2 toxin, β-zearalenol (β-ZEL). We also identified occurrence of MTs that are not currently regulated such as penicillic acid (PA), nivalenol (NIV) and emergent MTs such as sterigmatocystin (STC), mycophenolic acid (MPA), moniliformin (MO), citrinin (CIT), enniatins A and B (ENN A and ENN B), beauvericin (BEA), tenuazonic acid (TeA), tentoxin (TEN), alternariol (AOH), its methyl ether (AME) and altenuene (ALT).

Sample preparation. A sample was first powdered and mixed thoroughly; a portion weighing 10 grams was then put into a 50-ml centrifuge tube and added 10 ml of acetonitrile/water mix (80/20 % vol.) with acetic acid solution (1 % of volume). Extraction was performed during 30 minutes; first in a shaker (10 minutes), then in an ultrasound bath (10 minutes), and again in a shaker (10 minutes).

A resulting extract was centrifuged for 5 minutes at 7000 rpm. 1 cm^3 of the extract was put into an eppendorf tube and added 1 cm^3 of methanol, mixed, centrifuged again and then the supernatant was poured into chromatography vials for analysis. Each sample was analyzed in two replicates.

MTs were quantified with Waters Xevo liquid chromatography tandem mass spectrometry system with triple quadrupole mass spectrometer detector (electrospray ionization-ion trap) with the heated source. The device was controlled with MassLynx V4.2 software. The source had the following parameters: capillary voltage was 0.5 kV; cone voltage, 3 V; the source temperature, 500 ºC; desolvation temperature, 500 ºC; gas flow in the cone, 150 L/Hr; desolvation gas flow, 1000 L/Hr; collision gas flow, 0.15 cm^3/min; nebulizer pressure, 7 Bar. Analytes were divided onto a column filled with silica gel that used octadecylsilane as its stationary phase (Zorbax SB-C18, 150 × 4.6 mm, 3.5 µm, pore size was 80 Å, and carbon share was 10 %, Agilent). The column temperature was 30 °C; the autosampler temperature, 4 °C. The eluent flow rate was 0.5 cm^3/min. The injection volume was 10 mm^3. Table 1 provides data on parameters of MT detection within Selected Reaction Monitoring (parent and daughter ions, fragmentor voltage and collision energy).

The following mobile phases were used in the analysis: (A), water/methanol (95/5 % vol.); (B), methanol/water (5/95 % vol.); both phases were modified with 10 мM of ammonium acetate. The gradient scheme in positive polarity was as follows: the start at 10 % B; the 7th minute, 75 % B; the 17th–19th minutes, 100 % B; the column equilibration from 19.5th to 24th minute at 10 % B. The gradient scheme in negative polarity was as follows: the start at 0 % B; 1 minute, 0 % B; the 7th minute, 70 % B; the 15th–17th minute, 100 % B; the column equilibration from 19.5 to 22nd minute at 0 % B.

Table 1

MT detection parameters for HPLC-MS/MS

Analyte	Parent ion, m/z	Daughter ions*, m/z	Fragmentor voltage, V	Collision energy, V	Retention time, min
Negative polarity					
AME	271.112 $[M-H]^-$	**255.974**	74	22	15.7
		182.992	74	48	
		255.974	74	22	
PA	169.062 $[M-H]^-$	**109.982**	14	8	8.82
		92.951	14	14	
		125.059	14	8	
ZEN	317.445 $[M-H]^-$	**130.915**	82	28	14.75
		174.979	82	24	
		149.045	82	22	
CIT	281.180 $[M+CH_3OH-H]^-$	**249.073**	2	18	14.43
		205.077	2	26	
		130.063	2	36	
β-ZEL	313.232 $[M-H]^-$	**174.120**	90	24	13.17
		187.902	90	26	
		130.06	90	34	
TeA	196.217 $[M-H]^-$	**138.996**	54	18	13.73
		111.951	54	24	
MO	96.998 $[M-H]^-$	**40.913**	4	26	3.00
		68.938	4	24	
AOH	257.273 $[M-H]^-$	**146.978**	2	32	12.79
		212.993	2	22	
		185.006	2	28	
PAT	153.117 $[M-H]^-$	**80.941**	16	12	7.43
		52.976	16	14	
		108.968	16	10	
TEN	413.609 $[M-H]^-$	**271.176**	2	16	13.00
		141.038	2	20	
		108.968	2	22	
NIV	371.268 $[M-CH_3COO]^-$	**281.153**	12	14	7.17
		311.167	12	10	
		191.033	12	24	
Positive polarity					
PA	170.955 $[M+H]^+$	**125.000**	12	12	7.51
		97.050	12	16	
CIT	251.111 $[M+H]^+$	**233.129**	52	16	9.58
		205.060	52	26	
		191.025	52	24	
AME	273.070 $[M+H]^+$	**184.162**	44	36	15.16
		258.123	44	26	
ALT	293.202 $[M+H]^+$	**239.124**	2	20	10.24
		257.126	2	14	
DON	297.174 $[M+H]^+$	**249.138**	16	10	6.92
		231.135	16	12	
		203.126	16	42	
AFL B1	313.047 $[M+H]^+$	**241.080**	56	36	10.44
		284.948	56	22	
		213.132	56	42	
AFL B2	315.130 $[M+H]^+$	**287.104**	80	24	10.23
		259.039	80	28	
		243.120	80	36	

End of the table 1

Analyte	Parent ion, m/z	Daughter ions*, m/z	Fragmentor voltage, V	Collision energy, V	Retention time, min
MPA	321.306 [M+H]+	**207.129**	4	24	10.65
		159.046	4	32	
		102.965	4	42	
STC	325.140 [M+H]+	**310.033**	82	22	15.05
		253.122	82	42	
		196.927	82	50	
AFL G1	329.110 [M+H]+	**243.058**	72	24	9.72
		199.793	72	40	
		283.014	72	24	
AFL G2	331.1304 [M+H]+	**189.067**	22	38	9.53
		245.075	22	30	
		217.009	22	34	
OTA	404.1298 [M+H]+	**189.067**	2	22	10.40
		245.075	2	62	
		217.008	2	34	
TEN	415.4022 [M+H]+	**256.194**	60	28	12.06
		302.257	60	12	
		132.081	60	40	
HT-2	442.3404 $[M+NH_4]^+$	**215.114**	16	12	11.56
		302.257	16	6	
		263.188	16	12	
T-2	484.396 $[M+NH_4]^+$	**305.216**	54	12	12.54
		245.191	54	10	
		215.113	54	18	
ENN B	640.666 [M+H]+	**196.140**	70	24	19.76
		214.200	70	24	
		186.200	70	38	
ENN A	682.730 [M+H]+	**210.175**	90	22	21.91
		228.171	90	24	
		200.171	90	46	
FB1	722.634 [M+H]+	**352.430**	18	34	10.03
		334.374	18	40	
FB2	706.638 [M+H]+	**336.447**	10	36	12.29
		318.391	10	38	
		354.440	10	32	
BEA	784.730 [M+H]+	**134.089**	82	62	20.31
		244.217	82	28	
		262.213	82	22	

Note: * means that daughter ions used for MT quantification are given in **bold**; the rest are used for qualitative confirmation.

Standard solutions of 27 MTs were prepared from dried standards (Sigma-Aldrich; Fermentek, Jerusalem, Israel). Stock standards were prepared in acetonitrile (AFL, STC, CIT, trichothecenes of groups A and B, ZEN and its analogues, OTA, PA, PAT), methanol (*Alternaria* toxins, ENN A, ENN B, BEA, MO, MPA) or in an 'acetonitrile/water' mixture (50/50 % vol.) as it was the case with FB1, FB2 with a concentration equal to 100 or 500 μg/mm^3. Standard solutions were used to make a multi-standard and calibration solutions. All solutions were stored at -18 °C.

To quantify MTs, external calibrations on a 'clean' matrix were applied. 'Positive' samples were divided into two sub-groups; the first one included samples with MTs levels being higher than the limit of detection (LOD) but lower than the limit of quantification (LOQ); the second one was made of samples that contained MTs in concentrations being higher than LOQ. LOD and LOQ were calculated as per 3-σ and 10-σ criteria. MT recovery varied between 60 and 120 %.

The obtained data were statistically analyzed with IBM SPSS Statistics 23 (Statistical package for social sciences, USA) and Microsoft Office Excel 2007 (Microsoft Corp., USA). Data on MT content in total samples were given as arithmetic mean (mean) and 90-th percentile (90 %); contamination levels lower than the limit of quantification were taken as equal to 0. Data on MT levels in contaminated samples were given as a range of MT levels (range) and arithmetic mean (mean).

Results and discussion. We examined MT occurrence and levels of MT contamination in 185 samples of fresh fruits and berries. The structure and levels of identified MT contamination varied significantly depending on a culture. Strawberries, black currant and raspberries turned out to be the most contaminated with MTs among berries.

Table 2 provides data on MT occurrence and levels in the examined fruits and berries. Twenty three out of 27 analyzed MTs were identified in **strawberries**; some of them (CIT, ENN A and ZEN) were detected in trace quantities. PA prevailed in strawberries (53 % of the cases), followed by FB2 (43 %), AFL G2 (30 %) and NIV (25 %). Average levels in samples contaminated with these MTs reached 28–69 μg/kg (for PA and NIV). FB1 (18 % of the cases), AME (7.5 %) and BEA (5.0 %) were identified less frequently. Some samples were contaminated with fusariotoxins DON, ZEN, β-ZEL, ENN A and B and *Alternaria* toxins ALT, TEN and AOH though contamination levels were not high for them. TeA was not identified in any strawberry samples. C. Juan et al. [16] reported low levels of contamination with toxins produced by *Alternaria* fungi in strawberries.

MTs were less frequently identified in **raspberries** as compared with strawberries. PAT was the most frequent contaminant; 23 % out of 13 analyzed samples contained PAT in quantities between 5.82 and 7.29 μg/kg (Table 2) and this was by several times lower than the maximum permissible level (MPL) established for this toxin regarding several sorts of berries and fruits. Two raspberry samples (15 %) were contaminated with TEN in low quantities; NIV was identified with the same frequency (its average level was 5.4 μg/kg). *Alternaria* toxins AOH, ALT and TeA were identified in some samples; the latter has the highest acute toxicity in comparison with other *Alternaria* toxins [17, 18].

Table 2

MT occurrence and levels in fresh fruits and berries

Toxin	MT occurrence, %		MT content in total samples, μg/kg		MT levels in contaminated samples, μg/kg	
	total, including	higher than LOQ	average	90 %	range	average
Strawberries (40 samples)						
PA	52.5	50.0	13.89	42.09	1.31–131.81	27.78
FB2	42.5	32.5	0.77	2.10	1.50–5.10	2.37
AFL G2	30.0	30.0	0.76	1.28	0.25–20.0	2.54
NIV	25.0	25.0	17.24	62.74	28.67–200.90	68.98
FB1	17.5	7.5	2.12	0	4.60–66.00	21.24
AME	17.5	7.5	0.17	0	1.81–2.61	2.28
BEA	17.5	5.0	0.10	0	0.53–3.51	2.02
T-2	15.0	15.0	0.55	1.42	0.97–7.46	3.64
HT-2	12.5	12.5	0.649	0.91	2.25–19.92	5.16
ENN B	12.5	2.5	0.01	0	0.52	0.52
ENN A	12.5	0	< 0.5	< 0.5	< 0.5	< 0.5
ALT	7.5	7.5	0.32	0	0.72–10.71	4.24

End of the table 2

Toxin	MT occurrence, %		MT content in total samples, µg/kg		MT levels in contaminated samples, µg/kg	
	total, including	higher than LOQ	average	90 %	range	average
DON	5.0	5.0	2.51	0	2.44–98.09	50.26
AFL B2	5.0	5.0	0.33	0	0.23–12.91	6.57
MPA	5.0	2.5	0.01	0	0.36	0.36
TEN	5.0	5.0	0.25	0	0.57–9.44	5.01
OTA	5.0	2.5	0.16	0	6.55	6.55
AOH	2.5	2.5	0.03	0	1.37	1.37
β-ZEL	2.5	2.5	0.25	0	9.83	9.83
AFL G1	2.5	2.5	0.14	0	5.69	5.69
AFL B1	2.5	2.5	0.08	0	3.37	3.37
CIT	2.5	0	< 0.5	< 0.5	< 0.5	< 0.5
ZEN	2.5	0	< 1.0	< 1.0	< 1.0	< 1.0
Raspberries (13 samples)						
PAT	23.1	23.1	1.46	5.90	5.82–7.29	6.34
TEN	15.4	15.4	0.03	0.16	0.16–0.21	0.18
NIV	15.0	9.0	0.83	< 3.0	3.00–7.85	5.42
TeA	7.7	7.7	1.66	< 12.5	21.59	21.59
ALT	7.7	7.7	0.08	< 0.5	1.08	1.08
AOH	7.7	0	< 0.2	< 0.2	< 0.2	< 0.2
Black currant (14 samples)						
DON	100	100	22.02	58.9	0.53–92.5	22.02
TEN	28.5	21.4	0.49	0.64	0.45–5.70	2.26
AOH	14.3	14.3	0.26	1.10	1.10–2.60	1.85
TeA	7.1	7.1	0.94	< 5.0	13.12	13.12
ZEN	7.1	7.1	0.15	< 0.3	0.9	0.9
HT-2	7.1	0	< 0.5	< 0.5	< 0.5	< 0.5
MPA	7.1	0	< 0.2	< 0.2	< 0.2	< 0.2
Red currant (12 samples)						
HT-2	16.6	8.3	0.26	1.59	3.17	3.17
AOH	8.3	8.3	0.10	< 0.4	1.15	1.15
TeA	16.6	8.3	4.02	< 4.0	48.2	48.2
STC	8.3	0	< 0.2	< 0.2	< 0.2	< 0.2
Gooseberries (13 samples)						
TEN	30.8	30.8	0.09	0.38	0.16–0.42	0.31
DON	30.8	23.1	2.63	10.29	8.25–15.64	11.39
HT-2	15.4	15.4	0.34	1.78	1.78–2.63	2.21
AOH	7.7	7.7	0.07	< 0.5	0.93	0.93
T-2	15.4	0	< 1.0	< 1.0	< 1.0	< 1.0
FB2	7.7	0	< 7.0	< 7.0	< 7.0	< 7.0
AFL G1	7.7	0	< 0.2	< 0.2	< 0.2	< 0.2
TeA	7.7	0	< 25.0	< 25.0	< 25.0	< 25.0
Blueberries (2 samples)						
AOH	100	100	12.1	22.5	1.70–22.50	12.1
AME	50	50	1.75	1.75	3.50	3.50
Dogwood (6 samples)						
NIV	50	17	55.70	< 20.0	305.09	305.09
AME	17	17	0.7	< 0.7	2.29	2.29
Plum and blackthorn (4 samples)						
MO	100	100	105.31	149.9	79.50–149.95	105.31
Apples (17 samples)						
TEN	12	12	0.07	< 0.5	0.56–0.69	0.62

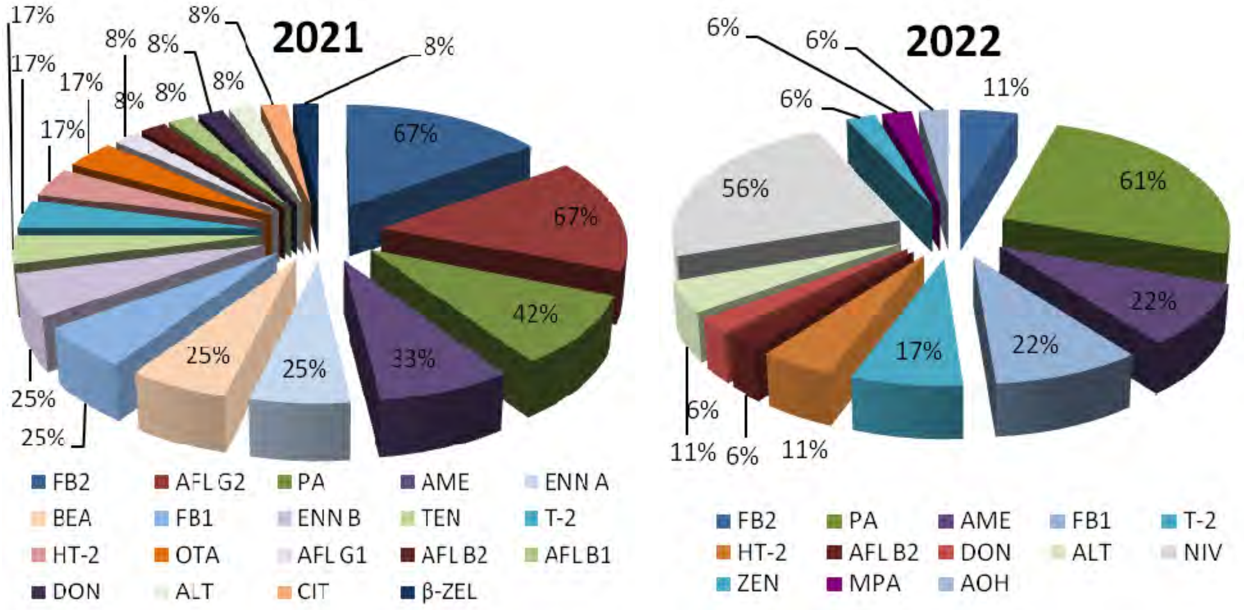


Figure 1. MT occurrence in strawberries grown in 2021 and 2022

Currant and dogwood samples were mostly contaminated with metabolites of *Alternaria* and *Fusarium* fungi. All the **black currant** samples contained DON though its levels were not high and varied between 0.5 and 92.5 µg/kg; still, this toxin is known to be able to damage genome even in the lowest concentrations. Emergent TEN, AOH and TeA were identified in half of the analyzed samples. **Red currant**, as opposed to black one, was contaminated with MTs much less frequently. Some samples were contaminated with HT-2, AOH and TeA. NIV was identified in 3 out of 6 **dogwood** samples (in one case its level was higher than 300 µg/kg); AME was another toxin identified in these berries. Eight out of 27 analyzed MTs were identified in **gooseberry** samples. Among them, TEN and DON occurred most frequently as they were identified in 31 % of the cases. **Blueberry** samples contained *Alternaria* toxins AOH and AME. MO was identified in all the **plum** and **blackthorn** samples with its average level being 100 µg/kg.

This study revealed that MT contamination was the lowest in apples and pears. Only TEN was identified in 12 % of **apple** samples and its levels were low; MTs were not detected in the analyzed **pear** samples.

We estimated a spectrum of MTs identified in strawberries. The results indicate that the greatest contribution to contamination in these products is made by toxicogenic microfungi from *Fusarium* genus and, to a lesser extent, by *Aspergillus* sp. and *Penicillium* sp. We analyzed taxonomy of molds identified in fruits and berries and compared the data with profiles of identified MTs. The results indicate there is high prevalence of MT producers from *Alternaria* genus in raspberry, gooseberry, currant, and plums (unpublished data).

We comparatively analyzed contamination of strawberries harvested in 2021 and 2022 to estimate influence exerted by climatic and seasonal factors on MT accumulation in berries. Strawberries harvested in 2021 contained 19 MTs (Figure 1); MT produced by 'storage fungi' prevailed among the identified ones, first of all, AFL G2, as well as some other AFL, OTA, emergent fusariotoxins ENN A and B and BEA. Only 13 MTs were identified in samples harvested in 2022; PA occurred by 20 % more frequently than in the previous year and in contrast AFL occurred much less frequently and no emergent fusariotoxins were identified. NIV was identified the most frequently among other fusariotoxins. Less diverse MTs were likely due to warmer weather (as compared with the previous year) in the Central region in Russia during a period when berries grew and ripened, harvesting included.

A significant number of the analyzed fruit and berry samples were simultaneously contaminated with several MTs: 75 % of strawberry samples, 50 % of black currant samples, 38 % of gooseberry samples, 23 % of raspberry samples and 8 % of currant samples contained more than one MT.

AFL G2+PA+FB2, PA+FB2, PA+FB2 and PA+AFL G2 were identified in **strawberry** samples. The remaining combinations included one or several of the above-listed MTs and fusariotoxins FB1, T-2, HT-2, NIV, ENN and BEA. *Alternaria* toxins TEN, AME and ALT occurred much less frequently. We should note that one of 40 analyzed strawberry samples contained 13 MTs including AFL B1 and OTA (6.55 µg/kg, which was higher than the hygienic standard established for OTA in other types of plant products) as well as several fusariotoxins including DON and FB1+FB2 (98.09 µg/kg and 66.00 µg/kg accordingly), MPA and CIT (in trace quantities). AFL contamination in strawberries was also reported by T. Klapec et al., 2022, who identified several AFL (except AFL B1) in 70 % of the analyzed samples; the maximum contamination level reached 3.185 µg/kg [19].

High levels of contamination with fusariotoxins in intact strawberry samples can partially occur due to the fact that phytopathogenic molds, while damaging the root system, are still able to penetrate other parts of a plant. As a result, MTs synthesized by them can be identified in berries and the process does not always involve any obvious spoilage. Other researchers also described cases when MTs were identified in berries, fruits and vegetables without any visible spoilage signs [20]. The study that focused on asparagus revealed that MTs were able to migrate from soils into edible parts of a plant through the root system [21].

PAT, NIV and TeA, metabolites of *Penicillium sp.*, *Fusarium sp.* and *Alternaria sp.*, were simultaneously identified in 23 % of **raspberry** samples. **Currant** samples were contaminated with various combinations of *Alternaria* toxins and fusariotoxins in 50 % of the cases. DON+TEN with AOH or without it were identified in black currant samples (29 %); DON+AOH and DON+TeA also occurred in these berries (7 %).

In addition, we compared MT contamination in visually intact berries and fruits and in the same sorts with visible damage and spoilage. The analyzed samples were divided into two groups: without visible damage and spoilage (127 samples) and with some signs of mechanical damage and/or molding (58 samples). When analyzing strawberries, we estimated an additional sub-group from which we had preliminarily excluded berried with visible spoilage in addition to a sub-group that contained intact berries (Table 3).

Higher levels of MT contamination in moldy and damaged samples against intact ones were typical for all the analyzed berries and fruits (Figure 2).

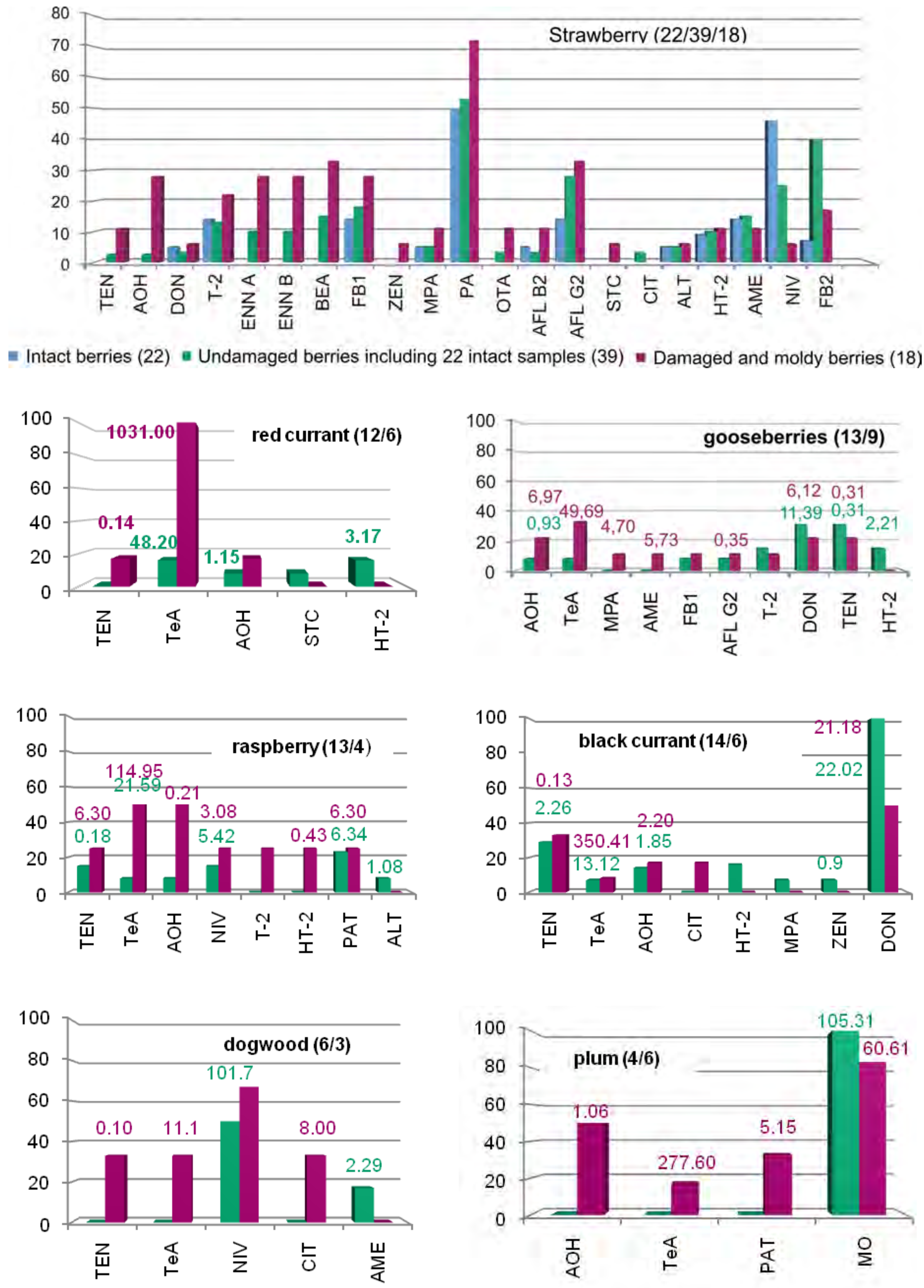
Fusariotoxins prevailed in intact strawberries; basic contaminants in this culture included NIV (46 % of the samples, the average level in contaminated samples was 68.98 µg/kg), FB2 (18 %, 1.75 µg/kg), T-2 and FB1 (14 %, 2.03 and 9.18 µg/kg accordingly), HT-2 (9 %, 1.10 µg/kg) and DON (5 %, 2.44 µg/kg). The samples were also contaminated

Table 3

Analyzed berries and fruits

Berries and fruits	Intact samples	With visible spoilage	Berries and fruits	Intact samples	With visible spoilage
Strawberries	22 + 17*	18	Raspberries	13	4
Apples	17	3	Dogwood	6	3
Black currant	14	6	Pears	5	3
Red currant	12	6	Plums, blackthorn	4	6
Gooseberries	13	9	Blueberries and huckleberries	3	0

Note: * means mixed samples with preliminarily removed damaged and moldy berries.



– intact berries and fruits, – berries and fruits with visible spoilage

Figure 2. MT contamination in intact and damaged berries and fruits

with MTs produced by 'storage fungi' *Penicillium* and *Aspergillus*: PA (50 %, 40.34 μg/kg), AFL G2 (14 %, 1.08 μg/kg), AFL B2 (5 %, 0.23 μg/kg) and MPA (5 %, 0.36 μg/kg); metabolites of *Alternaria* were less frequent: we identified AME (14 %, 2.51 μg/kg) and ALT (5 %, 1.18 μg/kg). The ratio between these three MT groups was as follows: 53 % (fusariotoxins) : 37 % (MT produced by 'storage fungi') : 10 % (*Alternaria* toxins).

An extended sampling was made of samples without visible damage but also included samples that contacted damaged and moldy berries (39 samples overall). We identified up to 20 different MTs in it. Additionally, OTA and STC were detected as well as such emergent MTs as ENN A and B, BEA, CIT, AOH and TEN. FB1 and FB2 prevailed among fusariotoxins (40 % and 18 % accordingly), followed by NIV (25 %) and BEA (15 %). It is noteworthy that NIV and DON occurred only in intact berries whereas EMTs (ENN A, ENN B and BEA), on the contrary, were identified in samples that contained berried of heterogeneous quality. The ratio between three MT groups remained practically the same: 54 % (fusariotoxins) : 36 % (MT produced by 'storage fungi') : 10 % (*Alternaria* toxins).

Twenty MTs were also identified in damaged and moldy strawberries (18 samples) including ZEN that did not occur in other subgroups of strawberry samples. Among other fusariotoxins, we identified greater frequency and higher levels of contamination with fumonisins, T-2 toxin, HT-2 toxin, BEA, ENN A and B. There was an increase in occurrence and levels identified for all the MTs produced by 'storage fungi' as well as a number of samples that were contaminated with *Alternaria* toxins.

It is noteworthy that tenuazonic acid (TeA), an emergent MT produced by *Alternaria* spp., was identified practically in all spoilt berries and fruits, excluding strawberry where penicillic acid (PA) occurred more frequently (in 72 % of the samples). The highest TeA levels were identified in moldy currant; all the currant samples were contaminated with this MT. The average TeA level equaled 1031 μg/kg in red currant (by 20 times higher than in intact samples); the level grew by more than 5 times in moldy raspberry and gooseberry and by 2.5 times in moldy black currant as opposed to intact samples (up to 350 μg/kg). Occurrence of TeA reached 50–100 % in these product groups whereas there were very few cases when it was identified in intact berries and fruits.

PAT detection in raspberry, plum and blackthorn, CIT in dogwood, AOH, AFL G2 and MPA in black currant also indicate the products were spoilt. T-2 toxin, HT-2 toxin and ENN A more frequently occurred in moldy raspberry samples; there was also more frequent contamination with *Alternaria* toxins TEN and AOH (together with TeA) whereas its levels remains relatively low. Quality of berries had practically no effects on occurrence and levels of contamination with PAT and NIV. We identified DON and emergent TEN, AOH, TeA and CIT in damaged and moldy black currant; the latter was identified only in poor quality berries.

Moldy and damaged gooseberry samples contained EMT much more frequently: AOH levels grew by 7 times and we also identified AME and MPA. NIV, CIT, TEN and TeA were identified in damaged and moldy dogwood berries. NIV occurred in such samples more frequently than in intact ones. Only poor quality dogwood turned out to be contaminated with CIT and TeA in quantities 8.0 and 11.1 μg/kg accordingly.

All the plum samples, both intact and moldy ones, contained EMT MO; AOH, PAT and TeA were identified only in moldy plums in quantities 1.06, 5.15 and 277.6 μg/kg accordingly. The data on PAT detection in plums which we obtained in this study are in line with the results obtained by N.H. Aziz et al., who found this toxin in 4 out of 10 analyzed samples at levels between 180 and 200 μg/kg [22]. It is worth mentioning that T-2 toxin was identified in one out of three moldy apple samples in quantity 134 μg/kg. This is higher than the hygienic standards for this toxin contents in some plant products.

Conclusions:

1. HPLC-MS/MS method for detection of mycotoxins (MTs and EMTs) in fruits and berries was developed. Conditions for chroma-

tographic separation of 27 analytes including poorly explored TeA and PA were optimized. Sample preparation procedure provided sufficient recoveries (over 60 %). Limits of detection and quantification were estimated.

2. This study was the first in the Russian Federation to concentrate on examining most popular fruits and berries among the mass consumer foods to identify a wide range of 27 MTs in them. Strawberries, gooseberries, black currants and raspberries turned out to be the most contaminated with MT; red currants, apples and pears were less contaminated. Each type of berries and fruits had its own typical contaminants; thus, PA, AFL G2 and FB1 prevailed in strawberries, MO in plums and blackthorn and DON in black currant.

3. Data obtained in this research indicate occurrence of not only regulated MTs in fruits and berries (T-2, FB1, FB2, DON, ZEN, OTA and AFL B1 in strawberry, PAT in raspberry, DON in gooseberry and black currant) but also their derivatives (AFL B2, G1, G2, STC in strawberry; NIV in dogwood and raspberry; HT-2 in gooseberry and red currant) and poorly explored EMTs (PA, ALT, TEN, MPA, ENN B, BEA and AOH in strawberry; TeA and TEN in black currant, raspberry and gooseberry AOH and AME in blueberry; MO in plums).

4. From MTs, TeA prevailed in the most of berries and fruits with visible spoilage excluding strawberry, apples and pears; PA prevailed in spoilt strawberry. The contamination levels of MT were by several times higher in spoilt and damaged berries and fruits against intact ones. The detection of PAT in plums and raspberry and CIT in black currant and dogwood as well as STC, OTA, ZEN and AFL B2 in strawberries also confirmed their fungal invasion.

5. The data obtained on the nature and levels of contamination of MT and EMT in fresh fruits and berries indicate the necessity to perform hygienic assessment of fruits and berries sold on the Russian market. This assessment should cover not only regulated PAT and AFL but also emergent mycotoxins and their producers. To calculate contribution made to intake with food by the most typical MTs and EMTs in such products, it is advisable to perform in-depth survey of penicillic acid, aflatoxins and fumonisin B1 contamination of strawberry and tenuazonic acid in raspberries, current, gooseberries and plums.

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Competing interests. The authors declare no competing interests.

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Research article

PUBLIC HEALTH RISKS CAUSED BY CONTAMINATION OF LOCAL FOOD PRODUCTS

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Food safety is a major concern around the world due to toxic metal contamination in food and associated health risks. Vegetables, cereals and baked goods make up a large part of a healthy human diet as vital sources of nutrients, minerals and fiber. Long-term intake of metals with food facilitates their accumulation in the human body.

The study aimed to assess health risks for adults and children caused by alimentary intake of chemical elements with local food products.

The study was conducted in the Republic of Bashkortostan, which is a territory with a developed agricultural and industrial complex. A total of 524 plant samples were selected and analyzed to identify levels of lead, cadmium, copper, zinc, iron, nickel and aluminum in them by using atomic absorption analysis. Samples included bakery and cereal products sold in the republic and vegetable products from private farms.

The article describes the results of hygienic assessment with its focus on contents of essential and toxic elements in locally produced foods. Intake of contaminants with local food products was established based on the data on average food consumption per capita. We determined contributions made by two groups of traditional mass-consumption products to the total exposure that influences health of people living in different districts across the republic. The study established the total hazard quotient of non-carcinogenic effects to be higher than its threshold vale and to equal 3.43 for children and 1.54 for adults. The greatest contributions to the total hazard quotients were made by copper (45 %) and cadmium (30 %). Our assessment of carcinogenic risks caused by intake of contaminants with foods revealed that the total health risk as per the median value corresponded to its permissible level.

Keywords: *risk assessment, metals, safety, quality, diet, vegetables, cereals, bakery products.*

Research on healthy diets clearly shows that they have considerable influence on people's health and quality of their life by providing the body with all the necessary nutrients. It is our diet that provides our bodies with enough energy to synthesize hormones, enzymes and vitamins and to recover cells and tissues properly [1]. Health of each individual as well as a nation as a whole depends on food quality and quantity. Food safety is a major issue in health protection [2–4].

Heavy metals are commonly recognized as food contaminants both on the global and regional scale [5]. Chemicals from this group are characterized with high prevalence and great ability to migrate in environmental objects. Some of them (cadmium, lead) are hazardous due to their high biological activity and

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toxic properties. Long-term intake of these elements even in low doses can result in impaired functioning of the cardiovascular, nervous and immune systems; it can cause factional disorders of the kidneys and liver and developing oncologic diseases. Some specific elements such as iron, copper, chromium, and zinc have a key role in maintaining proper functioning of the body and act as micronutrients whereas their excessive intake can affect metabolism [6].

Some studies have shown that traditional mass consumption products (cereals, bakery, milk products, vegetables, and potatoes) make the most considerable contributions to health risks caused by toxic elements in them [7–9]. Chemicals are usually contained in plant products in their ion form or are bound to a plant protein and pectin. Their levels are influenced by soil-related or geological natural factors as well as anthropogenic ones such as usage of fertilizers, industrial pollution and motor transport. It is rather difficult to reduce levels of toxic microelements in food products without changing their nutritional value since metal protein complexes are stable compounds; given that, it is especially vital to prevent food contamination [10, 11].

Researchers primarily pay attention to issues related to chemicals being dispersed at mining operation sites or contamination with heavy metals identified in agricultural soils [12, 13]. Much less attention has been given to burdens on public health created by contamination in locally produced agricultural foods, especially those produced on private farms; such products are often neglected in studies with their focus on food safety indicators. This aspect of the outlined problem is important due to growing consumption of vegetables and melons by the population in the Russian Federation [14], absence of standards on permissible levels of chemicals in foods that are priority contaminants in some regions and the system for monitoring of food quality and safety still being underdeveloped at the moment.

All the aforementioned gave grounds for conducting a study in the Republic of Bashkortostan, a leading industrial and agricultural region in Russia. A peculiar feature of the republican economy is that some oil-processing, chemical, mining and metallurgical enterprises are located in zones with developed agriculture; chemization of agriculture makes an additional contribution to technogenic dispersion of contaminants.

In this study, our aim was to assess health risks for adults and children caused by alimentary intake of chemical elements with food products produced locally in the Republic of Bashkortostan.

Materials and methods. To estimate levels of micronutrients in foods, we identified seven chemicals in bakery products and cereals (N = 228) sold in Bashkortostan and in plant products (N = 296) grown by locals on private farms. Levels of elements were identified by using the atomic absorption procedure with plasma and electrothermal atomization on Varian SpectrAA, models 240FS and 240Z (Australia).

Plant samples included open field potatoes, carrots and table beet; these vegetables were more eligible to examine contamination in them than, for example, greenhouse ones. Root vegetables were sampled directly at places where they were grown. To obtain one combined sample, not less than 8 to 10 spot samples of plant products were taken at different places within one selected land spot. Territories were selected for analysis considering specific economic activities performed there: the western part had oil and gas fields (5 districts); the south-eastern part, ore mines and ferrous and non-ferrous metallurgy (4 districts); the central part had both industry and agriculture (5 districts); and the northern part was the least industrialized (4 districts).

Daily alimentary intakes were calculated using data on average per capita consumption of basic food products in 2021 provided by the Federal State Statistics Service as well as data obtained by a survey that focused on actual diets consumed by children aged 7–11 years; the survey was conducted by round-the-clock diet tracking.

We assessed health risks caused by alimentary intake of contaminants and influence exerted on public health by the calculated exposure to chemical contaminants in food products in accordance with the Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals[1]. Risks of developing non-carcinogenic effects were estimated by calculating hazard quotients (HQ) and hazard indexes (HI). Risks of carcinogenic effects involved calculating individual carcinogenic risks (CR). HQs were calculated allowing for a median dose.

All the results were statistically analyzed with SPSS Statistics 21.0. The Kolmogorov – Smirnov test was applied to check normalcy of distribution in the analyzed groups. To estimate whether intergroup differences were significant, we took the non-parametric Kruskal – Wallis test for three or more groups and the non-parametric Mann – Whitney test for two groups. The data were given as the median with the 25th and 75th percentiles. Differences were considered significant at $p < 0.05$.

Results and discussion. Lead, mercury, cadmium and arsenic are considered toxic elements that are subject to hygienic control in food raw materials and food products. These aforementioned heavy metals are widely spread contaminants that occur in food raw materials and food products and affect human health [15]. Nutritional value of food products is no less important than their safety, in particular, levels of other microelements in them. Essential and conditionally essential elements, if taken in excessive quantities, may also induce substantial changes in health and adaptive capabilities of the body [16, 17].

Levels of chemicals in vegetables, cereals and bakery products are given in Figure 1.

Comparative analysis with the Kruskal – Wallis test revealed statistically significant differences between various vegetables as per levels of cadmium (H = 12.1; $p = 0.002$), iron (H = 16.8; $p = 0.0001$), zinc (H = 61.1; $p = 0.0001$), copper (H = 86.9; $p = 0.0001$) and aluminum (H = 16.8; $p = 0.0001$).

The highest levels of copper (Me = 0.84 mg/kg (0.64; 1.1)), zinc (Me = 3.3 mg/kg (2.6; 4.5)), cadmium (Me = 0.016 mg/kg (0.008; 0.028)) were identified in table beetroot; iron, in potatoes (Me = 4.3 mg/kg (3.2; 5.0)); aluminum, in carrots (Me = 0.51 mg/kg (0.28; 0.97)). We did not detect any statistically significant differences between lead and nickel levels in various root vegetables ($p > 0.05$). Median contents of lead and cadmium in vegetables did not exceed maximum permissible levels. Still, single samples contained cadmium in levels higher than the existing hygienic standards. The highest lead contamination was identified in table beet (0.16 mg/kg, the permissible level being 0.03 mg/kg).

Statistical analysis of two independent product groups (cereals and bakery products) with the Mann – Whitney test established significant differences as per levels of copper (U = 179; $p = 0.0001$), zinc (U = 271; $p = 0.002$), nickel (U = 247; $p = 0.002$), cadmium (U = 4361; $p = 0.018$) and lead (U = 7074; $p = 0.0001$). We did not detect any statistically significant differences in aluminum and iron levels ($p > 0.05$). The highest levels of zinc were identified in whole grain oat flakes (34 mg/kg); copper, in buckwheat cereal (5.7 mg/kg). The median levels of lead and cadmium did not exceed their maximum permissible levels. The highest lead contents were identified in bakery products (Me = 0.015 mg/kg (0.006; 0.029)); cadmium, in cereals (Me = 0.009 mg/kg (0.005; 0.013)).

We analyzed chemical contamination comparatively in different districts in the republic and established certain peculiarities typical for each of them. The established differences were statistically significant and were identified as per levels of iron (H = 24.0; $p = 0.0001$), copper (H = 15.7; $p = 0.001$), zinc (H = 12.8; $p = 0.005$), aluminum (H = 26.7; $p = 0.0001$), cadmium (H = 31.2; $p = 0.0001$) and lead (H = 99.9; $p = 0.0001$). Figure 2 provides the results.

[1] R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals (approved and validated by G.G. Onishchenko, the RF Chief Sanitary Inspector, the First Deputy to the RF Public Healthcare Minister on March 5, 2004). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200037399 (June 12, 2022) (in Russian).

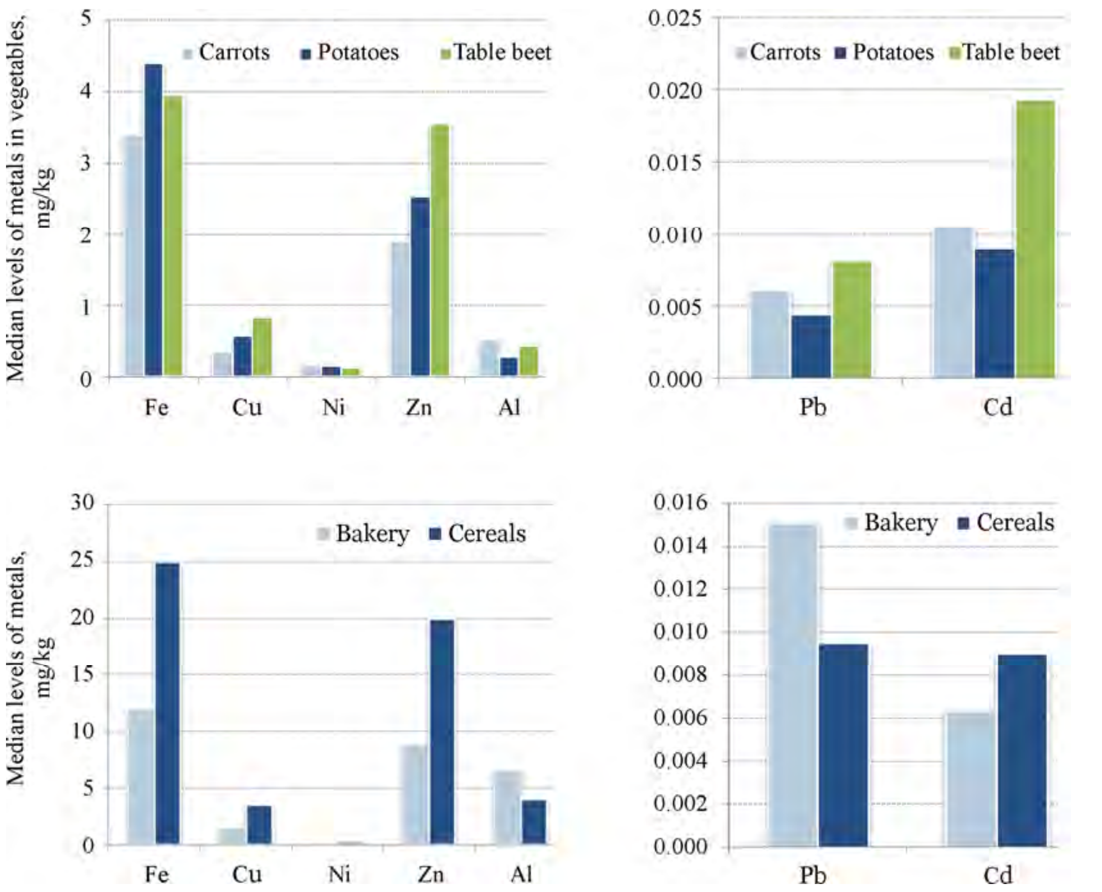

Figure 1. Microelements in food products

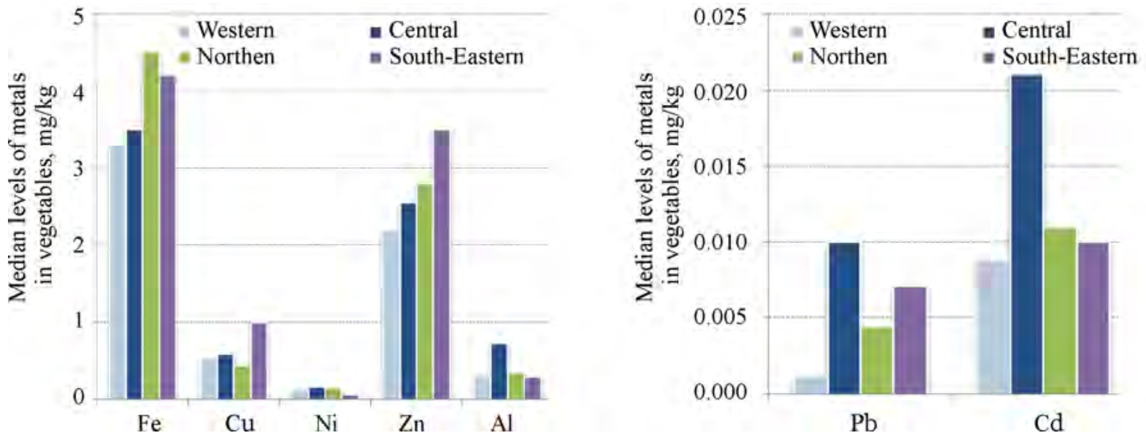

Figure. 2. Microelements in vegetables grown in Bashkortostan districts with different economic specialization

The highest levels of copper (Me = 1.00 mg/kg (0.47; 1.3)) and zinc (Me = 3.5 mg/kg (2.3; 4.2)) were identified in vegetables from the south-eastern part of the republic. They occurred due to ore mining enterprises located on this territory. The lowest levels of lead contamination were detected in vegetables from the western part. The higher median cadmium levels (up to 4 MPC) were identified in vegetables from the central part of the republic. A share of samples with cadmium levels being higher than MPC was also higher in the central part where it equaled 29 % whereas such shares did not exceed 10 % in other districts and the median levels to MPC ratios varies from 1.1 to 3.

Therefore, metal levels varied depending on a kind of a vegetable due to their different absorbability as well as due to regional peculiarities of soil and ambient air contamination.

The research data on levels of elements in bakery products, cereals and vegetables were used to calculate exposures to contaminants for children and adults (Table 1).

Daily intake of a specific metal depends on both its contents in food products and their consumed quantities. In Bashkortostan, average daily consumption of vegetables, potatoes included, amounts to 400 grams per person; cereals and bakery products, about 300 grams per person[2]. The RF Public Healthcare Ministry recommends daily consumption of vegetables to be about 340 grams per person; bakery products and cereals, 260 grams per person. The questioning [18] established that children aged 7–11 years consumed approximately 150 grams of vegetables and about 380 grams of cereals and bakery goods a day. Obviously, daily intake of vegetables was lower that recommended whereas cereals and bakery products were consumed in quantities that were by 1.5 times higher than the recommended norms.

The median exposure of adults to copper and zinc was considerably lower that their tolerable daily intake (500 and 1000 μg/kg of body weight a day for copper and zinc accordingly). Daily intake of these metals was higher for children and amounted to 6 % of tolerable daily intake of copper and 18.2 % of tolerable daily intake of zinc.

Calculated daily intake of iron amounted to 12.4 % of its tolerable daily intake for adults and 30 % for children (0.8 mg/kg of body weight per day). Iron was introduced with bakery products and cereals in higher quantities than with vegetables.

The median nickel intake with the analyzed food products amounted to 8.5 % and 16 % of its tolerable daily intake (0.02 mg/kg of body weight per day) for adults and children accordingly. Nickel contained in bakery products and cereals was established to make greater contribution to the total daily intake by children than nickel in vegetables. As for adults, differences in nickel intakes with these product groups were minimal for adults.

Daily intake of aluminum amounted to 1.6 mg for adults and 1.8 mg for children and was substantially lower than tolerable weekly intake. Its value equals 7 mg/kg of body weight per day, that is, 70 mg a day for adults and 30 mg a day for children. Bakery products made the greatest contribution to aluminum intake.

Tolerable weekly intake of lead recommended for adults by the FAO/WHO amounts to 3.6 μg/kg a day. If we take an average body weight to be 70 kg for an adult and 30 kg for a child, we will get tolerable daily intake of lead to be equal to 250 and 107 μg accordingly. According to our calculations, the median lead intake by children is the highest when they consume bakery products; it equals 0.18 μg and is considerably lower that the permissible level.

Table 1

Median levels of microelement intake with food products, μg/kg of body weight a day

Microelement	Adults			Children		
	vegetables	bakery products and cereals	Σ	vegetables	bakery products and cereals	Σ
Lead	0.032	0.053	0.085	0.029	0.15	0.18
Cadmium	0.072	0.033	0.11	0.065	0.093	0.16
Iron	22	77	99	20	219	239
Copper	3.6	9.5	6.9	3.3	27	30
Nickel	0.70	0.99	1.7	0.63	2.6	3.2
Zinc	16	59	75	14	168	182
Aluminum	2.4	21	23	2.2	57	59

[2] Potreblenie produktov pitaniya v domashnikh khozyaistvakh v 2020 godu po itogam vyborochnogo obsledovaniya byudzhetov domashnikh khozyaistv [Food consumption in households in 2020 as per the results of the sample survey with its focus on household budgets]. Moscow, The Federal State Statistics Service, 2021, 83 p. (in Russian).

Table 2

Hazard quotients (HQ) for microelements in food products

Microelement	Adults			Children		
	vegetables	bakery products and cereals	∑ HQ	vegetables	bakery products and cereals	∑ HQ
Lead	0.01	0.02	0.02	0.008	0.04	0.05
Cadmium	0.14	0.07	0.21	0.1	0.2	0.32
Iron	0.07	0.3	0.33	0.07	0.7	0.80
Copper	0.2	0.5	0.69	0.2	1.42	1.60
Nickel	0.002	0.002	0.00	0.001	0.005	0.01
Zinc	0.05	0.2	0.25	0.05	0.6	0.61
Aluminum	0.002	0.02	0.02	0.002	0.06	0.06

The total daily intake of cadmium is equal to 11 and 16 % of its tolerable daily intake (1 µg/kg of body weight a day) for adults and children accordingly. Bearing in mind, that cadmium is a toxic metal with long half-life, it is advisable to closely monitor its intake with other food products and drinking water.

We calculated hazard quotients (HQ) for each element allowing for intake with different food products. It was done to determine potential risks of developing non-carcinogenic health effects caused by exposure to metals. The results are given in Table 2.

The total non-carcinogenic risk caused by exposure to metals in vegetables turned out to be lower that the relevant permissible level (HQ < 1) as it equaled 0.47 for adults and 0.43 for children. The hazard quotient of each metal calculated for cereals and bakery products did not exceed its permissible levels for adults.

However, a health risk associated with copper intake equaled 1.42 for children and this was by three times higher than for adults. In general, children are more susceptible to contaminants in the environment due to their physiological peculiarities (elevated absorption of some chemicals in the gastrointestinal tract, lower body weight). Having ranked contributions made by the analyzed food products to exposures to chemicals, we established that the highest contribution to exposure to cadmium was made by vegetables (30 %); exposure to copper (45 %) and iron (22 %), by bakery products and vegetables.

We also calculated hazard quotients for non-carcinogenic effects caused by intake of metals with vegetables for people living in different districts in Bashkortostan (Figure 3).

According to the research results, a risk of harmful health effects caused by daily intake of lead, iron, nickel, zinc and aluminum with vegetables was permissible in the analyzed districts. The total hazard quotient varied between 0.36 in the western part of the republic and 0.57 in the central one. The highest contribution to the total hazard index was made by cadmium in all the districts in the republic. The calculated hazard quotient for cadmium was usually below 1 but approximately 25 % of all the analyzed samples had HQ between 1 and 2 for this metal.

Several organs and systems were established to be critical under simultaneous exposure to the analyzed chemicals in food products. They included the gastrointestinal tract and liver (the hazard index, or HI, equals 0.69 for adults and 1.60 for children), blood (HI = 0.59 for adults, HI = 1.41 for children), the immune system (HI = 0.33 for adults, HI = 0.80 for children), the hormonal system (HI = 0.23 for adults, HI = 0.37 for children), and the kidneys (HI = 0.21 for adults, HI = 0.32 for children).

A probability that malignant neoplasms would develop due to cadmium and lead intake with food products varied within 10^{-6}–10^{-4} thus corresponding to a conditionally acceptable (permissible) risk level (Table 3). However, as cadmium contents were by 4 times higher than MPC in some samples, in this case an average daily intake of the metal was beyond safe levels. As a result, an individual carcinogenic risk also grew and moved closer to its permissible limits. Our study highlights the necessity to conduct relevant monitoring of agricultural products as per safety indicators

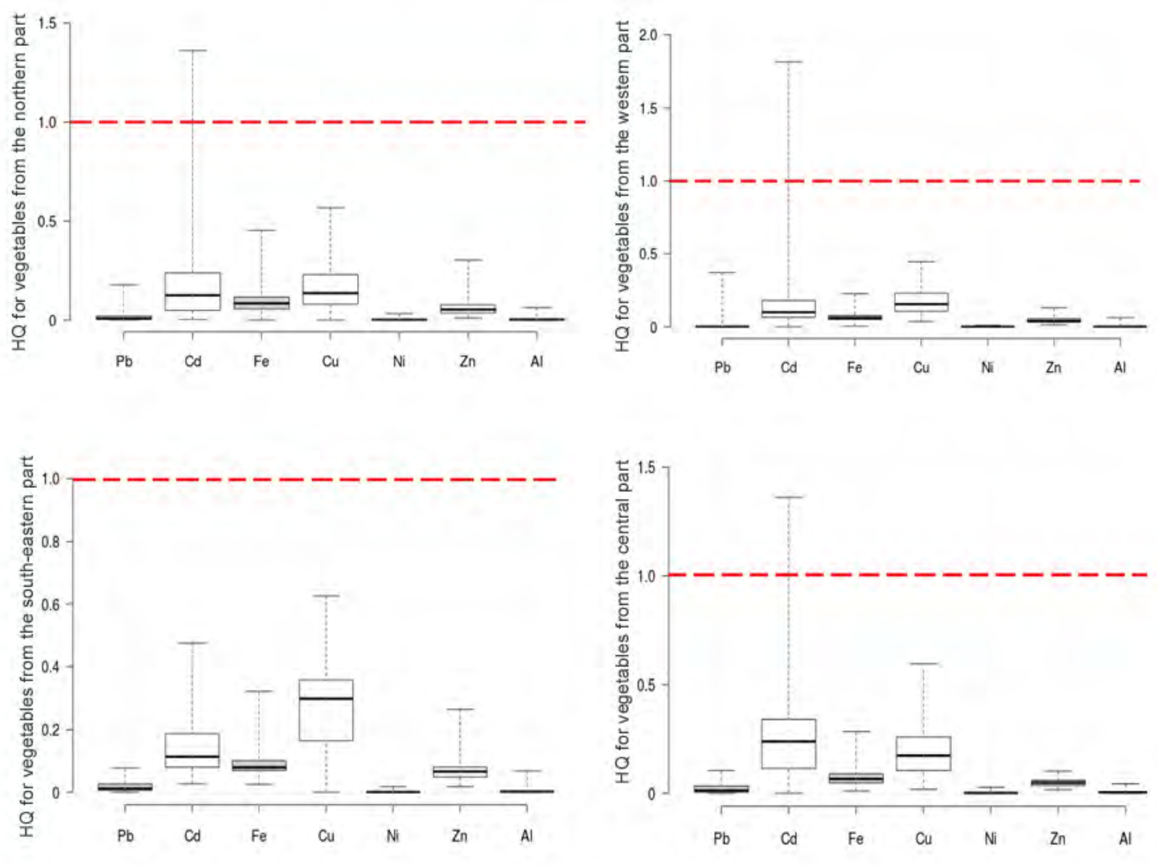

Figure 3. Hazard quotients for non-carcinogenic effects caused by intake of metals with vegetables for people living in Bashkortostan

Table 3

Assessment of carcinogenic risks for people of Bashkortostan caused by exposure to chemicals in food products

Microelement	adults		children	
	vegetables	bakery products and cereals	vegetables	bakery products and cereals
Lead	$1.5 \cdot 10^{-6}$	$2.5 \cdot 10^{-6}$	$1.4 \cdot 10^{-6}$	$7.0 \cdot 10^{-6}$
Cadmium	$2.7 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$	$2.5 \cdot 10^{-5}$	$3.5 \cdot 10^{-5}$
Total risk	$4.4 \cdot 10^{-5}$		$6.9 \cdot 10^{-5}$	

and to assess harmful effects produced on children since their bodies have much weaker abilities to metabolize, detoxify and excrete toxins [19–21].

Conclusion. This study established that in general agricultural products grown on private farms and cereals / bakery products sold in the Republic of Bashkortostan were not contaminated with extreme levels of toxic elements. Among all the analyzed product groups, vegetables contaminated with cadmium pose a relatively higher health risk, especially for people living in the central part of the republic (HQ = 0.57).

Critical organs and systems under simultaneous exposure to the analyzed contaminants in foods include the gastrointestinal tract and liver (the hazard index, or HI, equals 0.69 for adults and 1.60 for children), blood (HI = 0.59 for adults, HI = 1.41 for children). Non-carcinogenic risks for other organs and systems do not exceed their permissible levels.

The greatest contributions into non-carcinogenic health risks are made by copper (45 %) and cadmium (30 %) whereas those made by other elements account for less than 20 %. We have established that a potential health risk for children is associated with consumption of cereals and bakery products (3.01) whereas consumption of vegetables accounts for an insignificant share in this risk (0.43). The highest total hazard index has been established for chemicals that affect the gastrointestinal tract, liver and blood. The median value of the total carcinogenic risks associated with contaminants in food amounts to $4.4 \cdot 10^{-5}$ for adults and $6.9 \cdot 10^{-5}$ for children and was within its permissible levels. Therefore, potential health risks occur even under exposure to chemicals in concentrations lower than MPC.

Limitations of the study: the established risks occur only when locally produced foods are consumed; the research results should not be extrapolated to other territories.

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Competing interests. The authors declare no competing interests.

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Research article

ON SUFFICIENT SUBSTANTIATION FOR MAXIMUM PERMISSIBLE LEVEL OF ZILPATEROL IN MEAT PRODUCTS

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The Joint FAO/WHO Expert Committee recommends the maximum permissible level of zilpaterol in meat to be fixed at 0.5 µg/kg. This level is substantiated by results of analysis described in several research works. Nevertheless, substantiation provided for this recommended standard requires a detailed discussion.

In this study, we aimed to analyze substantiation of FAO/WHO suggestions on the maximum permissible level (MPL) of zilpaterol in meat as per health risks for consumers.

Our analysis of research results revealed that the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) were established allowing for negative effects on various organs and systems in the body. The lowest observed adverse effect level (LOAEL) under acute exposure was taken as a baseline for establishing MPL. This level produces negative effects on the nervous system (developing tremor). However, modifying factors used in MPL development have not been supported with solid argument. We also established that the LOAEL identified for the nervous system under acute exposure was much lower than NOAELs for other organs and systems under chronic exposure. Therefore, the aforementioned research results seem rather controversial.

It is necessary to consider another additional factor, which is wide prevalence of cardiovascular diseases among adult population and risk factors that cause their development. Therefore, potential adverse effects on the cardiovascular system are no less important and we should note that they have been reliably detected both in acute and chronic experiments.

In this study, we modeled a health risk caused by adverse effects of consuming meat products with residual zilpaterol levels; the risk was modeled in dynamics. The modeling experiment established that an impermissible health risk of adverse health outcomes in the cardiovascular system occurred even under exposure to zilpaterol in levels close to the lowest limit of sensitivity. Consequently, it seems rather premature to accept the maximum permissible level for zilpaterol in meat that is being suggested at present. It is recommended to cut its level down to the lowest limit of detection.

Keywords: *zilpaterol, food products, meat products, risk assessment, maximum permissible level, LOAEL, NOAEL, mathematic modeling.*

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At present, growth promoters are used in animal husbandry worldwide to enhance productivity. Along with hormonal stimulators (stilbenes or steroid hormones), non-hormonal growth promoters are also of practical interest. Among them, special attention is paid to beta-adrenergic agonists (ractopamine, zilpaterol, clenbuterol). Zilpaterol is the most interesting beta-agonist as regards its safety for human health.

In some countries, zilpaterol (in the form of zilpaterol hydrochloride) is applied as a food additive [1]. The basic purpose of its usage is to enhance muscle growth and increase carcass leanness; another aspect is making feed usage more effective (muscle growth enhancement due to using a food additive) in livestock breeding [2].

At present, zilpaterol hydrochloride as a food additive is considered safe for consumers in some countries (Brazil, Canada, Guatemala, Honduras, Nicaragua, Peru, the USA and some others) [3] whereas China and Taiwan prohibit to use it in agriculture and animal husbandry [4]. In the European countries, use of β-agonists, zilpaterol included, has been allowed since 1996 only for therapeutic use under direct veterinary supervision. Nevertheless, its residual quantities in meat and meat products are not regulated[1].

Zilpaterol can produce certain effects on β-adrenergic receptors of skeletal muscles and smooth muscles of the bronchi, uterus, heart, vessels and other organs [5–8]. Adverse effects on the human body caused by zilpaterol intake are associated with its pharmacological activity and can involve influence on the cardiovascular system (CVS) (with developing heart failure and future decrease in life expectancy at birth [9–12]), respiratory system, nervous system and also produce some systemic effects (a rise in the body temperature in particular) [13]. That is, when zilpaterol is administered with food even in residual quantities, it can create unacceptable (impermissible) public health risks.

Given that, in 2016 the Joint FAO/WHO Expert Committee on Food Additives (JECFA), relying on the analysis of some research works, recommended the maximum permissible level (MPL) of zilpaterol in meat to be fixed at 0.5 μg/kg [14]. Nevertheless, substantiation provided for this recommended standard requires a detailed discussion.

In this study, we aimed to analyze substantiation of FAO/WHO suggestions on the maximum permissible level (MPL) of zilpaterol in meat as per health risks for consumers.

Our analysis revealed that the JECFA relied on the results obtained by studies with their focus on acute effects; those studies were conducted with participating volunteers, asthmatic patients included (as the most sensitive population group). The results allowed establishing no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) allowing for adverse effects on various organs and systems in the body (Table 1). The LOAEL for cardiovascular effects was established to be equal to 0.25 mg/person; respiratory effects, 0.1 mg/person; effects on the nervous system, 0.05 mg/person. In its turn, the NOAEL for cardiovascular effects amounted to 0.1 mg/person and for respiratory effects, 0.05 mg/person. The experts failed to establish a NOAEL that could cause adverse effects on the nervous system within the analyzed exposure limits [14].

Chronic experiments on animals also made it possible to establish NOAELs and LOAELs for cardiovascular effects, blood and systemic effects (Table 2). Thus, the LOAEL

[1] Council Directive 96/22/EC of 29 April 1996 concerning on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. *Official Journal of the European Communities*, 1996, no. L 125/3, pp. 3–9.

Table 1

Research results obtained by the JECFA regarding influence exerted by zilpaterol on the human body under oral administration

Critical effect	NOAEL, mg/person*.	LOAEL, mg/person
Cardiovascular system:	0.10	0.25
Systolic blood pressure (elevated)	0.25	0.50
Diastolic blood pressure (elevated)	0.25	0.50
Cardiac output (elevated)	0.25	0.50
Elevated heart rate	0.10	0.25
The respiratory system: Bronchodilation	0.05	0.10
The nervous system: **Tremor**	–	**0.05**

N o t e : * means body weight is considered as equal to 70 kg.

Table 2

Research results regarding effects produced by zilpaterol on the body under chronic exposure

Species	Critical effect	NOAEL, mg/kg of body weight a day	LOAEL, mg/kg of body weight a day
Mice	Changed enzyme ratios in blood	0.02	0.05
Rats	Decreased heart rate	–	0.05
	Growing body weight	–	0.06
	Hypersalivation, growing body weight	0.2	2.0
Rabbits	Growing body weight	–	20.0
Dogs	Peripheral vasodilatation, increased heart rate, lower blood pressure	–	0.5
Javanese monkey	Increased heart rate	0.01	0.05

that causes adverse effects on the CVS and blood was established to be equal to 0.05 mg/kg of body weight; systemic effects, between 0.06 and 20.0 mg/kg of body weight. The NOAEL established for cardiovascular effects equaled 0.01 mg/kg of body weight; blood, 0.02 mg/kg of body weight; systemic effects, 0.2 mg/kg of body weight. The JEFCA materials do not provide any information on exploring chronic effects produced by zilpaterol on the nervous system.

Therefore, this information provided by the JEFCA indicates that no NOAEL was established for effects on the nervous system under acute exposure. At the same time, there are no data on negative effects produced on the nervous system by zilpaterol within the analyzed levels under chronic exposure.

The LOAEL for the nervous system under acute exposure was also established to be considerably lower than NOAELs established for other organs and systems under chronic exposure. Therefore, the aforementioned research results seem rather controversial. Nevertheless, the LOAEL that could cause adverse effects on the nervous system (developing tremor) under acute exposure was taken as a starting point to derive the MPL for zilpaterol. This dose is equal to 0.71 µg/kg [14] and the lowest one mentioned in the research. This critical effect was probably selected due to skeletal muscle tremor being the most frequently mentioned by experts as a critical adverse effect under short-term oral administration of zilpaterol [15–18].

An acceptable daily intake (ADI) was established as equal to 0–0.04 µg/kg of body weight by applying a default uncertainly factor of 10 (the results extrapolated on the most sensitive people) and an additional uncertainty factor of 2 for the use of a LOAEL instead of a NOAEL. Still, there are no data on applying an uncertainty factor associated with the extrapolation of the results obtained under short-term exposure on long-term exposure scenarios despite absence of no-effect exposure levels established for critical effects. Besides, an uncertainty factor of 2, which is used to account for using LOAEL instead of NOAEL, hardly seems sufficient.

At the same time, it is advisable to bear in mind that zilpaterol, given its elimination half-life from animals under oral administration varying between 3.69 to 4.81 hours [5], can still remain in the body in some quantities during a day. This may lead to up to 2 % of administered zilpaterol dose remaining in the body. Given that, we can conclude that daily consumption of meat containing zilpaterol in residual quantities can be considered a chronic exposure factor.

Wide prevalence of cardiovascular diseases among adults as well as risk factors that cause them is an additional factor, which, in our opinion, should be considered. Therefore, potential adverse effects on the cardiovascular system are no less important and we should note that they have been reliably detected both in acute and chronic experiments, anabolic impact of zilpaterol taken into account.

Since we were not able to find any relevant data on effects produced by zilpaterol on the cardiovascular system, we resorted to mathematical modeling to predict risks caused by functional disorders of the CVS under exposure to this additive. We created a mathematical model that described a health risk in dynamic under two scenarios involving daily intake of meat with zilpaterol contents being equal to an MPL suggested by the Joint FAO/WHO Expert Committee (0.0005 mg/kg) and the limit of detection (LoD) for it in meat products (0.0001 mg/kg) [19]. Prediction relied on using an evolution model that described accumulating risks of the CVS functional disorders in accordance with the methodical guidelines by the Eurasian Economic Commission[2].

We applied a recurrent relation to calculate accumulation of risks of functional disorders:

$$R_{t+1} = R_t + (\alpha \cdot R_t + \beta \cdot D)C \qquad (1)$$

with a preset initial risk level R_0, where

R_{t+1} is a risk of disorders at a time moment t+1;

R_t is a risk of disorders at a time moment t;

α is a risk evolution factor due to natural causes;

β is a factor of zilpaterol effects;

C is a time empirical coefficient ($C = 0.00274$ for daily averaging);

D is a zilpaterol dose, mg/kg.

[2] MR 2.1.10.0062-12. Kolichestvennaya otsenka nekantserogennogo riska pri vozdeistvii khimicheskikh veshchestv na osnove postroeniya evolyutsionnykh modelei: metodicheskie rekomendatsii. [MR 2.1.10.0062-12. Quantification of non-carcinogenic risk under exposure to chemicals based on evolution models: methodical guidelines]. Moscow, Rospotrebnadzor's Federal Center for Hygiene and Epidemiology, 2012, 36 p. (in Russian).

The factor of zilpaterol effects was established based on a model that described an 'exposure – likelihood of effect' relationship and was created considering data on influence exerted by zilpaterol on changes in likelihood of an effect:

$$P = b_0 + b_1 \cdot D \tag{2}$$

where P is likelihood of effect, D is a zilpaterol dose [mg/kg]; b_0 = 0.007, b_1 = 185.2 are model parameters.

Given the established relationship, the coefficient β value in the equation (1) is determined as per the following formula:

$$\beta = g \cdot b_1 \tag{3}$$

where β is the coefficient that describes zilpaterol effect on a risk of this effect;

g = 0.05 is a coefficient that shows severity of the effect;

b_1 = 185.2 is a coefficient that shows influence exerted by zilpaterol on changes in likelihood of an effect.

The applied values of the coefficient considered, the ultimate value of the coefficient β was equal to 9.26.

Ultimately, the recurrent equation to calculate a risk of an effect occurring under exposure to zilpaterol at the lower boundary of the estimated level was given as follows (4):

$$R_{t+1} = R_t + \left(0.0835 \cdot R_t + 9.29 \cdot D\right) \cdot 0.00274 \tag{4}$$

with the initial condition $R_t = 1.9 \cdot 10^{-4}$.

This equation was used to build a curve that showed evolution of a health risk caused by functional disorders of the CVS under exposure to the zilpaterol dose D (calculated risk) and an adjoining curve built without zilpaterol effects taken into account (D = 0) (background risk). An additional risk (ΔR_t) is determined for each time moment as a difference between the background and calculated risk levels.

An acceptable risk level was estimated as per the value of the reduced risk index according to the risk assessment methodology accepted by the Eurasian Economic Commission [20]. Meat consumption was estimated based on statistical data collected in the Russian Federation[3].

The modeling made it possible to establish that consumption of meat that contained zilpaterol in quantities equal to the MPL recommended by the Codex Alimentarius Commission created unacceptable health risks already by 35 years of age. At the same time, if zilpaterol was contained in consumed meat in a quantity equal to the lowest limit of its analytical detection in meat, a health risk would become unacceptable by 55 years of age (Table 3).

Therefore, daily zilpaterol intake with meat in quantities even equal to the level of sensitivity for the method of detection can create impermissible health risks.

Conclusion. The maximum permissible level of zilpaterol in meat suggested by the JECFA, which is equal to 0.5 μg/kg, is based on the ADI established on the basis of the LOAEL under short-term exposure. It does not allow for chronic effects on the nervous and cardiovascular systems; when modifying factors are used, extrapolation of the results obtained by exploring short-term effects on long-tern exposure is not considered.

This allows us to conclude that the MPL for zilpaterol in meat suggested by the JEFCA are not well-grounded. Besides, our modeling of health risks associated with adverse effects caused by consumption with meat with zilpaterol in residual quantities revealed that

[3] Ratsion pitaniya naseleniya 2013: statisticheskii sbornik [Population diets in 2013: statistical data collection]. *Rosstat*. Moscow, IITs 'Statistika Rossii', 2016, 220 p. (in Russian).

Table 3

The reduced health risk index caused by functional disorders of the cardiovascular system under exposure to zilpaterol under different scenarios of its intake with meat

Age, years	The risk index when zilpaterol is contained in consumed meat in quantity equal to suggested MPL	The risk index when zilpaterol is contained in consumed meat in quantity equal to lowest LoD
5	0.0014	0.0003
10	0.0046	0.0009
15	0.0092	0.0018
20	0.0147	0.0029
25	0.0231	0.0046
30	0.0358	0.0072
35	**0.0550***	0.0110
40	**0.0842**	0.0168
45	**0.1285**	0.0257
50	**0.1959**	0.0392
55	**0.2987**	**0.0597**
60	**0.4561**	**0.0912**
65	**0.6984**	**0.1397**

Note: * risk levels higher than acceptable ones are given in bold.

impermissible health risks could occur even under exposure to this food additive in a concentration close to the level of sensitivity for the method of detection.

Consequently, it seems rather premature to accept the maximum permissible level for zilpaterol in meat that is being suggested at present. It is recommended to cut its level down to the lowest limit of detection.

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Research article

ASSESSING INDEX OF ACCUMULATED CYTOGENETIC DISORDERS IN WORKERS EMPLOYED IN METALLURGY UNDER EXPOSURE TO ADVERSE OCCUPATIONAL FACTORS

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Metallurgy is a major economic branch in Russia with more than 4000 enterprises operating in it and seventy percent of them being city-forming ones. This study focuses on cytological assessment of the oral mucosa and secretion from the middle meatus mucosa in workers employed in metallurgy.

The aim of this study was to investigate cytological laboratory indicators in workers employed in metallurgy under exposure to adverse occupational factors.

A clinical and diagnostic examination of workers employed at a metallurgical plant in Bashkortostan was performed in 2019–2020; it involved cytological studies of the oral mucosa (buccal epithelium) and the middle meatus mucosa (rhinocytogram). In this study, we applied the Index of cytogenetic disorders accumulation (Iac) that allows for cellular kinetics indicators.

The overall hygienic assessment of working conditions for workers employed at the analyzed metallurgic plant corresponds to the hazard category 3.2–3.3 in accordance with the criteria outlined in the Guide R (harmful, class 2 or 3). The research results revealed cytogenetic disorders of buccal epithelial cells in the workers who had contacts with adverse occupational factors. Low likelihood of cytogenetic disorders was established for 66.67 % of the workers; moderate, 9.2 %; high, 23.81 %. We assessed rhinocytograms of the workers exposed to adverse occupational factors and revealed some signs of allergic inflammation characterized with high eosinophil count.

The research results confirm high significance of diagnostic procedures for developing an algorithm for screening examinations of working population as well as indicators of health disorders under exposure to adverse occupational factors (noise, heating microclimate, industrial dust, gaseous chemicals).

Keywords: *metallurgical production, hygienic assessment of working conditions, adverse occupational factors, Index of cytogenetic disorders accumulation, buccal epithelium cells, rhinocytogram.*

Metallurgy is a major economic branch in Russia with more than 4000 enterprises operating in it and more than seventy percent of them being city-forming ones [1–4]. More than a half million people are employed in the branch. Metallurgic productions rely on complete production cycles; this involves using multiple technological processes, different temperature regimes, binding substances and catalysts. Despite massive modernization which is now taking place in the industry, workers employed in it are still exposed to variable adverse occupational factors. They include noise, heating microclimate, industrial dusts, flammable, explosive and poisonous substances, vibration, ultra-

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sound, electromagnetic radiation as well as substantial physical loads and work in night shifts [1, 5–7]. Domestic studies have confirmed a substantial role that belongs to adverse occupational factors at metallurgic production in developing pathologies of various organs and systems [8, 9], including upper respiratory mucosa. They can progress latently and become a factor that induces various respiratory diseases [10].

At present, it is becoming more and more vital to search for the most informative physiological, biochemical, immunologic and other criteria to estimate effects produced by harmful factors [11, 12].

Rhinocytogram (RCG) studies that involve microscopic examination of imprint smears taken off the nasal mucosa are a simple atraumatic procedure for estimating the state of the respiratory mucosa [13, 14]. RCG provides an insight into the condition of the epithelial tissue in the upper airways and leukocyte occurrence as well as tentatively estimates microflora [15, 16].

Micronuclei assay of the buccal epithelium is a procedure for estimating diseases and processes associated with DNA damage induction; it is considered an effective biomarker [17]. This assay is becoming popular with researchers due to its low invasiveness in cell sampling, simple preparation and storage procedures. All this makes the buccal macronuclei assay an ideal option to conduct molecular-epidemiological examinations [18–20].

Some studies address using the buccal macronucleus assay at productions that involve exposure to formaldehyde, pesticides, and cytostatics; at iron and chromium productions; copper-smelting plants; clothing manufacture; in microbiological industry; auto repair shops etc. [21]. Still, we have not been able to find any studies with their focus on examining the buccal micronuclei assay and imprint smears taken off the nasal mucosa (rhinocytogram) of workers employed in metallurgy.

The relevance of this study is evidenced by detected pre-pathological and pathological conditions; they make it possible to diagnose severity of an occupational or a work-related disease and predict its possible clinical course.

In this study, we aimed to investigate cytological laboratory indicators in workers employed in metallurgy under exposure to adverse occupational factors.

Materials and methods. To achieve our aim and solve the research tasks within this study, we conducted a complex clinical and diagnostic examination of workers employed at a metallurgical plant in 2019–2020; the plant was located in the Republic of Bashkortostan. Laboratory indicators were analyzed relying on data of a periodical medical examination (PME). It was performed in conformity with the Order by the RF Labor Ministry No. 988n, the RF Public Healthcare Ministry No. 1420n issued on December 31, 2020[1]. The test group was made up of workers with various occupations employed at the analyzed enterprise; their occupational activities were likely to involve exposure to harmful occupational factors. We used the following criteria to include workers into the test group and to sample biomaterials: a chronic disease (with exacerbation over the last 12 months) or acute diseases (2 or more over the last 12 months) of the respiratory organs in a case history; exposure to predominantly fibrogenic aerosols at a workplace. The reference group included workers employed at the same plant who were not exposed to harmful production factors. Workers' average age was 53.76 ± 1.26 years and their average work records at the plant equaled 25.66 ± 1.22 years. Both groups were comparable as per age and sex; all the analyzed workers had long work records.

[1] Ob utverzhdenii perechnya vrednykh i (ili) opasnykh proizvodstvennykh faktorov i rabot, pri vypolnenii kotorykh provodyatsya obyazatel'nye predvaritel'nye meditsinskie osmotry pri postuplenii na rabotu i periodicheskie meditsinskie osmotry: Prikaz Mintruda Rossii i Minzdrava Rossii ot 31 dekabrya 2020 goda № 988n/1420n [On Approval of the list of harmful and (or) hazardous occupational factors and works that require mandatory preliminary medical examinations prior to recruitment and periodical medical examinations: The Order by the RF Labor Ministry and RF Public Healthcare Ministry issued on December 31, 2020 No. 988n/1420n]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573473071 (June 27, 2022) (in Russian).

We analyzed the results obtained by laboratory and instrumental control. It included examining air samples taken in closed premises, noise measurements, microclimate at workplaces, and artificial lighting. All the tests and measurements were performed by the Testing Laboratory of the Center for Hygiene and Epidemiology in the Republic of Bashkortostan. Hygienic assessment of working conditions relied on the results of workplace evaluation and industrial control, our own observations of production processes and job descriptions provided by the human resources department of the plant.

Cytological studies involved examining samples of non-keratinized stratified squamous epithelium of the oral cavity mucosa (buccal epithelium) and the middle meatus mucosa. Micronuclei were identified in the buccal epithelium as per the standard described by P.E. Tolbert [22]. We also considered binucleated cells, karyopyknosis, karyorrhexis, and karyolysis. To estimate cellularity of a smear and morphological picture of secretion from the middle meatus mucosa as a whole, we performed thin-section analysis similar to blood leukocyte count. The threshold value was set at 100 and the results were given in %. The count covered epithelium (separately cylindrical, with metaplasia, squamous, with degeneration signs, including "naked nuclei") and leukocytes (neutrophils, eosinophils, monocytes, and lymphocytes). When describing a preparation, we noted the total smear cellularity; it was scarce in case some sporadic cells were identified in the field of vision, moderate in case small groups made of 3–5 cells were identified and multi-cellular if layers or dozens of cells were visible. We estimated how epithelium was located (groups, layers or sporadic cells), signs of degenerative changes in cylindrical epithelium, occurring mucus and microflora (cocci and bacilli).

This procedure makes it possible to identify cellular changes caused by exposure to physical and chemical irritation in secretion from the middle meatus mucosa [23]. Glass slides were examined on Mikmed-5 microscope (Russia) under magnification 10×40; 10×100.

In this study, we applied a so called index of accumulation of cytogenetic damage (Iac), which considered cellular kinetic indicators [24]. We defined three groups as regards likelihood of risks that cytogenetic damages would occur: low (Iac ≤ 2), moderate ($2 <$ Iac < 4) and high likelihood (Iac ≥ 4). Laboratory tests were performed only after all the participants gave their informed consent to them in accordance with the ethical principles stated in the Declaration of Helsinki (2000).

The results were analyzed with Statistica 6.0 software; we determined simple mean (*M*), standard error of the mean (*m*), and authenticity of the results by using the parametric Student's t-test (*t*) and the level of significance (*p*). Age-related causality of health disorders was identified by using the correlation coefficient (*r*).

Results. Basic occupations employed at the analyzed plant include wire drawers, refractory workers, annealers, automatic cold upsetter operators, cable winding operators etc. The environment tends to change substantially during the whole work shift; temperature grows drastically in warm season and drops in cold one; there is also powerful radiation from heated and smelted metals. Air is heavily polluted with dusts at some production sections, especially where metal is being prepared for further production operations. These processes involve substantial emissions of carbon oxide, fibrogenic aerosols, etc.

Wire drawers are exposed to dusts with silicon dioxide; workplace air at their workplaces also contains elevated levels of di-sodium carbonate. The overall hygienic assessment of working conditions at a wire drawer's workplace ranks them as harmful, the hazard category 3.3.

Refractory workers are exposed to dusts from refractory materials, carbon oxide and their work involves a lot of physical strain. Workplace air contains carbon oxide, fibrogenic aerosols and asbestos-containing dusts in concentrations by several times higher than MPC. The overall hygienic assessment of working conditions at a workplace of a refractor worker who deals with thermal furnace lining ranks them as the hazard category 3.2 (harmful conditions).

Basic work operations performed by annealers over the whole work shift involve exposure to di-iron trioxide and dusts with silicon dioxide (up to 1.5 MPC). The overall hygienic assessment of working conditions at an annealer's workplace ranks them as harmful, the hazard category 3.3.

Automatic cold upsetter operators are exposed to high concentrations of fibrogenic aerosols at their workplaces. The overall hygienic assessment of working conditions at an automatic cold upsetter operator's workplace ranks them as harmful, the hazard category 3.3.

Workplace air at a workplace of a cable winding operator contains plant and animal dusts together with silicon dioxide in levels being by several times higher than permissible ones. The overall hygienic assessment of working conditions at a workplace of a cable winding operator ranks them as the hazard category 3.2 (harmful conditions)

The overall hygienic assessment of working conditions at the analyzed metallurgic plant ranks them as harmful (class 3), the hazard category 2–3, according to the Guide R 2.2.2006-05[2] (table).

The tests revealed cytogenetic damages to the buccal epithelium cells in workers exposed to harmful occupational factors (Figure).

We detected more frequent buccal epitheliocytes with micronuclei in 47.61 % of the examined workers. This value was by two times higher than in the reference group (p > 0.05). Morphological signs evidencing impaired proliferation were identified in 7.14 % of the analyzed workers. Signs of cell necrosis were statistically significant ones among indicators of nucleus destruction. Karyolysis as a result of necrotic cell destruction was identified in 35.71 % of the examined workers (p > 0.05); it was preceded by occurring perinuclear vacuole or perinuclear vacuolization. Karyopyknosis is considered a natural way of buccal epithelium cellular apoptosis; it was identified in 11.90 % of the examined workers. Cells with karyorrhexis signs were detected in 2.38 % of the examined production workers.

We applied the index of accumulation of cytogenetic damage to calculate likelihood of cytological abnormalities. Low likelihood of cytogenetic damage was identified for 66.67 % of the examined workers; moderate, 9.2 %; high, 23.81 %.

RCG assessment established moderate total cellularity in preparations of workers exposed to harmful occupational factors. Squamous epithelium cells were in groups and in layers and were identified in 52.38 % of the examined workers. Neutrophilia as a sign of non-specific, probably microbial, inflammation was detected in 85.71 % of the examined workers; elevated quantities of cylindrical

Table

Working conditions for workers employed at the analyzed metallurgical plant, ranked as per harmfulness and hazards

Occupation	Harmful factors, hazard category of working conditions					
	Noise	Plant and animal dusts	Chemicals (sanitary-hygienic profile)	Labor hardness	Microclimate in workshops	Overall assessment of working conditions
Wire drawer	3.1	3.1	3.1	3.1–3.2	3.1	3.3
Refractory worker dealing with thermal furnace lining	3.1	3.2	3.1	3.1–3.2	3.1	3.2–3.3
Annealer	3.1	3.1	3.1	3.2	3.1	3.3
Automatic cold upsetter operator	3.1	3.2	3.2	3.2	3.2	3.3
Cable winding operator	3.1	3.1	3.1	3.1–3.2	3.1	3.2

[2] The Guide R 2.2.2006-05. Guide on Hygienic Assessment of Factors of Working Environment and Work Load. Criteria and Classification of Working Conditions (approved by G.G. Onishchenko, the RF Chief Sanitary Inspector on July 29, 2005, became valid on November 01, 2005). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200040973 (July 09, 2022) (in Russian).

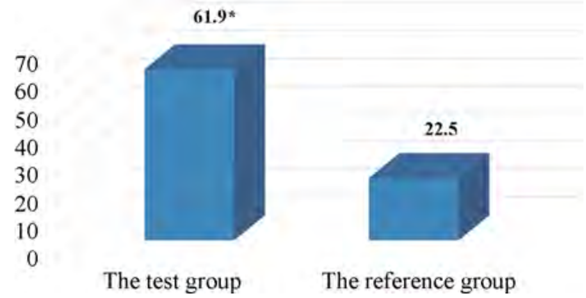

Figure. Cytogenetic damages to the buccal epithelium cells in workers exposed to harmful occupational factors (%): * means differences are authentic ($p < 0.05$)

epithelium, 28.57 %; slight metaplasia in epithelium, 9.52 % of the analyzed workers. Some insignificant quantities of microflora, predominantly cocci, were identified in smears. Some patients had signs of allergic inflammation evidenced by elevated eosinophil quantities in 28.57 % of the examined workers. Cytological preparations of the middle meatus mucosa in the reference group had cylindrical epithelium cells in rare fields of vision (5.00 % of the workers in this group); sporadic squamous epithelium layers and epithelial cells with metaplasia were identified in 15.00 % of the workers in this group.

Discussion. Metallurgic production typically involves emissions of multiple harmful chemicals into workplace air. These chemicals are applied in technological processes or are manufactured by using them and can produce variable effects on the human body. Industrial aerosols make cytogenetically abnormalities in the buccal epitheliocytes of the oral mucosa much more probable thereby moving workers from a group with moderate likelihood of such disorders into a group with high likelihood. Cytological and morphological signs of the process are elevated numbers of cells with impaired proliferation and cells with necrosis signs. By analyzing the index of accumulation of cytogenetic damage, we confirmed cytotoxic effects produced by industrial aerosols. More frequent occurrence of cells with cytogenetic changes in the oral cavity is considered the earliest sign of disrupted cytogenetic homeostasis and a decrease in workers' adaptation resources [25]. Degenerative (dystrophic) changes in the nasal secretion epithelium develop due to cytopathogenic effects produced by microflora, allergens, or chemical aerosols. Other signs of a degenerative process include cilia loss, nucleus and cytoplasm vacuolization, hypochromia, cytoplasm acidophilia, cytoplasm having fuzzy contours up to its complete destruction with 'naked" nuclei occurrence [20].

In this study, we analyzed the data obtained by laboratory tests and examination of workers employed at a metallurgical plant. The analysis established that exposure to industrial aerosols leads to much greater likelihood of developing cytogenetic abnormalities in the buccal epithelium cells and in the epithelial cells of nasal secretion. The research results confirm high significance of diagnostic procedures for developing an algorithm for screening examinations of working population as well as indicators of health disorders under exposure to adverse occupational factors.

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Research article

A CONCEPTUAL SCHEME OF A PREDICTIVE-ANALYTICAL MODEL FOR DESCRIBING INCIDENCE OF WEST NILE FEVER BASED ON WEATHER AND CLIMATE ESTIMATION (EXEMPLIFIED BY THE VOLGOGRAD REGION)

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The present study focuses on weather and climatic factors influencing the incidence of West Nile fever (WNF) in the Volgograd region. We aimed to describe a relationship between these factors and the WNF incidence and to create a conceptual scheme of a predictive-analytical model for making forecasts how an epidemiological situation would develop in future.

According to this aim, we selected an approach that involved identifying a statistical correlation between the analyzed factors and the WNF incidence in the Volgograd region and estimating the power of this correlation. The study primarily relied on using correlation analysis that was followed by assessing authenticity of the study results. The obtained data made it possible to establish that air temperature was a leading potentiating factor in the Volgograd region. It produced certain effects that varied in their intensity on a whole set of abiotic and biotic factors (water level and temperature, numbers and activity of vectors, how fast the virus amplifies in vectors, etc.).

The study established that use of comprehensive statistical data (average monthly indicators) allowed more precise estimation of correlations. We also considered and confirmed a hypothesis about a delayed effect produced by air temperature on population incidence and numbers of West Nile virus vectors in the Volgograd region; it was the most apparent in years with the maximum numbers of infected people (1999, 2010, and 2012). We revealed a statistical correlation between air temperature and average annual water level and the WNF incidence among population and the number of West Nile virus vectors. There was a strong correlation between the number of vectors and the WNF incidence. A conceptual scheme of a predictive model for determining rate of the WHF incidence in Volgograd region was created based on the statistical analysis results.

Keywords: *West Nile fever, West Nile virus, epidemic situation, predictive-analytical model, factor estimation, weather and climatic peculiarities, correlation analysis, WN virus vectors, Volgograd region.*

West Nile fever (WNF) is an acute transmissible natural focal arboviral disease. Its clinical picture usually involves fever and intoxication; in more severe cases, the disease can affect the central nervous system with appearing meningitis- or encephalitis-like symptoms.

West Nile virus (WNV) is the infectious agent that causes the disease. It belongs to *Flaviviridae* family, *Flavivirus* genus [1]. Mosquitos from *Culex* genus are its primary vectors but the virus can also be carried by mosquitos from *Aedes*, *Coquillettidia*, *Cu-*

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liseta, *Uranotaenia* genus and ticks from *Ixodes*, *Hyalomma*, *Dermacentor* genus [2–4]. The virus is predominantly transmitted by an infected mosquito's bite. Still, there have been some cases when WNV has been transmitted from a mother to a fetus through the placenta, with breast milk as well as by artificial transmission [5–7].

WNF has become a growing concern for public healthcare over the last decades due to its growing nosoarea, occurring outbreaks with severe clinical course and absence of any specific treatment or prevention procedures.

Since WNV was first identified in Uganda in 1937, its nosoarea has grown drastically. At present, WNF incidence is detected in America, Africa, South Asia, Western Pacific region and Europe, the Russian Federation included.

WNF is widely spread in America. Disease cases (between 700 and 3000) are registered annually in more than 48 states in the USA. Most infection cases are neuro-invasive[1].

WNF incidence is registered in 22 European countries; 4218 infected people were identified over 2010–2020. In Russia, the disease has been registered in 30 regions and markers of the infectious agent have been identified in 62 regions [8].

In the Volgograd region, WNF cases have been registered since 1999 and their total number accounts for 44 % of all the cases registered in Russia. WNF incidence is identified in most districts in the region (its maximum rates are detected in Volgograd and Volzhskiy). Given that, it is quite a vital trend in epidemiological studies to identify major factors that have their influence on the WNF incidence in the Volgograd region as well as to examine correlations between them. Data obtained by using tools of statistical and mathematical analysis can provide certain grounds for creating predictive-analytical models. Such models make it possible to estimate how an epidemiological situation as regards WNF would develop in future.

Many researchers believe that it is rather difficult to model and predict outbreaks of transmissible diseases comprising WNF. The major obstacles include complicated transmission mechanisms that involve interactions between an infectious agent, vector and a susceptible host and we should also remember that the whole complex is influenced by variable external factors.

Building up dynamic time series is a common approach applied to model an epidemic process of arboviral diseases. Within this approach, incidence among population is considered a function of delayed environmental indicators [9–11]. An advantage this statistical approach has against imitation models is its relative simplicity. Models can be easily parameterized by using dynamic time series of epidemiological data and data on the environment. However, a major limitation of such models is that they rely on linear approximations for non-linear systems and this leads to multiple simplifying assumptions. In other words, data on external factors are sufficient for calculating a risk of transmission but certain significant effects can be produced by other non-environmental phenomena (for example, after several years when WNF has been intensively transmitted in bird populations, they may develop immunity to it and this, in its turn, can potentially limit WNF transmission [12]). To describe these effects, it is necessary to apply complex non-linear mathematical functions. Nevertheless, a complex approach should be implemented to examine such relationships; it can only be developed after all the factors that influence the intensity of a given epidemiological process have been investigated by using primary tools of statistical analysis, correlation one included.

According to the existing insight into regularities of an epidemic process typical for arboviral infections, it is primarily influenced by nature and climatic factors (air temperature, precipitations, a water level in natural basins and a number of vectors). These factors are typically considered in attempts to predict how an epidemiological situation would develop. In

[1] West Nile virus. Final Cumulative Maps & Data for 1999–2019. *Centers for Disease Control and Prevention*, 2020. Available at: https://www.cdc.gov/westnile/statsmaps/cumMapsData.html (April 14, 2022).

addition, some ecological (air humidity) and social factors (changes in a landscape due to wider use of land, disinsection procedures, local infrastructural peculiarities on a given territory, national habits, etc.) can produce certain effects on transmission of an infectious agent and, consequently, on incidence [13, 14]. Besides, such demographic indicators as infected people's age and sex are potentially connected with severity of an infection and its detectability and can thereby determine intensity of an epidemic process. It is noteworthy that when predictive-analytical models are created, it is important to describe and estimate effects produced by these factors. But, as a rule, such descriptions and estimations require profound mathematical analysis since the analyzed indicators are heterogeneous and can be both quantitative and qualitative. Given that, these groups of indicators have not been given any attention at this stage in our research and have not been analyzed or included into the concept of the model being developed in this study.

To create models for assessing risks of infectious agent transmission (WNF included) it is most common to use retrospective and operative climatic and / or meteorological indicators. However, such a dataset does not always give an opportunity to reliably predict probable time and localization of potential outbreaks [15].

Time invariance is another difficulty in developing such predictive models. Use of time series in analysis is based on an assumption that delayed effects produced by environmental factors on a risk of infection are static (for example, last month precipitations correlate positively with a today risk) and do not change over time (for example, last month precipitations can have a positive correlation with a risk in July but a negative one with a risk in August). The outlined problem concerns WNF as well due to impacts exerted by multiple environmental factors on numbers of the primary vectors, their activity and infection rate and, consequently, on incidence. Certain approaches applied by researchers to predict how an epidemiological situation with WNF would develop were described in one of our previous studies [16].

In this study, our aim was to describe a relationship between natural, climatic and ecological factors and the WNF incidence in the Volgograd region and to create a conceptual scheme of a predictive-analytical model for making forecasts how an epidemiological situation would develop in future.

Materials and methods. We chose an approach that involved identifying statistical correlations between a set of factors and the WNF incidence in the Volgograd region and estimating their intensity. This region of the Russian Federation was selected due to high representativeness of the provided data, which was associated with the infection being registered in the region for a long period and its greatest rates in the Russian Federation (44.0 % of the total number of the cases registered in the country). To achieve our aim, we applied correlation analysis (linear regression was used in all cases) with the subsequent estimation of the obtained results. It is noteworthy, that correlation analysis is a statistical tool and is widely used both in Russian and foreign studies. Results obtained by correlation analysis make it possible to examine correlations between factors by applying proper mathematical procedures at the next stage in the present research.

We selected some factors that could possibly influence WNF transmission, namely, air temperature, the number of vectors and a water level detected at hydrological posts. Given that, we took the following retrospective data:

1) average winter, average summer and average annual temperatures and WNF cases over the period 1999–2019;

2) average annual and average seasonal (spring-autumn period between April and October) numbers of vectors (mosquitoes *Culex spp.*) over the period 1999–2018, average annual water levels and water temperature detected at hydrological posts in Volgograd and the urban settlement of Srednyaya Akhtuba over the period 2001–2017 (we were not able to find data on a longer period in available information sources);

3) average monthly air temperatures and numbers of vectors as well as monthly air temperatures in 1999, 2010, and 2012 (these

years were selected due to a combination of high average seasonal air temperatures and the greatest number of WNF cases registered in the Volgograd region in them).

We performed correlation analysis of effects produced by variable factors on the WNF incidence using Microsoft Excel 2016 16.0.13628.20128 (Microsoft, USA). We applied a free-access JASP software package, version 0.14.1 (The University of Amsterdam, Netherlands) to determine how the analyzed indicators were distributed and to estimate statistical significance.

To determine whether an identified correlation was statistically authentic, we compared the obtained values with critical values of Pearson's rank correlation coefficients (*p*) with statistical significance taken as equal to 0.05.

Prior to the analysis, we checked whether all the data conformed to the normal distribution by using Kolmogorov – Smirnov test. As a result, we established that values of all the analyzed factors were distributed normally; therefore, the further analysis involved calculating Pearson's correlation coefficients.

Results and discussion. We performed multi-factor correlation analysis aiming to identify priority groups of indicators for further investigation. Data used in the analysis included average annual air temperatures, water levels detected at hydrological posts in Volgograd and Srednyaya Akhtuba, numbers of vectors as well as the WNF incidence over the period 2001–2017. Table 1 provides the results.

The obtained values of the correlation coefficient for an annual average air temperature indicate there is a strong correlation between this factor and the WNF incidence. At the same time, correlations between water levels and numbers of vectors were either weak or even very weak.

Therefore, given these results, we can assume that the air temperature is a factor with the most significant influence on the WNF incidence in the Volgograd region. This might be due to effects produced by air temperatures on several other factors such as a development rate, a growth in quantity, activity and infection rate of primary WNV vectors in natural and anthropogenic infection foci as well as levels to which natural and artificial basins heat up; the latter is significant since such basins are major places for mosquito reproduction.

However, use of averaged data in correlation analysis does not fully allow estimating influence produced by each separate factor. Given that, the further analysis with different datasets was performed for each analyzed factor separately.

Estimating intensity of the correlation between air temperature and the WNF incidence. Having estimated influence exerted by average winter (December-February) and average summer (June-August) temperatures on the WNF incidence, we established that the value *R* was negative (-0.094 (p = 0684)) for average winter temperatures. This indicates there is no statistically significant correlation in this case. At the same time, the correlation coefficient value equaled 0.631 ($p = 0.002$) for average summer temperatures and this means there is an average correlation with quite high reliability (Figure 1).

We cannot state that average winter temperatures have zero influence on the WNF incidence in the Volgograd region since they produce certain effects on survival rates of primary vectors. Thus, favorable conditions for mosquito larvae to survive a winter season can persist in basements where air temperatures are higher than outside. In addition, there are data in literature indicating that some mosquito species are able to survive the winter in places of their natural reproduction at temperatures below -10 °C [17]. Given the aforementioned and based on average monthly temperatures in January, February and December and the WNF incidence in the Volgograd region in 1999, 2010, and 2012, we established that average temperatures in February and December had a strong correlation with the WNF incidence (*R* equaled 0.809 for February, $p = 0.4$; 0.824 for December, $p = 0.384$) whereas an average air temperature in January in the same years had only a weak correlation with the WNF incidence ($R = 0.125$, $p = 0.92$). Overall, it is necessary to examine influence exerted by air temperatures in winter on the incidence and number of vectors in greater detail.

For further analysis, we also chose years with the highest WNF incidence and months

Table 1

Results of multi-factor correlation analysis

Influence on the WNF incidence	*R*	*p*
Average annual water level (a hydrological post in Volgograd)	0.086	0.744
Average annual water level (a hydrological post in Srednyaya Akhtuba)	0.135	0.606
Average annual air temperature	0.721	0.001
Average annual number of vectors	0.069	0.792

N o t e : *R* is Pearson's correlation coefficient, *p* is statistical significance.

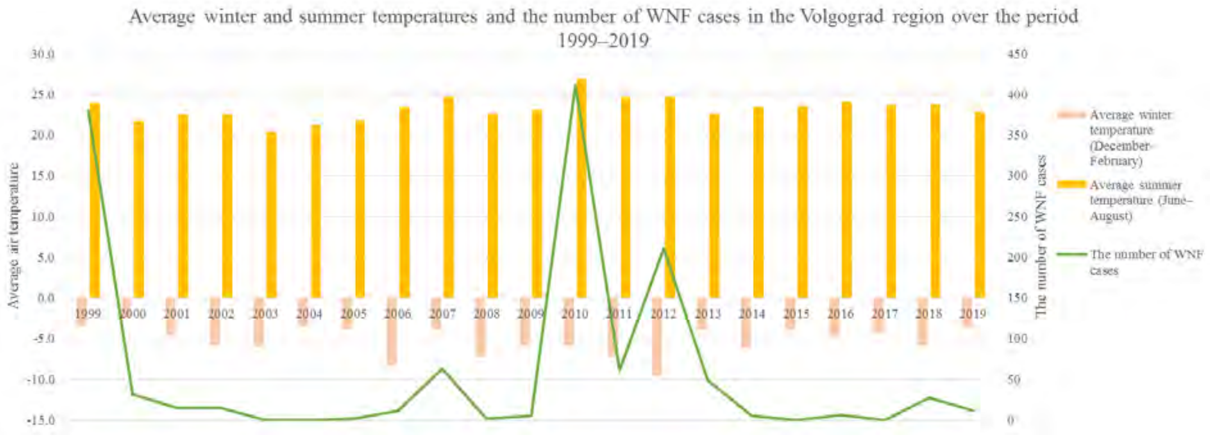


Figure 1. Correlations between average winter and summer temperatures and the WNF incidence

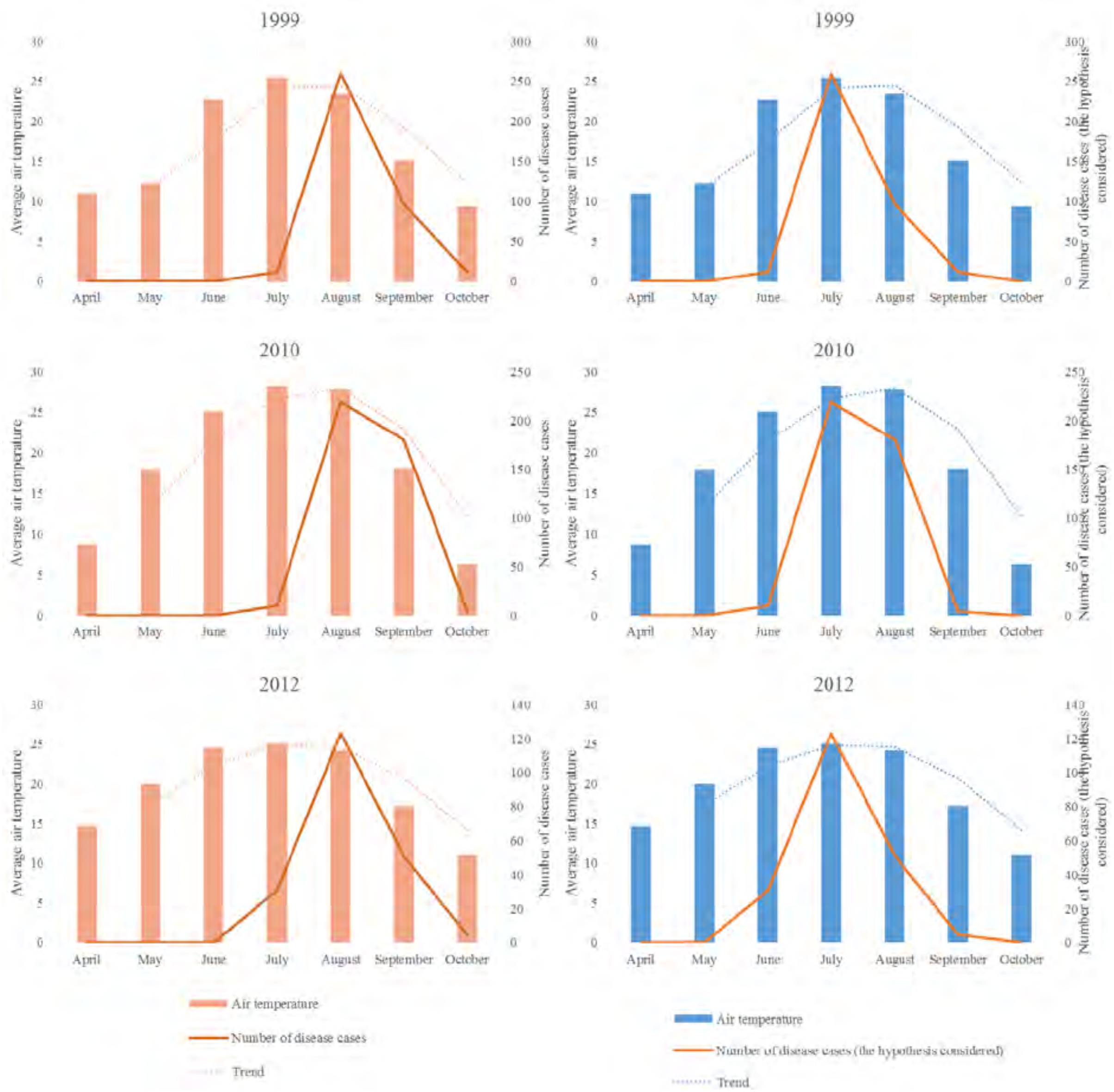
when average air temperatures were above +3...+5 °C thereby helping *Culex* mosquitos, primary WNV vectors, fly away from places where they usually spend the winter [18].

We took data on average monthly air temperatures and registered WNF cases in the Volgograd region over the period between April and October in 1999, 2010 and 2012 and performed correlation analysis; as a result, we established a moderate correlation between these temperatures and the WNF incidence (*R* was within a range between 0.36 and 0.395 (Figure 2A, Table 2)).

Given that the analyzed data were discrete, we considered a hypothesis on a cumulative effect produced by air temperatures on the WNF incidence. In particular, when analyzing time series, we made an assumption that effects produced by temperature on the incidence were delayed, that is, air temperature in the previous month had a positive correlation with the current rate of the WNF incidence. Therefore registered disease cases were shifted one month back along the time scale (Figure 2B). This time delay can be explained, first of all, by a time period necessary for the development cycle of primary vectors to be completed. Thus, according to data available in literature, *Culex pipiens* mosquitos need approximately 30 days under a temperature equals 16 °C for their eggs to develop into the imago stage; if a temperature rises to 24 °C, the full cycle takes approximately 12 days [19]. Second, this delay appears due to a long incubation period (between 3 and 14 days) when a person is infected with the WNF infectious agent.

When correlation analysis was performed considering a time delay (by 12, 21 and 30 days), a correlation became more intense and turned from average to strong and the greatest intensification was detected for the 30-day period (Table 2). Moreover, when we analyzed data within the temperature range between 22 and 29 °C (we considered linear regression), the correlation intensity was very high and became linear with high reliability in 1999 and 2010. In 2012 we detected an insignificant increase in the correlation intensity; this might be due to, among other things, effects produced by other factors during the epidemic season in that year.

The offered hypothesis was extrapolated onto the whole period during which the WNF incidence was registered in the Volgograd region. The delayed effects produced by air



A B

Figure 2. Estimating intensity of correlations between average monthly air temperatures and the WNF: *A* shows average monthly temperatures and the WNF incidence in 1999, 2010, and 2012; *B* shows average monthly temperatures and the WNF incidence (with all the values being shifted 1 month backward) in 1999, 2010, and 2012

Table 2

Results of correlation analysis with its focus on correlations between average monthly temperatures and the WNF incidence

Year	*R (p)*	R_c *(p)*	R_d *(p)*
1999	0.386 (0.393)	0.735 (0.06)	0.998 (0.037)
2010	0.36 (0.427)	0.724 (0.066)	0.998 (0.037)
2012	0.395 (0.38)	0.713 (0.072)	0.832 (0.375)

Note: R_c is the correlation coefficient with the incidence being shifted by 1 month; R_d is the correlation coefficient with the incidence being shifted by 1 month within a temperature range between 22 and 29 °C; *p* is significance of a correlation.

temperatures on the WNF incidence were identified in all the observed years: the correlation coefficient varied from 0.599 (2013) to 0.837 (2008); the correlation was linear within the temperature range between 22 and 29 °C in those years when the registered WNF incidence was higher than its long-term average rate. Nevertheless, absence of a linear correlation in the remaining years requires exploring a set of factors that mitigate risks of infection.

According to the results, the hypothesis was confirmed for the Volgograd region that air temperatures higher than 22 °C in the current month increase a risk that the WNF incidence would grow next month.

Estimating intensity of a correlation between air temperatures and the number of vectors. Correlation analysis that relied on average annual values of these two indicators over the period 1999–2018 became the first stage in estimating a correlation between air temperatures and the number of vectors (Figure 3).

As a result, we established an authentic absence of statistically significant correlations between the analyzed factors ($R = -0.153$, $p = 0.52$).

Further correlation analysis was performed using data on average monthly temperatures in winter and spring-autumn periods as well as average monthly numbers of vectors in 1999, 2010, and 2012.

Having analyzed average monthly temperatures in January, February and December in 1999, 2010, and 2012 and the average annual number of vectors, we established a very strong correlation between the analyzed factors In January and February as well as absence of any statistically significant correlation between an average monthly temperature and the average annual number of vectors in December in the aforementioned years. The obtained results had high statistical authenticity in all the analyzed cases (Table 3).

We analyzed average monthly ait temperatures and average monthly numbers of vectors in 1999, 2010, and 2012 (Figure 4A, Table 4). The resulting values of the correlation coefficient indicated that the correlation intensity varied between average and strong with high statistical authenticity.

In our further analysis of time series, we considered a hypothesis that average monthly air temperatures had a cumulative effect on the number of WNF vectors in the Volgograd region.

Bearing in mind some specific features of primary vectors' life cycle and the incubation period typical for WNF infection (both were considered in the previous section), we assumed that an air temperature that was observed last month would have a positive correlation with the number of WNF vectors in the current month. To confirm the hypothesis, we shifted monthly numbers of mosquitos in 1999, 2010 and 2012 one month back on the time scale (Figure 4B). In this case the results obtained by correlation analysis with linear regression taken into account established that the analyzed correlation was very strong (Table 4). Having analyzed data within the temperature range above 22 °C, we established that the calculated values had lower statistical significance. This might be due to an

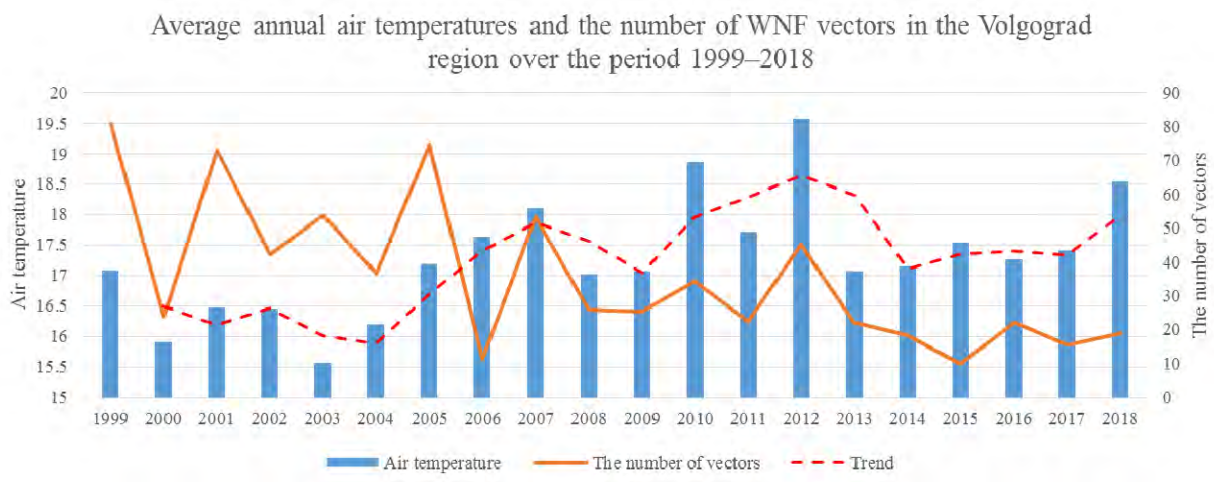


Figure 3. Estimating intensity of a correlation between air temperatures and the average annual number of WNF vectors in the Volgograd region

Table 3

The results of correlation analysis with its focus on average monthly temperatures and the average annual number of vectors

Months	*R*	*p*
January	0.9995	0.019
February	0.706	0.501
December	-0.434	0.714

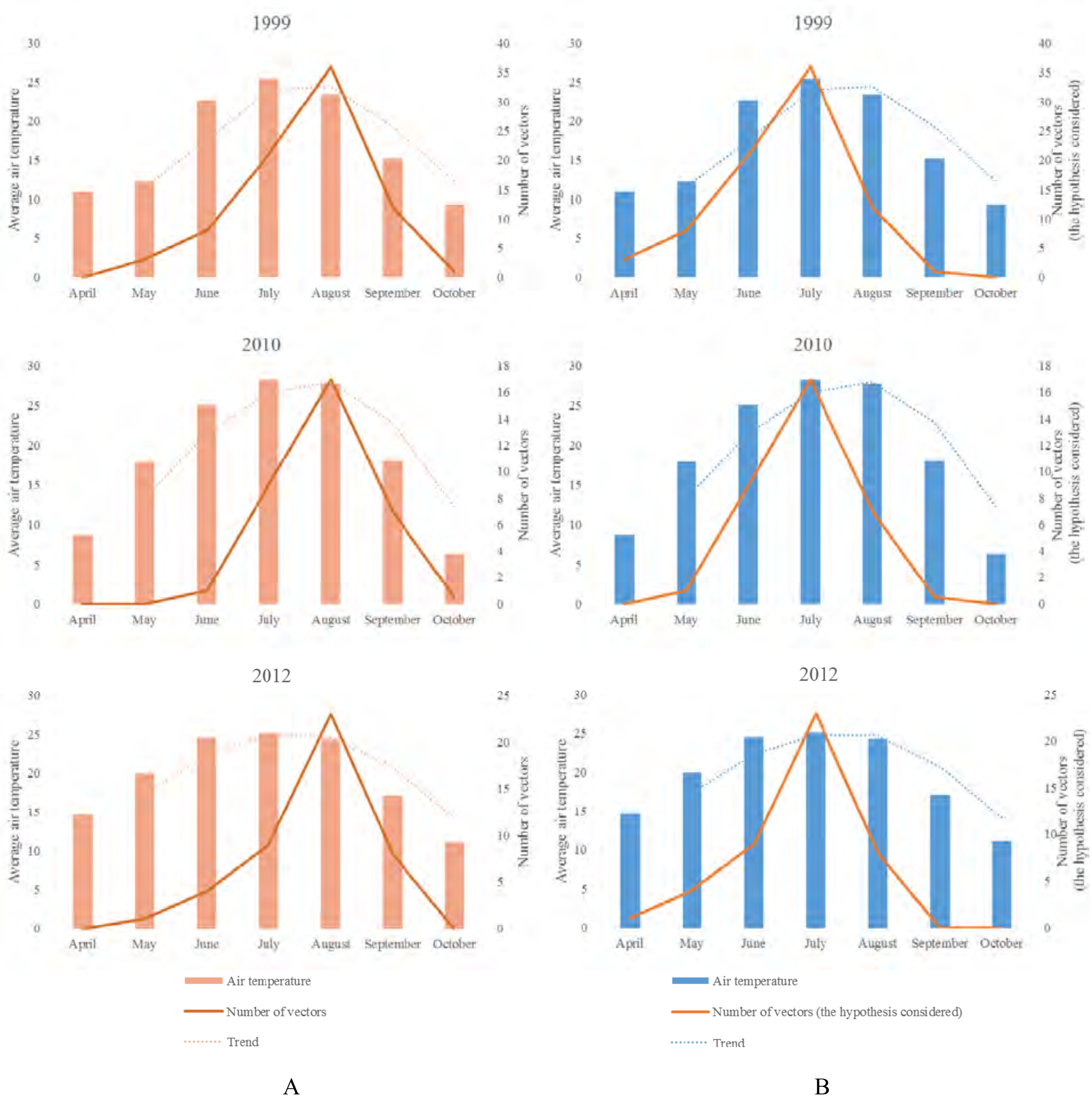


Figure 4. Estimating intensity of the correlation between average monthly air temperatures and the number of WNF vectors in the Volgograd region: *A* shows average monthly air temperatures and the number of WNF vectors in 1999, 2010, and 2012; *B* shows average monthly air temperatures and the number of WNF vectors (with the values being shifted 1 month backward) in 1999, 2010, and 2012

Table 4

The results of correlation analysis with its focus on average monthly temperatures and the number of WNF vectors

Year	*R (p)*	*Rc (p)*	*Rd (p)*
1999	0.784 (0.037)	0.844 (0.017)	0.789 (0.421)
2010	0.672 (0.098)	0.807 (0.028)	0.437 (0.712)
2012	0.596 (0.158)	0.771 (0.042)	0.949 (0.204)

Note: R_c is the correlation coefficient with a 1-month shift of the number of vectors; R_d is the correlation coefficient with a 1-month shift of the number of vectors within the temperature range above 22 °C; *p* is statistical significance of a correlation.

increase in the number of vectors in previous months (April – June).

Therefore, the results obtained by the accomplished analysis are highly reliable and indicate that the hypothesis is true for the Volgograd region: air temperatures higher than 22 °C in the current month have certain effects on an increase in the number of primary WNF vectors in the following month. Influence exerted by air temperatures on the growing number of WNF vectors occurs in a period between April and June when an average monthly air temperature is below 22 °C. This indicates that a threshold temperature level necessary for mosquito populations to grow is lower than a level required for a growth in the WNF incidence. Given that, we examined research articles that addressed a correlation between an air temperature and WNV accumulation as a factor determining the infection rate in vectors.

Estimating intensity of a correlation between air temperatures and the rate of virus accumulation in vectors (based on data available in literature). According to various research data, an air temperature is a basic factor for virus accumulation in vectors. The lowest temperature for the virus development in a mosquito equals +14 °C. A growing temperature makes for faster virus accumulation. Thus, if a temperature equals +14 °C, the quantity of viruses necessary to infect a human is accumulated in 58 days; +18 °C, in 22 days; +23.5 °C, in 15 days; +30 °C, in 11 days [20] (Figure 5A).

We estimated how intense a correlation was between an air temperature and infection rate in vectors; estimations were based on data available in literature. This analysis made it possible to establish an average correlation (within its upper limits). The obtained results were highly reliable (Table 5).

Within further analysis, we excluded air temperatures below +14 °C since it takes the virus about two months to accumulate in a mosquito under such a temperature and in most cases it is longer than an average life span of basic WNV vectors (Figure 5B). Besides, temperatures range between +18 and +25 °C is optimal for vectors' activity.

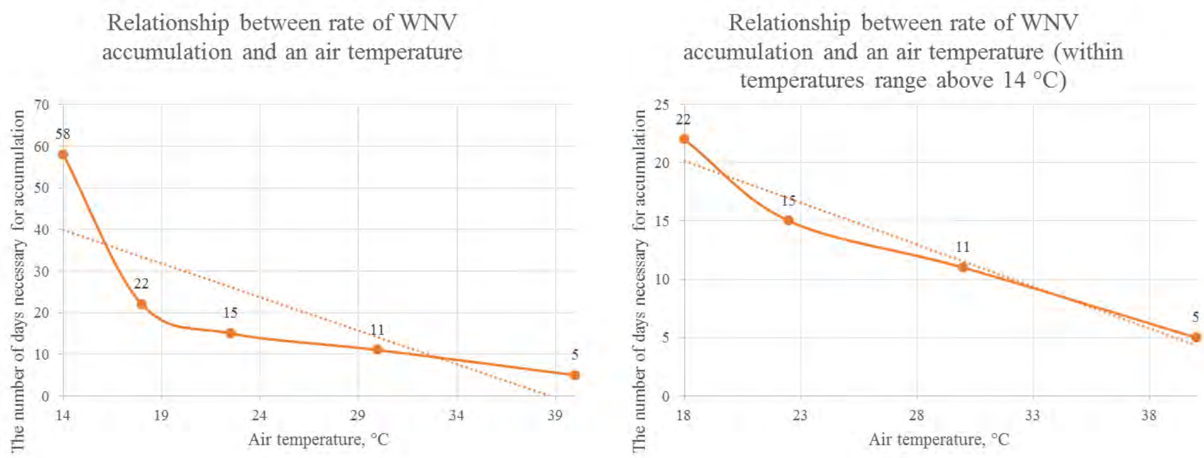
The accomplished correlation analysis (with linear regression) established a string correlation between air temperatures and the rate of WNV accumulation in mosquitos (Table 5).

Therefore, we can assume that if temperatures are above 14 °C, the rate of WNV accumulation in mosquitos will grow; consequently, a risk of infection spread among people will also increase. The higher an air temperature, the higher is a risk of infection. However, more detailed description of this correlation requires further investigation.

Estimating intensity of a correlation between the number of vectors and the WNF incidence. We performed correlation analysis to estimate intensity of a correlation between the number of vectors and the WNF incidence based on average seasonal numbers of mosquitos and data on the annual WNF incidence in the Volgograd region over the period 1999–2018. The analysis revealed a weak correlation and low reliability of the obtained results (Figure 6A).

Further analysis employed data on average seasonal numbers of vectors and the WNF incidence in 1999, 2010, and 2012. The correlation coefficient value indicated the correlation was very weak ($R = 0.081$; $p = 0.948$).

However, despite apparent absence of any correlation between the number of vectors and the WNF incidence, we cannot completely deny its existence since it can be more complicated in its essence and cannot be identified by



A B

Figure 5. Estimating intensity of the correlation between air temperatures and rate of virus accumulation in a mosquito

Table 5

The results of correlation analysis with its focus on the correlation between air temperatures and rate of virus accumulation in a mosquito

Factor	*R*	*p*
Accumulation rate	-0.796	0.107
Accumulation rate within temperatures range above 14 °C	-0.974	0.026

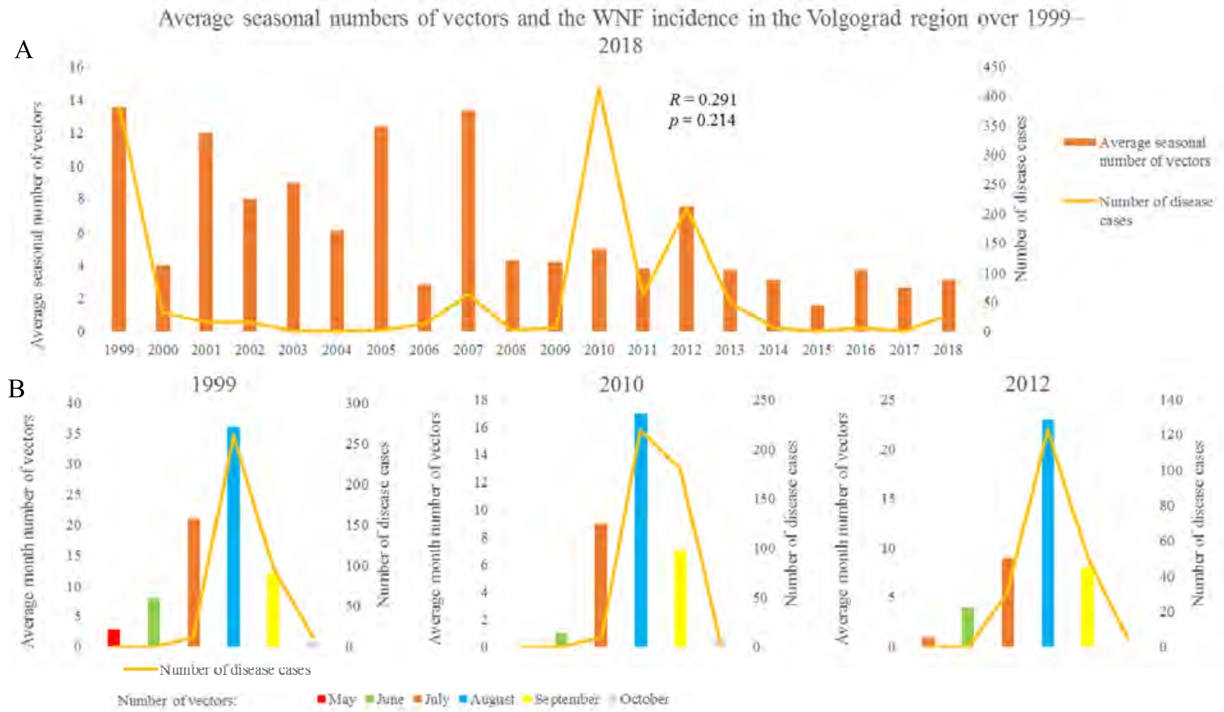


Figure 6. Estimating intensity of the correlation between the number of vectors and the WNF incidence

estimating average seasonal values. Given that, we investigated a correlation between average monthly numbers of mosquitos and monthly WNF incidence in 1999, 2010, and 2012. We established that the correlation intensity varied between high and very high in different years (Figure 6B, Table 6). We should note that this factor made the leading contribution to the WNF incidence rate in 2012.

Given all the obtained data, we can state that the great number of primary vectors increases a risk of people getting infected with WNF in the Volgograd region with high reliability.

Estimating intensity of a correlation between the number of vectors and other natural and climatic factors. Apart from air temperatures, the number of vectors can be influenced by a water level and temperature. Since comprehensive statistical data were not available, we performed our analysis using average annual water levels and temperatures detected at hydrological posts in Volgograd and the urban settlement of Srednyaya Akhtuba over the period 2001–2017 as well as the number of mosquitos in the Volgograd region (Table 7).

The results gave an opportunity to establish a strong correlation between average annual water levels at the hydrological post in Volgograd and average annual numbers of WNF vectors as well as an average correlation between these factors for the hydrological post in Srednyaya Akhtuba. At the same time, average annual water temperatures did not produce any effects on the number of vectors.

Therefore, we can state that an increase in a water level of the Volga River makes for growing numbers of WNF vectors in the Volgograd region. However, more profound examination of correlations between the analyzed factors requires more comprehensive research with wider sets of necessary statistical data.

The conceptual scheme of the predictive-analytical model. Considering all the established correlations between the analyzed factors as well as the offered hypotheses, we developed a conceptual scheme of a predictive-analytical model (Figure 7).

The developed concept requires further mathematical description that would employ various approaches, calculations based on retrospective and operative analytical data as well as estimating predictive value of obtained results.

Conclusion. The performed correlation analysis made it possible to confirm that air temperatures are the most significant factor that influences the WNF incidence in the Volgograd region. Its influence is characterized with combined effects produced on heating of water in natural basins, which are the basic place for WNV vectors' reproduction. This factor also influences the number of mosquito populations, their daily activity and infection rate with WNF infectious agent.

Correlation analysis also allowed us to establish that use of detailed statistical data (such as average monthly air temperatures, average monthly numbers of vectors, etc. against average annual ones) gave an opportunity to

Table 6

The results of correlation analysis with its focus on average monthly numbers of mosquitos and the WNF incidence

Year	*R*	*p*
1999	0.841	0.018
2010	0.811	0.027
2012	0.97	0.001

Table 7

The results of correlation analysis with its focus on the number of vectors, average water levels and average annual water temperatures over the period 2001–2017

Indicators	Hydrological post in Volgograd, *R (p)*	Hydrological post in Srednyaya Ahktuba, *R (p)*
Water level	0.609 (0.009)	0.503 (0.04)
Average annual water temperature	-0.12 (0.645)	-0.172 (0.509)

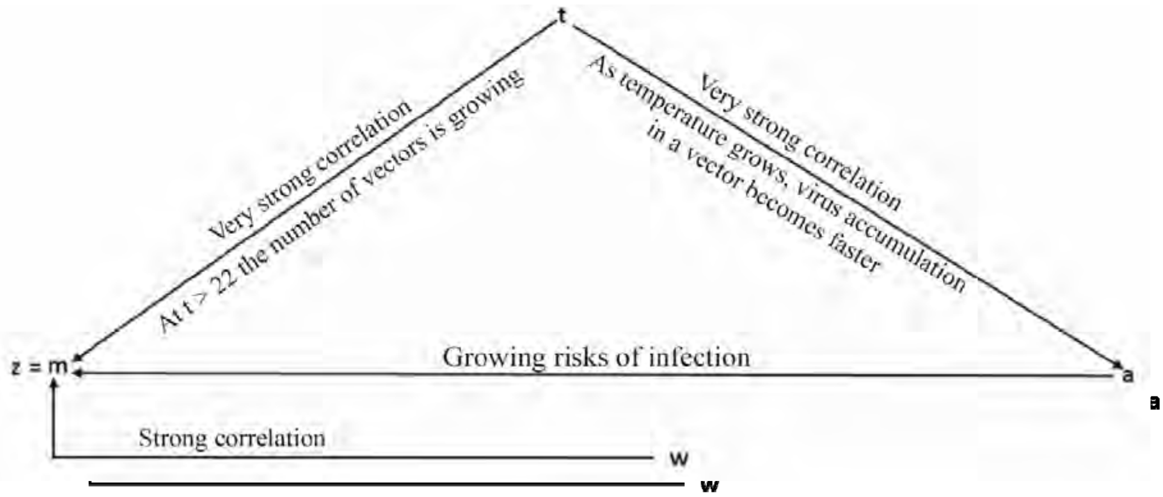


Figure 7. The conceptual scheme of the predictive-analytical model: **z** is the WNF incidence in the current month, **t** is the air temperature in the previous month, **w** is average seasonal water level, **a** is the virus accumulation rate

achieve more precise estimations of correlations between the analyzed factors.

In this study, we were able to confirm the hypothesis on cumulative effects produced by an air temperature on the WNF incidence and the number of WNF vectors in the Volgograd region. These effects were apparent in those years when the WNF incidence rates were the highest (1999, 2010, and 2012).

Also, the study results made it possible to establish correlations between the number of WNF vectors, air temperatures, average annual water level and the WNF incidence; these correlations had different intensity.

Overall, correlations between air temperatures and other factors analyzed in this study are rather complicated since they have different directions (can produce both positive and negative effects on the number of vectors and their natural biotopes); their effects can differ in their intensity, which is determined by existing optimal temperature limits (the number of vectors and infection rate grow faster within them); and influence exerted by air temperatures is delayed over time.

The obtained results gave grounds for developing the conceptual scheme of the predictive-analytical model described above. This model makes it possible to estimate how an epidemiological situation regarding the WNF would develop in the Volgograd region. Our future studies will address mathematical description of the developed scheme together with estimating its predictive value. This will allow achieving more precise forecasts and outlining prospects for this approach to be used in other regions in the Russian Federation.

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Competing interests. The authors declare no competing interests.

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Research article

NFAT5 GENE POLYMORPHISM AS A RISK FACTOR OF KNEE OSTEOARTHROSIS

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Knee osteoarthrosis (OA) is a multifactorial disease with genetic factors playing an important part in its development. Our research goal was to examine associations between polymorphic variants rs1060105 and rs56116847 of SBNO1 gene, rs6499244 of NFAT5 gene and rs34195470 of WWP2 gene and developing stage 4 knee osteoarthrosis in people living in the Central Chernozem Region in Russia.

Genotyping of polymorphic loci of candidate genes was accomplished in 95 patients with stage 4 knew osteoarthrosis and 500 people without the disease who were included into the reference group. We estimated associations between polymorphic loci of candidate genes and knee OA by using logistic linear regression within the allele, additive, recessive and dominant genetic models with gPLINK software.

As a result, we replicated an association between a GWAS-significant rs6499244 polymorphism of NFAT5 gene and knee OA in people living in the Central Chernozem Region in Russia. An allele variant A of rs6499244 of NFAT5 gene was established to be "a risk factor" regarding stage 4 knee OA within the additive (OR = 1.61, p_{perm} = 0.02) and recessive (OR = 2.07, p_{perm} = 0.02) genetic models. The rs6499244 locus of NFAT5 gene is located in an area of DNAse I hypersensitivity; it increases DNA affinity to four transcription factors (CDP_6, RFX5_known1, RORalpha1_2, TCF4_known1); it is localized in functionally active promoters and enhancers; it is associated with expression of nine genes (CLEC18A, COG4, EXOSC6, NFAT5, NOB1, NPIPB14P, NQO1, PDXDC2P, SMG1P7) and alternative mRNA splicing of three genes (NOB1, NPIPB14P, NQO1) in various organs and tissues in the body including those that are pathogenetically significant for OA (fat tissue, tibial nerves and arteries, and skeletal muscles).

Keywords: *knee osteoarthritis, NFAT5 gene, polymorphic locus, associations, candidate genes, risk factor, nuclear factor of activated T cells 5, evaluation of functional effects produced by polymorphism.*

Knee osteoarthrosis (OA) or gonarthrosis is a complex disease that involves degenerative, dystrophic and sometimes inflammatory changes in tissues forming the knee joint (cartilage, menisci, subchondral bone, synovial membrane, ligaments, and fibrous capsule) as well as in tissues beyond it, which are still an integral part of functions performed by the knee joint and are located close to it (tendons and muscles) [1]. According to data available in literature, OA prevalence is different in different populations across the world [2–5]. OA prevalence varies from 1.5 to 29.1 % in European countries [2, 3, 6–8] whereas this indicator is higher in Asia where it can reach 44 % [9–12]. According to official statistics, in Russia 13 % of people who are older than 18 have OA of the knee or hip joint [5, 13] and this corresponds to the disease prevalence in European populations. Gonarthrosis is also known to become more frequent among older people and its prevalence is higher among women [6, 14]. We should remember that knee OA develops in working age population thus deterio-

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rating their health and resulting in untimely disability [15].

Osteoarthrosis, just like some other human diseases, is considered a multifactorial disease that occurs due to interactions between several environmental, epigenetic and genetic risk factors [16]. As it has been stated in research works, a contribution made by the genetic component to OA development may vary from 40 to 65 % [17, 18]. Genetic grounds for OA development are being actively studied by various research teams who perform whole-genome sequencings aimed at detecting relevant associations. According to the GWAS Catalog, over a period from 2008 up to now 24 whole-genome sequencings have been accomplished with their focus on the knee and hip joints as well as hand joints[1]. As a result, researchers have detected more than 250 GWAS-significant single-nucleotide polymorphisms (SNPs) associated with OA development. We should note that 13 out of 24 studies have concentrated on examining a role that belongs to genetic factors in knee OA development; they made it possible to establish more than 80 polymorphic loci associated with risks of knee OA development ($p \leq 5 \cdot 10^{-08}$). The majority of these loci that are GWAS-significant for knee OA (more than 95 %) were established by analyzing samplings made of Caucasian people. But we should also note that Russian people have never been included in samplings analyzed in the aforementioned whole-genome sequencings.

In addition to whole-genome sequencing of OA, we should mention replication studies that are also significant. They give an opportunity to establish a role played by specific polymorphic markers that are GWAS-significant for OA in development of the disease in a given population across the world, Russian ones included. According to data available in literature, by now there has been a rather limited number of replication studies with their focus on knee OA and OA with other localizations performed in various populations worldwide [19–26]. These replication studies have established associations with the disease for isolated GWAS-significant polymorphic loci: OA in general, 4 SNPs (rs2302061, rs7639618, rs4836732, rs3204689); knee OA, 7 SNPs (rs4867568, rs143383, rs3884606, rs10947262, rs7639618, rs6976, rs2302061). Three out of seven SNPs (rs4867568, rs143383, rs3884606) had a confirmed association with knee OA in Asian populations and four SNPs (rs10947262, rs7639618, rs6976, rs2302061) in European ones or in mixed samplings that included both Europeans and Asians.

It is important to point out that in Russian literature there has been only one replication study focusing on OA among women in Bashkortostan [26]. The authors analyzed associations between OA in women and nine GWAS-significant polymorphisms including rs4836732of *ASTN2* gene; rs1298744 and rs2302061 of *DOT1L* gene, rs3204689 of *ALDH1A2* gene, rs6976 of *GLT8D1* gene, rs11177 of *GNL3* gene, rs6094710 of *NCOA3* gene, rs11841874 of *MCF2L* gene, and rs7639618 of *DVWA* gene. The analysis was performed in two groups, the test one made of 256 patients and the reference one made of 161 women without the disease. The study confirmed associations with OA with different localizations only for five SNPs (rs2302061 *DOT1L*, rs7639618 *DVWA*, rs4836732 *ASTN2*, rs6976 *GLT8D1*, and rs3204689 *ALDH1A2*); an association with knee OA was confirmed only for three SNPs, namely rs2302061 *DOT1L*, rs7639618 *DVWA*, and rs6976 *GLT8D1*.

Therefore, there are more than 80 known polymorphic loci that are GWAS-significant for knee OA. Still, any associations with the disease among European populations are confirmed by replications studies only for four of them, namely rs10947262 of *BTNL2* gene, rs7639618 of *COL6A4P1* gene, rs2302061 of *DOT1L* gene, and rs6976 of *GLT8D1* gene and this is extremely insufficient. We should note that these replication studies performed

[1] GWAS Catalog. The NHGRI-EBI Catalog of human genome-wide association studies. *National Human Genome Research Institute*. Available at: https://www.ebi.ac.uk/gwas/search?query=osteoarthritis (May 20, 2022).

on European populations (or mixed samplings) have not confirmed any associations with knee OA for 10 SPNs. All the aforementioned facts call for conducting further replication studies of knee OA in various populations, Russian included.

Our research goal was to examine associations between polymorphic variants rs1060105 and rs56116847 of *SBNO1* gene, rs6499244 of *NFAT5* gene, and rs34195470 of *WWP2* gene and developing stage 4 knee osteoarthrosis among people living in the Central Chernozem Region in Russia.

Materials and methods. To conduct our study, we created two samples. The first one, or the test group, was made of 95 patients with knee OA (their average age was 52.69 ± 5.67 years); the second one was the reference group made of 500 people (their average age was 52.96 ± 6.72 years). All the patients had radiological stage 4 of the disease characterized with the most apparent destruction of various joint tissues, first of all, cartilage and bone [2]. Clinical and instrumental examinations of the patients were performed and their diagnosis was verified by certified physicians at the Traumatology Department No. 1 of the Belgorod City Hospital No. 2. We relied on the following criteria when including patients into our test group: a) a patient had primary knee OA diagnosed in accordance with clinical and radiological criteria developed by the American College of Rheumatology [27]; b) a patient had stage 4 knee OA as per Kellgren – Lawrence scale [28]; c) a patient suffered from knee joint pains (pain syndrome intensity was ≥ 40 mm as per the visual-analog scale when walking); d) movement was limited in the knee joint. The reference group included people without any pathology of the musculoskeletal system (its absence was identified during periodical medical examinations). All the participants were born in the Central Chernozem Region, were native Russians and were not blood relatives; they all gave their consent to participate in the study prior to it. People with severe hypertension, ischemic heart disease, diabetes mellitus, liver and kidney failure or oncological diseases were excluded from the analyzed samples.

We examined genome DNA in our study. It was isolated from leukocytes by phenol chloroform extraction. DNA samples were genotyped by performing the polymerase chain reaction with TaqMan probes.

We selected polymorphic loci of candidate genes to analyze them in our study using the following criteria: 1) an association with knee OA established in European population by previous whole-genome studies (GWAS); 2) apparent functional significance of a polymorphism (regulatory potential, an association with expression and alternative gene splicing) [29, 30]; 3) frequency of a polymorphism being not less than 5 %. Four SPNs of three genes were selected for the present study in accordance with these criteria. They were rs1060105 and rs56116847 of *SBNO1* gene, rs6499244 of *NFAT5* gene and rs34195470 of *WWP2* gene.

We determined empirical distribution of genotypes for the analyzed samplings and checked whether it corresponded to a theoretically expected one in accordance with the Hardy – Weinberg law (deviations were considered statistically significant at $p \leq 0.05$). When comparing frequency of alleles and genotypes in the test group (patients with OA) and the reference group, we applied χ^2 criterion with Yates's correction for continuity and contingency 2×2. Intensity of associations was estimated using odds ratio (*OR*) with 95 % confidence interval (95 % CI) with STATISTICA for Windows 6.0. Associations between OA and the analyzed polymorphic loci of candidate genes were estimated by using logistic linear regression (we considered four genetic models, namely, allele, additive, recessive and dominant one), such covariants as age and BMI taken into account. All the calculations were performed with gPLINK software [31]. We performed multiple comparison correction allowing for a permutation test ($p_{perm} < 0.05$ was considered statistically significant) [32].

We estimated functional effects produced by polymorphism of candidate genes that had significant associations with OA by using

HaploReg (v4.1)[2] to examine regulatory potential [29, 33] and GTExPortal[3] to examine influence exerted on gene expression and alternative splicing [30].

Results and discussion. We established that the actual distribution of genotypes corresponded to the expected one according to the Hardy – Weinberg equilibrium for all the analyzed polymorphic loci of candidate genes in the test and reference groups ($p > 0.05$) (Table 1).

We analyzed associations between alleles and genotypes of examined candidate genes polymorphism and developing stage 4 knee OA and established an association between rs6499244 locus of *NFAT5* gene and the disease (Table 1). The logistic regression analysis revealed that the minor allele A in rs6499244 of *NFAT5* gene had certain "risky" significance for OA within the additive (OR = 1.61, 95 % CI = 1.09–2.38, p = 0.02, p_{perm} = 0.02) and recessive (OR = 2.07, 95 % CI = 1.10–3.90, p = 0.02, p_{perm} = 0.02) genetic models.

Therefore, we identified a genetic risk factor of knee OA development in our study. This factor is the allele A in rs6499244 of *NFAT5* gene.

The next stage in our study involved analyzing regulatory effects produced by rs6499244 of *NFAT5* gene (using up-to-date bioinformatics resources). The analysis revealed that this locus had critical functional importance in the body.

Use of HaploReg (v4.1)[2] made it possible to establish that rs6499244 was located in the 3'-non-translated area in *NFAT5* gene, in an evolutionary conservative area of DNase I hypersensitivity in ovary tissues, in a region of regulatory motifs to four transcription factors (CDP_6, RFX5_known1, RORalpha1_2, TCF4_known1). We identified that rs6499244 of *NFAT5* gene was localized in a DNA area associated with histones (H3K27ac) that marked active enhancers in chondrocyte cell culture, brain, fat tissue, and peripheral blood cells. It was also localized in a DNA-area interacting with H3K9ac type histones that marked functionally active promoter sections in fat tissue cells and primary mononuclear cells in peripheral blood. The allele A, which is "risky" as regards OA development, increases affinity to four transcription factors: CDP_6 (ΔLOD = 3,3), RFX5_known1 (ΔLOD = 10,9), RORalpha1_2 (ΔLOD = 12), TCF4_known1 (ΔLOD = 5).

Use of GTExPortal[3] made it possible to establish that the locus rs6499244 had a significant association with expression of nine genes (*CLEC18A, COG4, EXOSC6, NFAT5, NOB1, NPIPB14P, NQO1, PDXDC2P, SMG1P7*) and alternative mRNA splicing of three genes (*NOB1, NPIPB14P, NQO1*) in cell cultures, tissues and organs that had pathogenetic significance for OA development (fat tissue, skeletal muscles, tibial arteries and nerves) (Table 2). We revealed that rs6499244 was associated with expression of *CLEC18A, EXOSC6, NPIPB14P, SMG1P7* genes and alternative mRNA splicing of three genes *NOB1, NPIPB14P, NQO1* in fat tissues. Fat tissue is known to produce adipokines that not only participate in metabolic processes but also make for chronic inflammatory reactions in the body. Adipokines and some other cytokines act together and these combined actions lead to cartilage tissue degradation [34]. T.N. Boer with colleagues (2012) showed that levels of adipokines were substantially higher in patients with radiological stage 4 osteoarthrosis than in the reference group (p < 0.001) [35]. We should note that *NFAT5* gene also participates in regulating inflammatory reactions in the body due to activation of immune cells, especially T-cells and macrophages [36].

We established that the allele A in rs6499244 of *NFAT5* gene was associated with low expression of *CLEC18A, NOB1, NPIPB14P* genes and alternative mRNA splicing of *NOB1* gene in skeletal muscles (Table 2). The condition of muscle tissues, muscle weakness included, is known to make a substantial contribution to OA development and progressing [37, 38]. Muscle weakness can be caused not only by absence of

[2] HaploReg v4.1. Available at: http://compbio.mit.edu/HaploReg (May 18, 2022).
[3] GTExPortal. Available at: http://www.gtexportal.org/ (May 18, 2022).

Table 1

Frequencies of alleles and genotypes established for polymorphic loci of candidate genes in the patients with stage 4 knee OA and the reference group

Polymorphism	Alleles, genotypes	Patients with stage 4 knee OA (*n* = 95), abs.(%)	Reference group (*n* = 500), abs.(%)	*OR* (95 % CI)	*p*
rs1060105	C	149 (78.42 %)	788 (78.80 %)	0.98 (0.66–1.95)	0.98
	T	41 (21.58 %)	212 (21.20 %)	1.02 (0.69–1.52)	
	C/C	58 (61.05 %)	315 (63.00 %)	0.92 (0.57–1.48)	0.81
	C/T	33 (34.74 %)	158 (31.60 %)	1.15 (0.71–1.87)	0.63
	T/T	4 (4.21 %)	27 (5.40 %)	0.77 (0.22–2.39)	0.82
	H_o/H_e (P_{HWE})	0.347/0.338 (1.000)	0.316/0.334 (0.229)		
	Minor allele T (allele model)			1.02 (0.70–1.49)	0.91
	C/Cvs. C/Tvs. T/T (additive model)			1.24 (0.77–1.98)	0.38
	C/Cvs. C/T + T/T (dominant model)			1.37 (0.78–2.42)	0.28
	C/C + C/Tvs. T/T (recessive model)			0.95 (0.25–3.65)	0.94
rs56116847	G	119 (62.63 %)	640 (64.26 %)	0.93 (0.67–1.30)	0.73
	A	71 (37.37 %)	356 (35.74 %)	1.07 (0.77–1.50)	
	G/G	37 (38.95 %)	213 (42.77 %)	0.85 (0.53–1.37)	0.56
	A/G	45 (47.37 %)	214 (42.97 %)	1.19 (0.75–1.90)	0.50
	A/A	13 (13.68 %)	71 (14.26 %)	0.95 (0.48–1.87)	1.00
	H_o/H_e (P_{HWE})	0.474/0.468 (1.000)	0.430/0.459 (0.145)		
	Minor allele A (allele model)			1.07 (0.78–1.48)	0.67
	G/G vs. A/G vs. A/A (additive model)			0.84 (0.56–1.28)	0.42
	G/Gvs. A/G + A/A (dominant model)			0.82 (0.47–1.45)	0.50
	G/G + A/Gvs. A/A (recessive model)			0.76 (0.33–1.77)	0.53
rs6499244	T	94 (49.47 %)	543 (54.30 %)	0.82 (0.60–1.14)	0.25
	A	96 (50.53 %)	457 (45.70 %)	1.21 (0.88–1.68)	
	T/T	26 (27.37 %)	157(31.40 %)	0.82 (0.49–1.38)	0.51
	A/T	42 (44.21 %)	229 (45.80 %)	0.94 (0.59–1.49)	0.86
	A/A	27 (28.42 %)	114 (22.80 %)	1.34 (0.80–2.26)	0.29
	H_o/H_e (P_{HWE})	0.442/0.500 (0.304)	0.458/0.496 (0.087)		
	Minor allele A (allele model)			1.21 (0.89–1.66)	0.22
	T/T vs. A/T vs. A/A (additive model)			**1.61 (1.09–2.38)**	**0.02**
	T/T vs. A/T + A/A (dominant model)			1.74 (0.91–3.32)	0.09
	T/T + A/Tvs. A/A (recessive model)			**2.07 (1.10–3.90)**	**0.02**
rs34195470	G	101 (53.72 %)	523 (52.40 %)	1.05 (0.76–1.46)	0.80
	A	87 (46.28 %)	475 (47.60 %)	0.95 (0.67–1.31)	
	G/G	24 (25.53 %)	136 (27.25 %)	0.92 (0.54–1.56)	0.83
	A/G	53 (56.38 %)	251 (50.30 %)	1.28 (0.80–2.04)	0.33
	A/A	17 (18.09 %)	112 (22.45 %)	0.76 (0.42–1.39)	0.42

End of the table 1

Polymorphism	Alleles, genotypes	Patients with stage 4 knee OA (*n* = 95), abs.(%)	Reference group (*n* = 500), abs.(%)	*OR* (95 % CI)	*p*
	H_o/H_e (P_{HWE})	0.564/0.497 (0.222)	0.503/0.499 (0.929)		
	Minor allele A (allele model)			0.95 (0.69–1.30)	0.74
	G/G vs. A/G vs. A/A (additive model)			0.97 (0.64–1.47)	0.89
	G/Gvs. A/G + A/A (dominant model)			1.05 (0.55–2.01)	0.88
	G/G + A/Gvs. A/A (recessive model)			0.87 (0.42–1.77)	0.69

N o t e : *OR* is odds ratio, 95 % CI is 95 % confidence interval of odds ratio, *p* is the significance level, H_o/H_e is observed / expected heterozygosis, P_{HWE} is the significance level of a deviation from the Hardy – Weinberg law.

Table 2

Data on associations between rs6499244 of *NFAT5* gene expression (eQTL) and alternative mRNA splicing (sQTL) of genes in cells, tissues and organs pathogenetically significant for OA

Gene	Tissue/organ/cell culture	The significance level *p*	Linear regression coefficient β
eQTL analysis			
CLEC18A	Thyroid gland	1.9e-9	-0.35
	Fat tissue	2.4e-7	-0.30
	Skeletal muscles	0.000057	-0.18
COG4	Fibroblast cell culture	0.000041	-0.10
EXOSC6	Fat tissue	0.0000038	-0.21
	Thyroid gland	0.00040	-0.14
NFAT5	Thyroid gland	9.1e-19	0.18
	Fibroblast cell culture	0.0000012	0.083
NOB1	Skeletal muscles	3.9e-10	-0.11
	Thyroid gland	3.3e-7	-0.14
	Brain	0.000020	-0.33
NPIPB14P	Tibial nerves	4.7e-15	-0.31
	Fibroblast cell culture	1.4e-13	-0.28
	Aorta	3.0e-10	-0.27
	Tibial artery	3.8e-10	-0.21
	Blood	1.0e-9	-0.17
	Brain	2.0e-9	-0.42
	Skeletal muscles	6.7e-9	-0.21
	Fat tissue	7.6e-7	-0.17
NQO1	Brain	0.000061	-0.21
PDXDC2P	Blood	0.000029	0.11
SMG1P7	Fat tissue	0.00013	-0.16
sQTL analysis			
NOB1	Skeletal muscles	3.8e-36	-0.61
	Fat tissue	8.1e-33	-0.66
	Tibial artery	2.0e-30	-0.62
	Thyroid gland	7.9e-30	-0.56
	Fibroblast cell culture	3.4e-29	-0.68
	Tibial nerves	3.6e-26	-0.63
	Blood	1.2e-16	0.42

End of the table 2

Gene	Tissue/organ/cell culture	The significance level *p*	Linear regression coefficient β
NPIPB14P	Blood	2.8e-9	-0.34
	Fat tissue	0.0000032	0.29
	Fibroblast cell culture	0.0000059	-0.28
NQO1	Fibroblast cell culture	2.9e-8	0.30
	Fat tissue	1.6e-7	0.31
	Tibial nerves	0.000023	0.25

Note: eQTL and sQTL are data taken from GTExPortal [30].

physical loads in muscles or their low levels but also by inflammation in them [38] which can be regulated, among other things, by *NFAT5* gene [36]. S. Muraki and others (2015) showed that weakness of the quadriceps muscle of thigh was associated with pains in patients with knee OA [37]. B.E. Oiestad with colleagues performed a systemic review and meta-analysis (2015) and obtained certain data on weakness of knee joint muscles being associated with elevated risks of knee OA both in men and women [38].

S.J. Rice and others (2019) provided data on associations between the locus rs7359336 that was not in equilibrium regarding coupling with rs6499244 ($r^2 = 0.91$), which we analyzed in our study, with elevated DNA methylation in *WWP2* gene in patients with OA [39]. The authors also established a substantial increase in *CLEC18A* gene expression in cartilage tissue in patients who had arthroplasty to treat OA ($p = 0.02$).

NFAT5 gene is known to code a transcription factor that regulates expression of genes participating in osmotic stress in mammals. The latter can become a basis for developing pathological processes in various hyperosmolar tissues and organs (the kidneys, intestinal epithelium, cornea and skin epidermis, and skeletal muscles)[4] [40, 41]. Some data confirm a role *NFAT5* gene plays in creating inborn immunity by activating gene expression in macrophages during TLR (Toll-like) receptor ligation [36, 42, 43]. We should note that immune disorders and inflammation of the synovial membranes (synovitis) are believed to contribute significantly to OA pathogenesis [2, 44, 45]. In future this may lead to “activation” of matrix metalloproteinases [46] that produce apparent proliferative and pro-inflammatory effects. *TLR2* and *TLR4* are known to be expressed intensely in macrophages of synovial fluid and to be responsible for macrophages activation [47]. *NFAT5* gene also plays an important role in proliferation of synoviocytes [48]. Inflammatory infiltrate of synovial fluid taken from patients with gonarthrosis contains large quantities of macrophages and T-lymphocytes [44]. According to data available in literature, macrophages mostly prevail in this infiltrate accounting for 65 %, followed by T-lymphocytes with their share being up to 22 % and B-lymphocytes up to 5 % [49].

There is a rather limited number of studies with their focus on how the locus rs6499244 of *NFAT5* gene (which is associated with knee OA as we have established in the present study) is involved into development of various human diseases. The GWAS Catalog provides data on the results produced by only two whole-genome sequencings that revealed significant associations between the analyzed locus and knee osteoarthrosis [50] and an age when menarche occurs [51]. Thus, I. Tachmazidou with colleagues performed a GWAS study (2019) on samples made of 77,052 patients who had OA with different localizations, knee OA included, and 378,169 people in the reference group. The study estab-

[4] GeneCards: The Human Gene Database. Available at: http://www.genecards.org/ (May 20, 2022).

lished an association between the allele A in rs6499244 of *NFAT5* gene and knee OA in an European population [50] and this is completely in line with our results.

Conclusion. Our study confirmed (replicated) an association between the GWAS-significant polymorphic variant rs6499244 of *NFAT5* gene and knee OA in people living in the Central Chernozem Region in Russia. We established that the allele A in rs6499244 *NFAT5* was a risk factor of stage 4 knee OA (*OR* = 1.61–2.07). rs6499244 polymorphism of *NFAT5* gene produces apparent functional effects (it is located in an area of DNase I hypersensitivity, increases DNA affinity to four transcription factors, is localized in functionally active promoters and enhancers and is associated with expression of nine genes (*CLEC18A, COG4, EXOSC6, NFAT5, NOB1, NPIPB14P, NQO1, PDXDC2P, SMG1P7*) and alternative mRNA splicing of three genes (*NOB1, NPIPB14P, NQO1*)) in various tissues and organs (tibial nerves and arteries, fat tissue, skeletal muscles and others) that are involved into pathophysiology of the disease.

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Review

IMMUNE STATUS AND CYTOKINE SPECTRUM AS PREDICTORS OF THE RISK OF SEVERE DISEASE AND PERFORMANCE INDICATORS OF INTENSIVE THERAPY IN PATIENTS WITH CORONAVIRUS INFECTION COVID-19

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The pandemic caused by a new strain of the SARS-CoV-2 coronavirus has swept the whole world but effective methods for treating this severe pathology have not yet been created. It has now been established that a risk of a severe course of COVID-19 is not so much a patient's age itself, but so-called age-related diseases; the renin-angiotensin system (RAS) is directly or indirectly involved into their development. The SARS-CoV-19 virus interacts with one of the main regulatory elements of this system, ACE2, and disrupts the balance between the two RAS branches. This ultimately manifests itself in an increase in levels of angiotensin II, which, through binding to the angiotensin type 1 receptor (AT1R), causes a number of pathological conditions, including hypertension, atherosclerosis, and cardiovascular diseases, enhances cell proliferation, apoptosis, death of vascular endothelial cells, etc. This process has been described in many reviews by Russian and foreign authors [1, 2]. However, cells of innate and adaptive immunity are another less well-described but no less important target of angiotensin II. The consequences of this interaction are analyzed in detail in this review. With COVID-19, dendritic cells are activated, macrophage proliferation and neutrophil infiltration increase with further involvement of CD4-lymphocytes and other cellular elements of the adaptive immunity in this process. Hyperactivation of the immune system is accompanied with the release of a large amount of pro-inflammatory cytokines, which can lead to the occurrence of a cytokine storm. The picture is aggravated by the inhibitory effect produced by the virus itself on the synthesis of signaling interferons at initial stages in its internalization into the cell. A separate section in the review addresses the problem how to predict a risk of a developing serious condition and search for its predictors by analyzing the state of the RAS and ratios of key cellular elements in the immune system. This is extremely important for making decisions concerning the amount of necessary medical care and strategies for subsequent treatment.

Keywords: *COVID-19, SARS-CoV-2, cytokine profile, cytokine storm, immune cells, immunodysregulation, predicting factor, immune status, renin-angiotensin system (RAS).*

The SARS-CoV-2 is an RNA-containing coronavirus. It is highly contagious and is spread both by sick people and asymptomatic carriers with a positive PCR-test with their share varying between 15 and 25 % [3]. The spike (S) protein in the coronavirus allows it to bind to angiotensin-converting enzyme 2 (ACE2) on target cell surfaces in an area with protease activity. The virus then penetrates a cell by endocytosis [4]. To enter a cell, the SARS-CoV-2 virus also employs the host's type II transmembrane serine protease, which

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acts as S-protein activation co-factor, as well as splitting ACE2 thereby facilitating the merger of virus cells with membranes. It is noteworthy that a receptor responsible for ACE2 binding to the coronavirus is a vital component in the renin-angiotensin system (RAS) functioning. This system plays the key role in maintaining proper homeostasis in the body through regulation of interactions between the cardiovascular and respiratory systems, water-salt and carbohydrate metabolism and blood pressure regulation [5]. The SARS-CoV-2 virus creates considerable imbalance in functioning of this vital system by interacting with the ACE2 receptor. We should not neglect the fact that the virus not only starts to reproduce actively after it has penetrated a cell but also produces certain effects on a response given by a cell to its invasion. By now, these two aspects in effects produced by the virus on the host body have been described in many reviews by Russian and foreign authors; however, there are hardly any works that concentrate on analyzing a close interrelation between these two systems. Therefore, the aim of this review was to analyze outcomes of the RAS impairments caused by the SARS-CoV-2 virus for the elements in the inborn and adaptive immunity as well as to estimate ability to predict a clinical course of the disease considering changes in them.

The RAS physiology and its relation with the immune system. In its normal conditions, the RAS supports proper homeostasis by regulating interactions between the cardiovascular and respiratory systems, water-salt and carbohydrate metabolism and blood pressure regulation [5].

The classic RAS consists of two branches. The first one includes angiotensin / renin / angiotensin I / angiotensin-converting enzyme (ACE) / angiotensin II (ANG II) and its receptors AT1R and AT2R. Interaction between ANG II and AT1R is accompanied with elevated blood pressure, vascular constriction and growing ADAM17 [6] activity. All this leads to occurring circulating TNF-a with subsequent induction of pro-inflammatory cytokines, more intense proliferation, infiltration and apoptosis [5, 7]. At present, it is a well-established fact that not a patient's age itself is a risk factor of severe COVID-19 but so-called age-related diseases that develop due to either direct or indirect involvement of the 1st RAS branch activation and high ANG II levels [8].

The second RAS branch has been discovered only recently. It includes ACE2/ANG 1-7/Mas receptor (Mas R) [9]. Its main function is to inhibit the 1st branch hyperactivity. ANG-(1–7) interacts with Mas receptor thereby inhibiting NF-κB pathway and consequently producing anti-inflammatory and anti-apoptotic effects [10] as well as activating the inborn immunity. ACE2 is a linking component in these two RAS branches and a key enzyme that converts ANG II (1-8) – ligand AT1R into ANG (1-7) with subsequent MasR activation [11]. Obviously, the virus induces a decline in ACE2 receptor density and this leads to imbalance between activity of two RAS branches together with a decrease in ANG 1-7 levels and elevated ANG II activity with all the following negative outcomes. This underlies a more severe clinical course of the disease in elderly people with concomitant pathologies who typically have initially lower ACE2 receptor expression [12]. Naturally, elevated ACE/ACE2 ratio is a predictor of developing acute respiratory distress syndrome that can be prevented by ANG 1-7 administration [13]. However, wide use of ANG 1-7 in clinical practice is limited due to its rapid degradation in the body and, probably, difficulties in delivering it to lung and brain tissues by intravenous introduction. Recently new data have become available that MasR can be activated not only by ANG 1-7 but also by Neuropeptide FF, Alamandine, Angiotensin III and IV as well as by Angioprotectin and other

MasR agonists. Their development is a promising trend in anti-COVID-19 therapy. At present, more than 7 compounds are being clinically tested, which either activate or enhance MasR expression, as well as agonists of this type of receptors [14–19]. As we can see from literature review, it is advisable to rely on using persistent ANG-1-7 analogues that activate MasR to correct any RAS impairments when treating patients with COVID-19 and other viral infections caused by viruses that use ACE2.

AT1R is localized on membranes of many cells. Cells that are included into the inborn and adaptive immunity are no exception. Figures 1 and 2 provide schemes that describe events happening in a patient infected with COVID-19 against elevated angiotensin II levels.

Not only dendritic cells become active, and macrophage proliferation and neutrophil infiltration occur in the inborn immunity. Another effect is intensified synthesis of pro-inflammatory cytokines against elevated sensitivity of membrane Toll-like receptors, subtypes 2, 4, 7 and 9, responsible for recognizing microbial and viral pathogens. Similar events happen to the adaptive immunity cells (Figure 2). T-helpers additionally activate elements in this system through releasing multiple pro-inflammatory cytokines and this happens against weaker inhibitory effects produced by T-regulatory cells and a decline in the released quantity of anti-inflammatory cytokine IL-10.

The SARS-CoV-2 virus was established to be able to penetrate the brain and interact with ACE2 receptors localized on astrocyte and microglia membranes [20] where multiple AT1R and TLRs receptors can also be found [21]. Figure 3 provides the scheme showing outcomes of interactions between the virus and ACE2 and TLR against elevated angiotensin II levels in the brain. Microglia acquires a pro-inflammatory phenotype corresponding to neural inflammation,

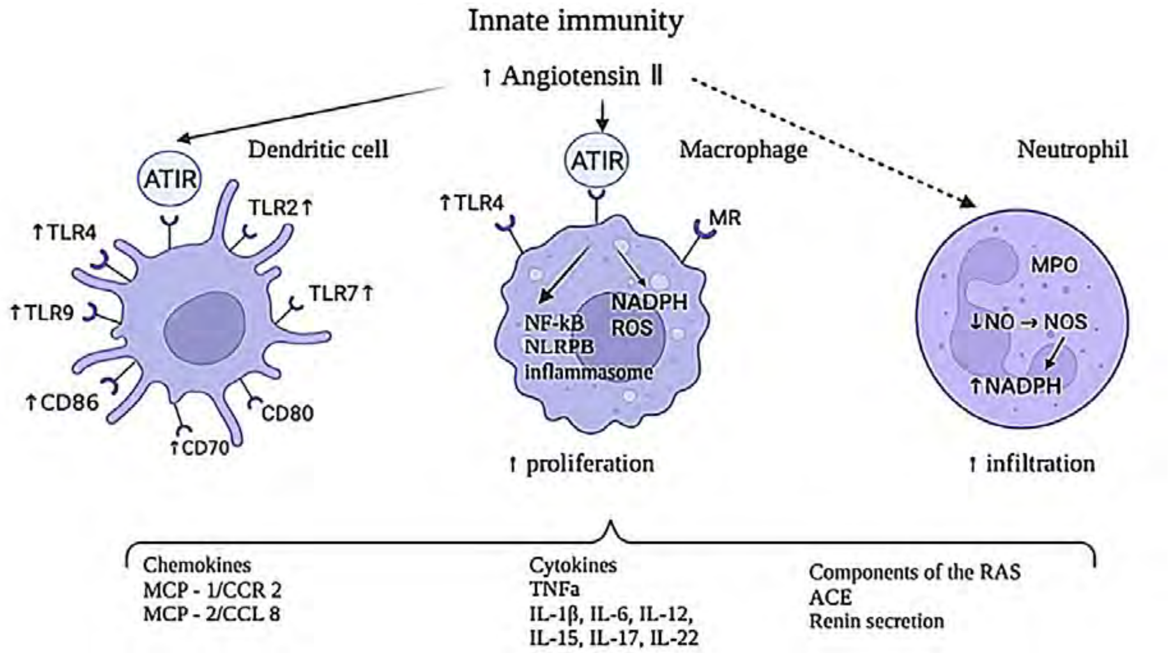

Figure 1. Angiotensin II excess influences inborn immunity cells through ATIR and TLR receptors. Dendritic cells become more active, macrophage proliferation and ROS and NF-kB production grow. Although neutrophils do not have a direct receptor for interaction, they still become able to infiltrate and increase NADPH production

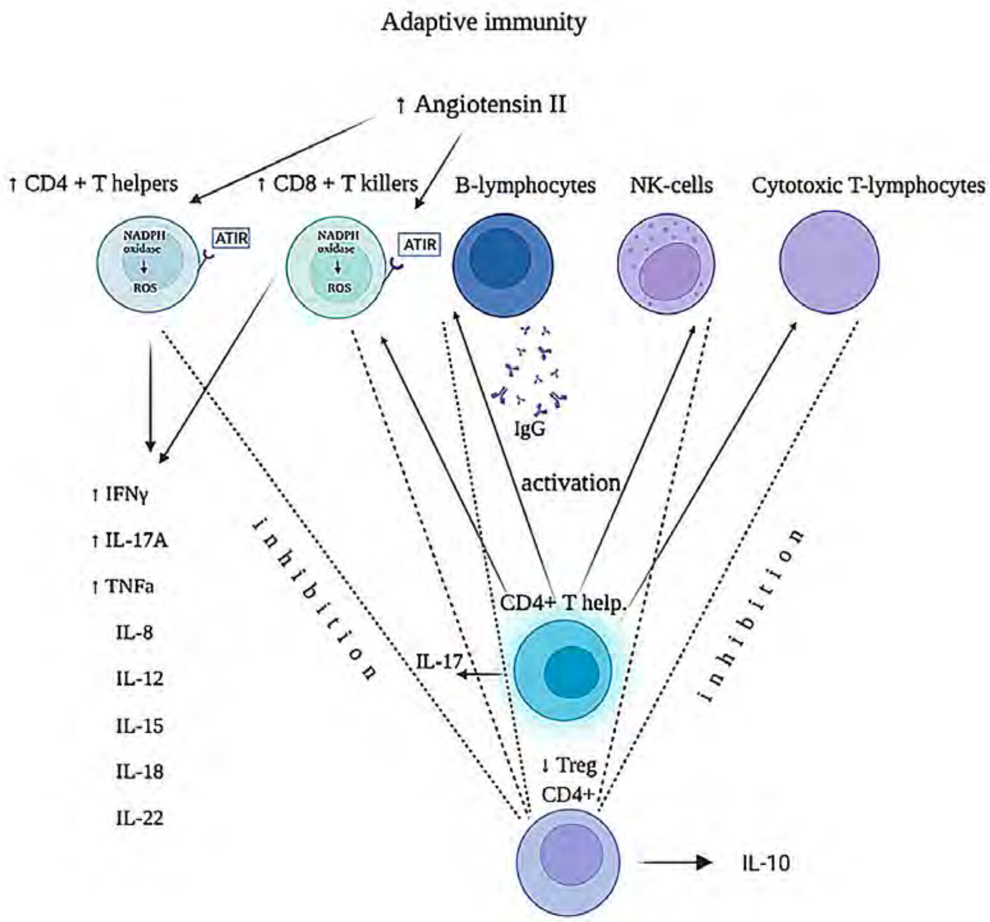

Figure 2. Angiotensin II excess activates CD4-lymphocytes, which induce the further reaction cascade. The quantity of regulatory T-cells with inhibitory effects goes down; as a result, all the cells become hyperactive and a cytokine storm occurs

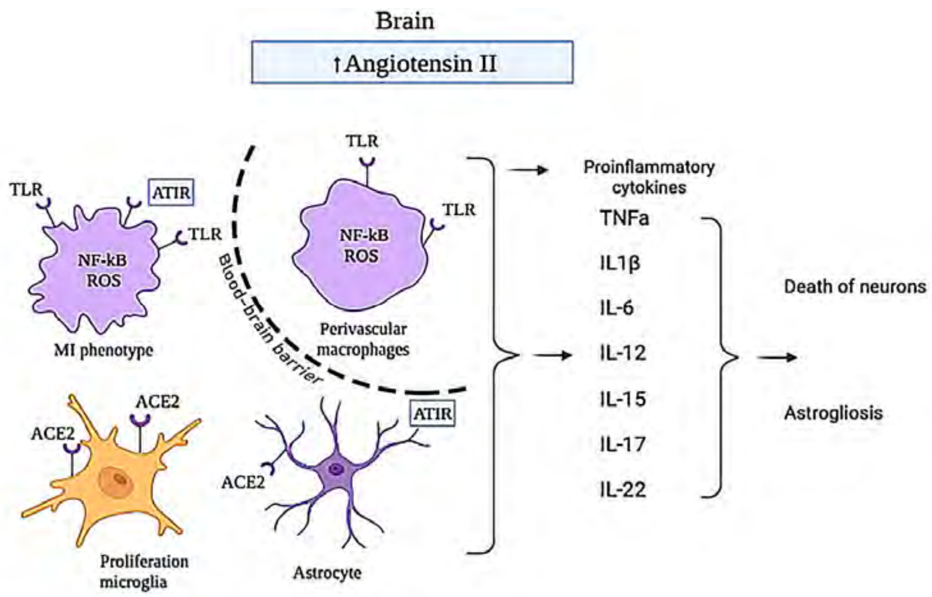

Figure 3. Angiotensin II accumulation in the brain in case ACE2-receptor is blocked by the SARS-CoV-2 virus produces adverse effects on AT1R and TLR receptors. This leads to pro-inflammatory way activation, formation of reactive oxygen species, synthesis of multiple pro-inflammatory cytokines and death of neurons against astrogliosis.

the blood-brain barrier becomes more permeable, neurons die and astrogliosis occurs. Macrophages in the vascular bed are activated and ROS and pro-inflammatory cytokines are released thereby making the blood-brain barrier more permeable and leading to death of neurons. In future, this will become obvious through varied neurological complications after recovery.

It is noteworthy that when soluble extra-membrane sACE2 was used as a medication to bind the virus, there was no significant improvement in COVID-19 patients. This was probably due to formation of such immune complexes as 'recombinant sACE2+SARS-CoV-2', which induced an autoimmune response in patents with formation of antibodies not only against the virus but also against parts of the ACE and ACE2 receptors with subsequent damage to all organs and tissues where these receptors were expressed. This only exacerbated the disease [22].

How the immune response to the SARS-COV-2 infection is impaired. Superficially, the SARS-CoV-2 interactions with the host body hardly seem different from other viruses: first, the virus binds to endosomal Toll-like receptors, 3rd and 7th types, and cytoplasmic RNA-receptors. Then a cascade of inborn immunity reactions is induced, NF-κB and IRF pathways are activated, interferons, IL-1 and IL-6 are produced, the adaptive cellular immunity and, consequently, the humoral immunity are activated. However, the SARS-CoV-2 virus induces an inflammatory cascade without letting the body to form a proper immune response; this is accompanied with intensive release of pro-inflammatory cytokines thereby exacerbating inflammatory reactions and leading to massive lesions in various tissues done by immune complexes.

If we analyze the issue in greater detail, we can see that the coronavirus is able to activate synthesis of alpha- and gamma-interferons in the host cells; the latter can interact with a regulatory site located in the *Ace2* gene promoter. This leads to intensified ACE2 synthesis thereby prolonging a situation when the virus is likely to spread further in an infected body [23]. Therefore, when the interferon system activated by the virus is overloaded with immune stimulators, this may lead to an inverse effect; namely, induce a 'cytokine storm' and the necessity to use immune suppressors [24] including antibodies to pro-inflammatory cytokines or their receptors, such as IL-6 and tumor necrosis factor alpha (TNF-α) or their receptors. It is assumed that a 'cytokine storm' develops due to step-by-step involvement of some intracellular pathways in the host body. Virus RNA that enters a cell is recognized by host endosomal RNA receptors, TLR3 and TLR7, as well as by RIG-I/MDA5, cytosolic RNA sensor. This activates NF-κB and IRF3 pathways together with translocation of these transcription factors into the nucleus and stimulation of type I interferon expression (alpha- and gamma-interferons) with their subsequent release into the extracellular space. Interferons interact with IFNAR membrane receptors and activate the JAK-STAT system. Next, there is phosphorilation of STAT1 and STAT2 proteins that penetrate the nucleus due to forming unified complexes with IRF9. There they stimulate certain genes (ISGs) with the IRSE element located on their promoter. This regulates the response intensity. Therefore, the coronavirus disrupts formation of protection responses rather crudely against the background interferon synthesis and at the stage when CTAT1 phosphorilation occurs. Initially delayed ISGs stimulation is then realized through hyperactivity of pro-inflammatory cytokine expression [24]. Bearing in mind that patients infected with the new COVID-19 infection typically have their immune system reacting too strongly to it, we can assume that the immune status of a given patient plays a significant role in making early predictions of how severe the disease will be.

Complications and predictors of severe COVID-19. The clinical picture of COVID-19 patients varies from mild to severe; the latter variant is becoming prevalent steadily all over the world together with a growing number of deaths due to the infection. Given that, there is an acute need to have reliable predictors of severe COVID-19 [25]. Having analyzed reviews on relevant predictors, we concluded there were at least 49 variables that would provide valuable predictive information about mortality due to COVID-19 among infected patients or its severe clinical course. These identified variables include socio-demographic indicators, data from medical histories on concomitant diseases, results of a physical examination, laboratory and X-ray data. It is necessary to select the most informative factors at the early stage in order to identify what treatment procedures would be the most effective [26].

Elevated IL-6, IL-10 and C-reactive protein (CRP) levels as well as a lower lymphocyte quantity are the earliest predictors of the disease exacerbation in COVID-19 patients [27]. Thus, it was established that the acute respiratory distress syndrome (ARDS) developed in 3–4 % of patients with bilateral pneumonia; the syndrome was combined with thrombotic coagulopathy in ¾ of such patients. In terminal cases, these complications would lead to thrombosis and a 'cytokine storm' caused by systemic release of pro-inflammatory cytokines such as interleukins IL-2, IL-6, IL-7, granulocyte colony-stimulating factor, C-X-C motif chemokine 10 (CXCL10), chemokine (C-C motif) ligand 2 (CCL2) and tumor necrosis factor alpha (TNF-a). The process is accompanied with leukopenia that indicates the cellular immunity is weak [28].

At present there are no doubts that there is an unbreakable internal link between the immunity and cytokine secretion; their high levels against lower lymphocyte populations were associated with elevated risks of death due to COVID-19. A prediction model was created based on IL-8 levels and quantities of CD4 + T-cells and NK-cells. It yielded good results in predicting death of COVID-19 patients. When a threshold equal to 0.075 was used, the model sensitivity and specificity amounted to 90.20 % and 90.26 %. Neutrophils, IL-6, $CD3^{-}CD56$, $CD16^{-}$ cells and leucocytes were the most powerful among all the factors in severe cases whereas CD, CD56, $CD16^{+}$ cells, $PD\text{-}1^{+}$ NK-cells, NK-cells, $CD4^{+}/CD8^{+}$ and perforin were the first five variables in milder ones. At the same time, neutrophils made the greatest contribution to this model and this is in line with a stronger inflammatory response in patients with severe COVID-19. Besides, measuring of NK-cell subsets proved to be useful at an early stage in hospitalization for identifying how the disease would develop [29]. Dynamic monitoring of cytokines and lymphocyte subpopulations potentially provides some useful data for more effective management of the disease. Large-scale meta-analyses identified a negative correlation between the declining cellular immunity and growing levels of pro-inflammatory cytokines for predicting transitions from a mild clinical course to a more severe one [28]. In this case, there is a signifiacnt drop in quantities of lymphocytes, monocytes, CD4 + T-cells, CD8 + T-cells, CD3-cells, CD19 cells and natural killers (NK) as well as an increase in interleukin-2 (IL-2), IL-2R, IL-4, IL-6, IL-8, IL-10, TNF-α and gamma-interferon levels in peripheral blood (INF-γ) [30]. Growing plasmin and plasminogen concentrations in blood are also biomarkers of elevated susceptibility to SARS-CoV-2 since plasmin can cut the relevant site in the S-protein of SARS-CoV-2 thereby increasing its virulence [31]. Growing neutrophils to lymphocytes ratios (NLR) and neutrophils to CD8 + T-cells (N8R) ratios are a powerful predictor of severe COVID-19 [32].

As it was stated above, pathological hyperactivity of the immunity is among key signs of the severe clinical course in COVID-19 patients. The central role in this state belongs to neutrophil activation. Proteomic profiling of plasma in cross-sectional and longitudinal cohorts of hospitalized COVID-19 patients revealed a prominent signature of neutrophil activation, including resistin, lipocalin-2, hepatocyte growth factor, interleukin-8, and granulocyte colony-stimulating factor, which were the strongest predictors of critical illness. A most significant finding was the fact that the signature of neutrophil activation was elevated already on the first hospitalization day in those patients whose state was after exacerbated down to critical levels and they were to be moved to intensive care units. The researchers assumed that high G-CSF levels stimulated emergency granulopoiesis to increase neutrophil production and IL-8 (CXCL8) regulated neutrophil migration into the lungs and, possibly, other tissues. There neutrophils were activated and then they released RETN, LCN2, HGF, MMP8 and other proteins with anti-microbial and other inflammatory functions that also induced considerable side lesions in the lungs, vessels and other organs [33]. NK-cells and T-cells are known to play a vital role in the anti-viral immunity. Studies with participating COVID-19 patients revealed that that the quantity and frequency of $CD4^{+}$T-cells, $CD8^{+}$T-cells and NK T-cells was considerably lower in severe cases than in mild ones. Significantly elevated expression of PD-1 and CD244 on $CD8^{+}$T-cells indicates that T-cells are depleted in COVID-19 patients and there is lower CD27 expression on $CD8^{+}$T-lymphocytes in severe cases against mild ones. Therefore, it is quite effective to use immune cell profiles and cytokine profiles as risk predictors to identify critically ill patients.

It is extremely vital to be able to predict a risk of a critical condition since this enables making correct decisions on relevant scope of healthcare and future treatment strategies. The current research in the sphere concentrates on several predictors among clinical and laboratory data (4C Deterioration and 4C Mortality Score). However, it should be noted that covariance between 4C Deterioration and 4C Mortality predictions did not differ as per sex or ethnicity and did not get weaker in younger age groups [34]. A research team from the University of Arizona, Harvard University and The National Center for Biotechnology Information (USA) has developed a mathematical model that makes it possible to estimate a probability of a cytokine storm in COVID-19 patients depending on how intensively cytokine production is stimulated by immune cells; the model relies on using multiple indicators. Unfortunately, a probability that the acute respiratory distress syndrome would develop in a COVID-19 patient is estimated in some clinics relying on simpler indicators such as high fever, C-protein levels, lower leucocyte count, developing lesions in the lungs, and lower saturation. Research data indicate that estimation of the disease severity and predictions of a possible fatal outcome should always consider effects produced by immunogenetics of a given patient, in particular, sex-specific immune genetic differences. Thus, for example, at present there is solid evidence that AB0 locus in the HLA genes is associated with susceptibility to the disease and we should remember that these genes regulate expression of DNA sequences coding cytokines and chemokines [35]. In addition, it was established that elderly males were a population group with elevated risks of the severe disease, developing pneumonia with the acute respiratory distress syndrome and death. Age is a key factor in COVID-19 prevalence and mortality; therefore, it is important to get an insight into age-specific immune profiles of patients and the condition of the both RAS branches together with analyzing ACE/ACE2 ratios if we want to select relevant prevention and treatment strategies. Thus, research results made it possible to identify the leading typical age-specific immune indicator associated with the disease severity, namely, certain circulating factors were determined including CXCL8, IL-10, IL-15, IL-27 and TNF-α. They all had a positive correlation with elderly age, longer hospitalization and a more severe clinical course of the disease [36].

Conclusion. We have analyzed multiple research articles that address variable impairments in the RAS and immune system functioning in COVID-19 patients. This allowed us to conclude there is a close interrelation between these two systems. ACE2 receptor is a binding element here and it plays the major role in the virus internalization in a cell. Elevated angiotensin II levels due to ACE2 receptor blockade and interactions with AT1R receptors localized on several cellular elements in the inborn and adaptive immunity lead to the latter becoming hyper-activated. This is accompanied with synthesis and intense release of multiple variable pro-inflammatory chemokines and cytokines into the extracellular space thereby posing a threat of a developing cytokine storm. Obviously, SARS-CoV-2 has a peculiarity which is its ability to induce an inflammatory cascade before a proper protective immune response occurs due to delayed interferon synthesis. Our analysis of research articles on predictors of the severe disease gave an opportunity to make a certain conclusion that ACE/ACE2 levels ratio and the immune status of a given patient allow estimating a risk of a severe clinical course even at initial stages in the disease development. The closest attention should be paid to changes in neutrophils. Therefore, our analysis of all the materials outlined in this review is of great importance not only for fundamental medicine but also for practical one since it contains data necessary for practical estimation of the disease prognosis and selection of the optimal therapeutic approach.

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Research article

PATIENT-SPECIFIC 0D-3D MODELING OF BLOOD FLOW IN NEWBORNS TO PREDICT RISKS OF COMPLICATIONS AFTER SURGERY

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Abnormal developments of the cardiovascular system are common congenital malformations. Computational fluid dynamics and mathematical modeling can be used to perform quantitative predictive assessments of hemodynamic properties in varied conditions.

This article addresses the development of a coupled 0D-3D model of blood flow in newborns to predict risks of complications after surgery. The 0D-model of systemic circulations is created by using the analogy between the blood flow in vessels and the flow of current through an electric circuit. A shunted section of the aorta and pulmonary artery is replaced with a 3D-model with two-way fluid-solid interaction (FSI). A section in a vessel with the aortic valve is examined in a separate 3D-model. Three-dimensional geometry is based on real CT-scans of a patient. The algorithm for coupling models of different levels relies on meeting the condition that pressures and volumetric blood flows are equal at the interaction boundary.

We have developed an algorithm for identifying personal parameters from the results obtained by solving an optimization problem. Computational experiments with different individual geometry of the aorta and aortic valve made it possible to analyze blood flow velocities, near-wall stresses, flows, and valve deformations. Observable near-wall stresses can be considered risk factors that could cause calcification on valve leaflets and other valve diseases.

Computational solutions in the "aorta – shunt – pulmonary artery" 3D-system allowed obtaining spatial distributions of velocities, pressures, near-wall stresses and other parameters that are significant in respect to probable pathology development. The developed approaches are primarily relevant for decision-making in surgical practice to predict risks of postoperative complications. In future, our plans are to develop the model so that it covers also saturation and oxygen exchange. This is necessary for assessing whether oxygen supply to the lungs is adequate.

Keywords*: 0D-3D model of blood flow, coupling algorithm, identification of parameters, patient-oriented, aorta, heart valve, newborn, shunt, risk of postoperative complications.*

Obstructive lesions of the right ventricular outflow tract, both isolated and combined with other congenital heart diseases, account for 25–30 % of all congenital malformations of the heart [1]. When an inter-system shunt was first introduced (in particular, a modified Blalock – Taussig shunt), it became a real breakthrough in surgical treatment of cyanotic heart diseases such as Tetralogy of Fallot, pulmonary atresia and some others [2, 3]. It is noteworthy that the modified Blalock – Taussig shunt remains a risky procedure that may result in extreme volume loads and acute thrombosis [4, 5]. To select an optimal shunt

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size is a vital task that hasn't been solved so far [6, 7].

Aortic valve disease is a most widely spread cardiovascular pathology. Valve disorders can be congenital when a valve with two leaflets is formed; or they can develop later when the valve leaflets become calcified [8, 9]. Valve disease is usually diagnosed by a physician who examines electrocardiography images of a specific patient and this estimation is rather subjective [10]. Exact methods are needed in this area since they allow quantitative assessments of functions performed by the aortic valve. Computer modeling makes it possible to simulate the aortic valve motion with precision and to obtain relevant data that are necessary for qualitative and quantitative assessment of the valve functioning [11].

Combined use of computational hydrodynamics and mathematical modeling has several advantages. First, we can predict blood flow properties under varied scenarios of surgery. Second, modeling results allow identifying critical health parameters of a specific patient that can be used as an indication to consider whether a surgery is advisable or not. Besides, biomechanical modeling can predict certain fundamental regularities that are typical for pathological processes.

At present, there is common understanding that it is necessary to develop complex multi-scale models to solve such tasks [12]. Experts have created basic principles for 0D, 1D, 3D coupling to identify blood flow properties [13, 14]. The study [15] addressed coupling of 1D – 3D models with vascular walls rigidity considered in the process. The authors of the study [16] coupled a finite-element model of the aorta and left ventricle and a 0D-circulation model for a patient with diagnosed pulmonary arterial hypertension. The study [17] concentrated on properties of coupled 3D-solid state two-ventricle heart model and 0D-closed-loop circulation model based on CircAdapt application. The research work [18] presented a model that described blood flow under aortic coarctation; the aortic arch itself was considered as a three-dimensional area whereas all the other vessels were described with 0D and 1D models. If we want to create models that describe complex hydrodynamics and movements of the aortic valve leaflets, we should rely on an approach that involves interaction between a fluid and a solid body (fluid-solid interaction or FSI) [19, 20]. Two-dimensional (2D) FSI studies have certain limitations due to the aortic valve being highly turbulent. These 2D-models should be adapted to realistic 3D-model geometry [21, 22].

A challenge that doctors often have to face is related to the necessity to objectify a surgery to treat the aorta coarctation and to estimate impacts exerted on blood flow by properties of a shunt and a place where it is installed in order to secure proper lung development in children with congenital heart diseases. To solve this, it is advisable to create a patient-specific blood flow model at several scale levels. The previous stage in our research involved developing a conceptual 0D-3D scheme of systemic circulation in newborns with the modified Blalock – Taussig shunt (Figure 1); we considered the results of 0D-model in detail [23]. The 0D-model of systemic circulation is created by using the analogy between the blood flow in vessels and the flow of current through an electric circuit. A shunted section of the aorta and pulmonary artery is replaced with a 3D-model with two-way fluid-solid interaction (FSI). A section in a vessel with the aortic valve is examined in a separate 3D-model. The present study concentrates on algorithms for coupling developed models of different levels and some results of 3D-modeling that make it possible to predict whether shunting is effective as well as risks of complication after surgery.

In this study, we aimed to develop a coupled 0D-3D model of blood flow in newborns to predict risks of complications after surgery.

Materials and methods. The mathematical statement of the 0D task includes several tens of differential and algebraic equations. We applied Runge – Kutta 4^{th} order method to find a numeric solution to them [23].

Geometry of the 3D-aortic valve model was created by using real CT-scans of a patient (Figure 2). The scans were transformed into a three-dimensional solid-state model with InVesalius software. A computational mesh was created with Meshmixer software package. The fluid equations were solved by using CFD FLUENT software package; we applied Navier – Stokes equations and Continuity equations for an incompressible and homogeneous fluid. Any impacts exerted by gravity or heat transfer between blood and the aortic valve were neglected in the models since their influence on the leaflet deformity was very slight. We applied the *k–ε* model [24] to simulate turbulence in the aortic valve. The aorta and valve leaflets were modeled as hyper-elastic and the elasticity law was set with the Ogden 1st order hyper-elastic model [24].

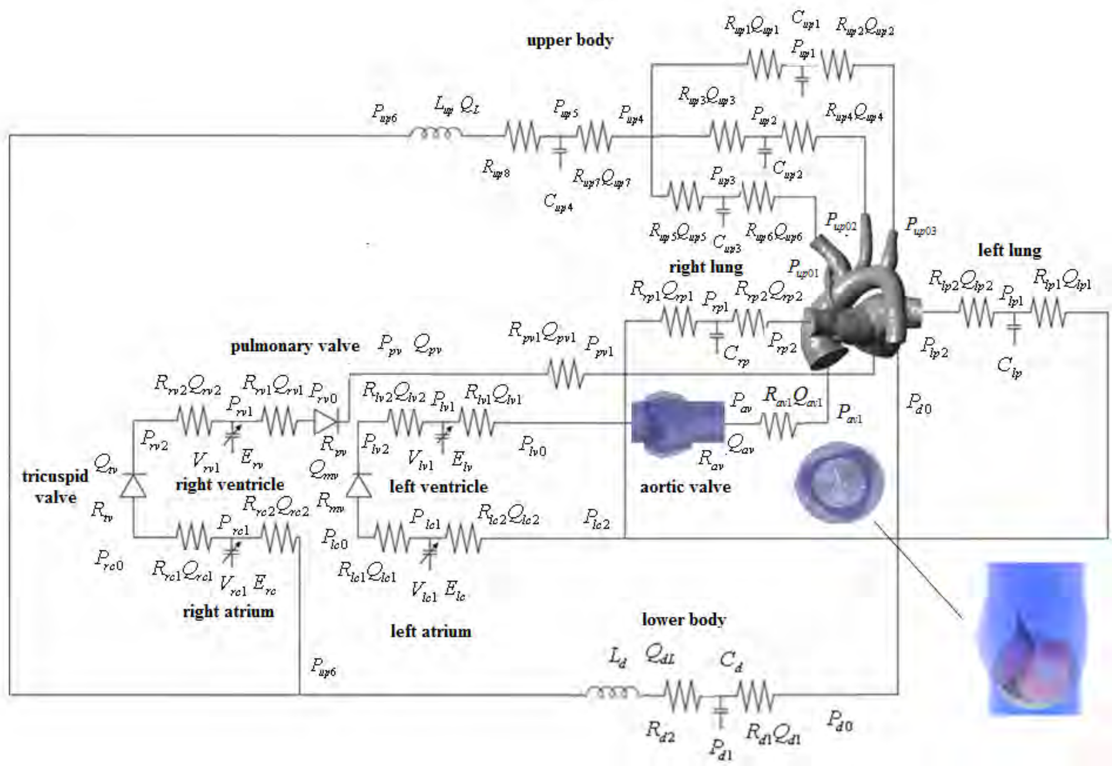
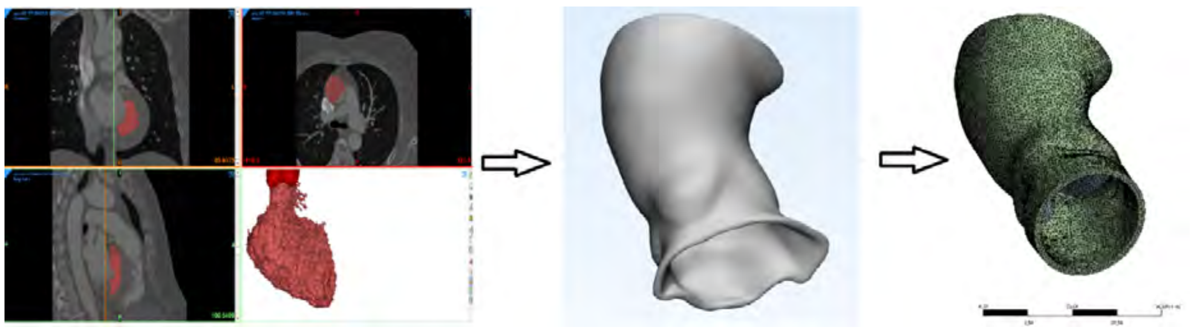


Figure 1. The conceptual scheme of systemic circulation [23]



a *b* *c*

Figure 2. *a* shows CT scans of a patient's chest; *b* is a solid-state model; *c* is a created computational mesh

A similar approach was used to simulate a three-dimensional flow in the shunted section of the aorta and pulmonary artery.

The algorithm for coupling models of different levels relies on meeting the condition that pressures and volume blood flows are equal at the interaction boundary [25]. Let us consider stages in the iteration algorithm: 1) blood flows and pressures that were identified at the initialization stage are fed into the 3D-model for solution; 2) pressures at the inlet and blood flows at the outlet are calculated within the stationary task in the 3D-model; 3) values calculated at the stage 2 are again returned into the 0D-model as boundary conditions to solve the equations and determine blood flows at the inlet to the aorta and pulmonary artery and pressures at the outlets; 4) convergence conditions at the boundaries are checked:

$$\left|P_{(i)}^{(0)(k)} - P_{(i)}^{(0)(k-1)}\right| < \delta P_{(i)};$$

$$\left|Q_{(i)}^{(0)(k)} - Q_{(i)}^{(0)(k-1)}\right| < \delta Q_{(i)}, \qquad (1)$$

where $P_{(i)}^{(0)(k)}$ is the pressure at the i-th boundary at the zero moment at the k-th iteration ($k = 1$ for the 1st iteration);

$Q_{(i)}^{(0)(k)}$ is the volume flow at the i-th boundary at the zero moment at the k-th iteration;

$\delta P_{(i)}$, $\delta Q_{(i)}$ are values of convergence criteria.

If the criteria (1) are met, we consider that the solution has been found at the zero step and we can move to the next time step. Otherwise, the values $Q_{(i)}^{(0)(k)}$ at the inlets and $P_{(i)}^{(0)(k)}$ at the outlets are again fed into the 3D-model, and the algorithm is repeated starting from the stage 2.

The algorithm for identifying patient-specific parameters is based on finding such a solution to the optimization task that would provide the periodicity of the solution on blood flow and pressure at any point in the 0D-model. We are planning to consider this algorithm in detail in our next articles.

Results and discussion. A time-dependent velocity at the inlet to the aortic valve was set as a sinusoid with the maximum flow velocity being 0.4 m/sec at peak systole. Shear strain velocity exceeded 50 sec^{-1} for large arteries and blood viscosity was almost constant due to high shear velocity [26]. Therefore, blood was considered a Newtonian fluid with constant density being 1050 kg/m^3 and dynamic viscosity being 0.0035 Pa·sec [20].

Computational experiments made it possible to analyze several properties of flow with different individual geometry of the aorta and valve. Figure 3 shows the most eligible computational mesh considering better convergence for a case when peak values of flow properties are observed. Such values are typical for pathological states.

Figure 4a shows how velocities are distributed in the aorta. The peak velocity reaches 1.874 m/sec at the narrowest sections and this is by 4.68 times higher than flow velocity at the inlet to the aorta. Turbulence zones are also visible. The highest wall shear stress value is at the valve leaflets from the incoming flow (Figure 4b). The highest value is 209.4 Pa whereas was shear stress at the aorta wall reaches only 15 Pa. Figure 4B shows shifts against the initial state (at the zero moment) up to its position after computations. The Figure 4d has sections painted red that means they are the most susceptible to elastic deformations. They are interleaflet triangles. The results show shifts of the valve leaflets and aorta walls. We can also see blood turbulences in the area close to the semilunar cusps. These observable wall shear stresses can be considered risk factors that can induce calcification of the valve leaflet and other valve diseases.

A model where the shunt was in the central position was taken as a computational area within the 3D "aorta – shunt – pulmonary artery" system. The solution to the task made it possible to identify distribution of some hemodynamic parameters that had medical significance. These parameters were velocity properties of blood flow, pressure on vessel

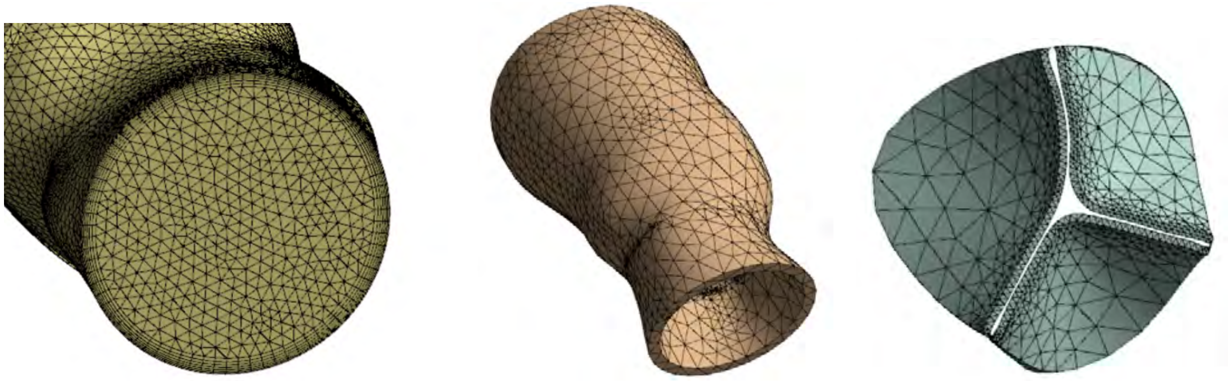


Figure 3. Visual images of computational meshes
(from left to right: aorta cross section, aorta wall, valve leaflet)

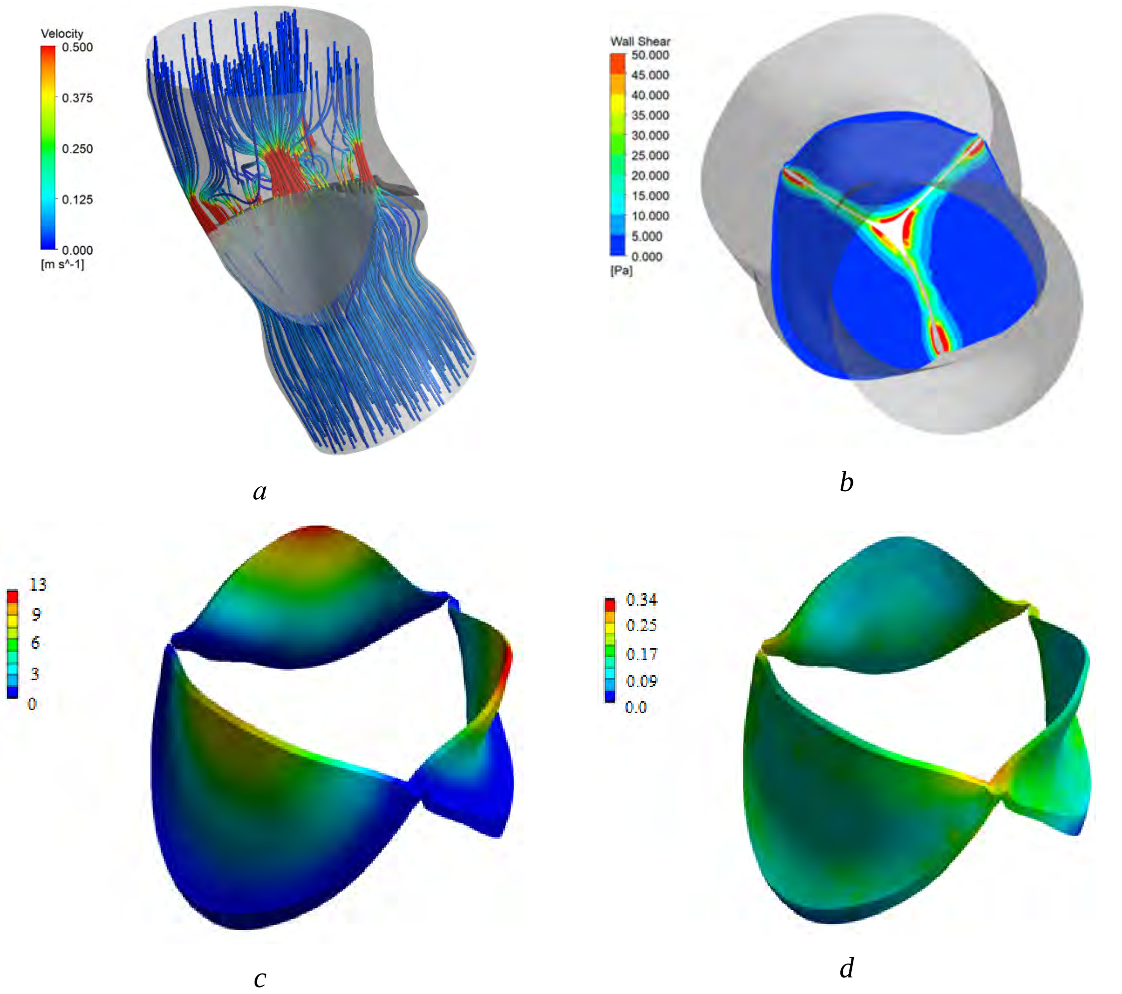


Figure 4. Distribution of flow properties in the aorta: *a* is for velocities; *b*, wall shear stresses;
c, shifts of the aortic valve leaflets; *d*, equivalent elastic deformations of the aortic valve leaflets

walls, wall shear stresses, and wall shear stresses averaged over the cardiac cycle. The results were obtained at a moment that corresponded to the maximum value of the volume blood flow $t = 0.125$ sec. The velocity distribution under using 0D-boundary conditions is well in line with the data available in other research works [25] (Figure 5a) in spite of different model geometries. The maximum velocities are observed in the area where the shunt and the artery are joined and in the shunt itself; the lowest ones are detected in the pulmonary artery area. The maximum blood flow values are detected in the shunt area (values vary within 6 m/sec). As for shear stress distribution, it is noteworthy that the results are consistent with those available in literature [25] (Figure 5b) both qualitatively and quantitatively. The maximum values are detected in the area where the aorta branches and in the shunt and vary within the limit of 100 Pa. The lowest values are mostly observed in the pulmonary artery and the ascending and descending part of the aorta. When comparing the results, we should note that there were also some differences detected in the modeling. The velocity distribution is different from those described in literature where the maximum velocities reached only 3.6 m/sec [27]. The same goes for distribution of shear stresses and pressures averaged over the cardiac cycle. The maximum shear stress values differ by more than twice: 40 Pa in the articles [27, 28] and 100 Pa as in the Figure 5b. The maximum pressure values also differ by 2 times: 13.89 kPa in the works [27, 28] and 26 kPa in our study (Figure 5c). The maximum values of shear stresses averaged over the cardiac cycle differ by more than 3 times: 45 Pa in the works [27, 28] and 150 Pa in the Figure 5d. These differences arise solely due to different approaches to simulating blood flow, namely,

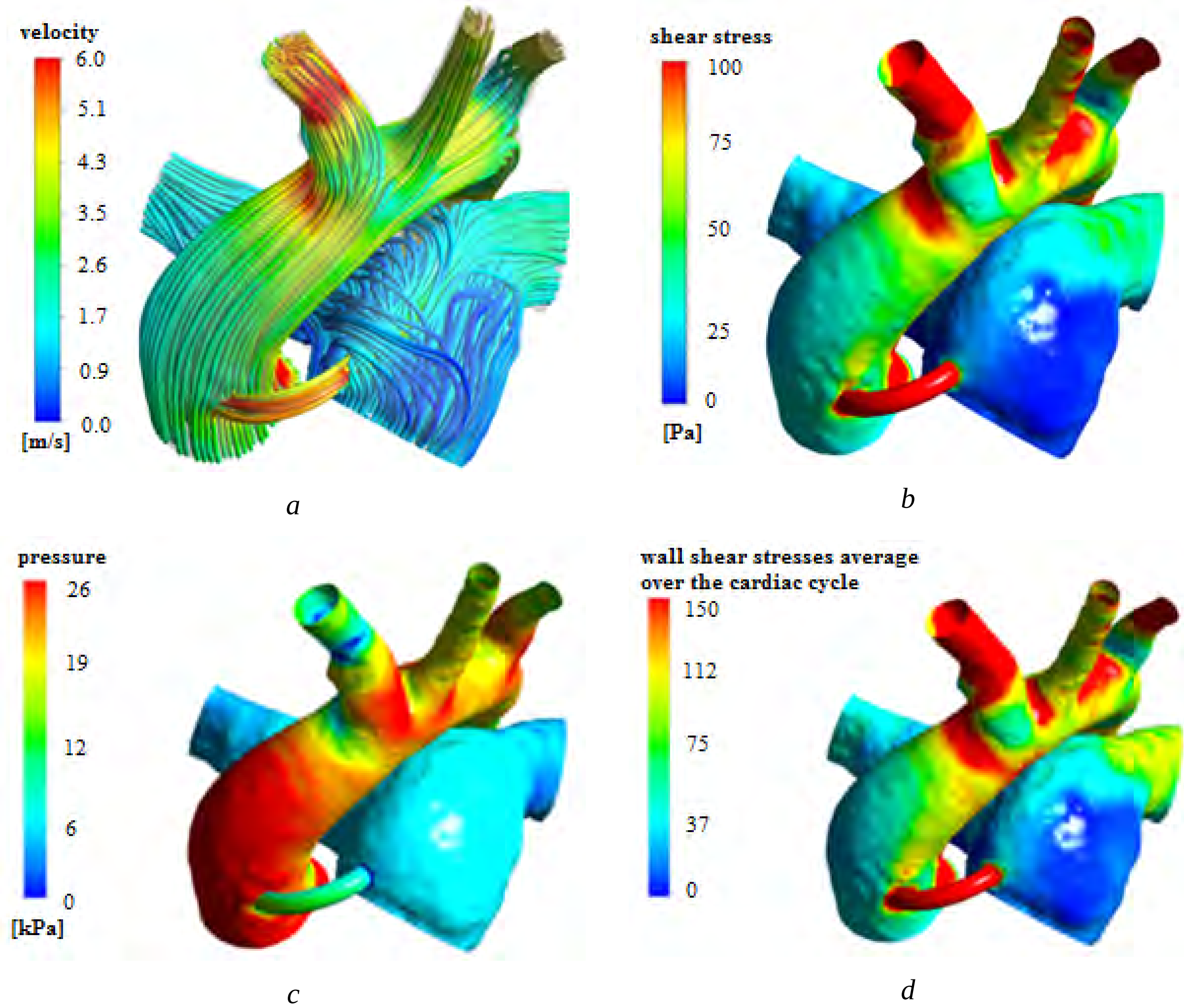


Figure 5. Distribution of blood properties in the 3D "aorta – shunt – pulmonary artery" system: *a* is velocity; *b*, shear stress; *c*, pressure; *d*, wall shear stresses average over the cardiac cycle

different boundary conditions. In the work [27], some average velocity profiles are set at the inlet and some constant pressures are set at the outlets. In our study, these boundary conditions are determined by finding a joint solution to the 0D-3D systemic circulation model. A result obtained for a specific patient can be interpreted as a high risk of complications developing in the middle term when the shunt is placed in the central position.

Conclusion. We have developed a coupled 0D-3D model of blood flow in newborns to predict risks of complications after surgery. The results obtained by 0D-modeling make it possible to predict how blood flow would be distributed in varied body parts and to estimate changes in blood flow into the lungs after shunting. On the other hand, three-dimensional tasking to simulate blood flow in the aortic valve and "aorta – shunt – pulmonary artery" system allows predicting significant hemodynamic parameters with spatial distribution thereby making it possible to visualize the most critical points.

The results obtained by patient-specific modeling have been shown to establish considerably different hemodynamic properties when coupled models are used. This emphasizes the importance of using patient-specific model parameters. The developed approaches, first of all, can be useful for decision-making in surgical practices to predict risks of complications under different variants of surgery. At present, this task might be too difficult to solve due to huge computational powers necessary to accomplish the required calculations.

In future we plane to develop an algorithm for selecting optimal shunting parameters such as a place where a shunt would be located and its size by performing a relevant numeric experiment. It is also advisable to develop approaches that could be used to assess risks of shunt thrombosis. We plan to develop the model so that it covers saturation and oxygen exchange. This is necessary for assessing whether oxygen supply to the lungs is adequate.

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Research article

RISK OF ALLERGY AND ITS IMMUNE PHENOTYPES IN CHILDREN WITH *MMP9 Q279R* GENE POLYMORPHISM

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Scientific research with its focus on allergic diseases relies on up-to-date molecular-genetic methods for identifying individual genetic variability; it seems an important stage in the implementation of programs with their aim to early detect and mitigate risks of such diseases.

In this study, our aim was to identify features of immune regulation associated with Q279R MMP9 gene polymorphism (rs17576) and benzene contamination in biological media in children with allergic diseases.

The test group included 33 children with allergic diseases; the reference group consisted of 40 relatively healthy children. CD-markers were identified with flow cytometry. Genotyping was performed with a real-time polymerase chain reaction.

The research revealed elevated levels of total IgE, IL-4 and TNFalfa under elevated benzene contamination in biological media that were by 1.2–4.2 times higher in the examined children with allergic diseases than in the reference group (p = 0.006–0.03). Q279R MMP9 gene polymorphismin in children from the test group had authentically more frequent occurrence of the GG and AG genotypes, by 1.7 times higher than in the reference group. This allows considering the allele G of the MMP9 gene as a sensitivity marker in children with allergic diseases (OR = 2.34; 95 % CI = 1.17–4.65). We established a growth by 2.8 times in total IgE level and greater IL-4 and TNFalfa expression, by 1.4 and 1.3 times accordingly, in carriers of the allele G against those carrying the homozygote AA genotype among the examined children with allergic diseases (p = 0.020–0.042). Logistic regression analysis established the adequacy of the dominant model (p = 0.01) and revealed a possible association between carriage of the AG and GG genotypes of Q279R MMP9 gene polymorphism and developing allergy (OR = 3.61; 95 % CI = 1.34–9.71).

A risk of developing allergy combined with benzene contamination in biological media and gene polymorphism of matrix metalloproteinase MMP9 (rs17576) is by 2.1 times higher for the allele G carriers against the AA genotype carriers (RR = 2.08; 95 % CI = 1.13–3.83). This allows considering the allele G of the MMP9 Q279R gene as a sensitivity marker in children with allergic diseases.

***Keywords:** genetic polymorphism, MMP9 Q279R, hypersensitivity markers, polymerase chain reaction, dominant model, CD-markers, a risk of developing allergy, IL-4, TNFalfa.*

Starting from the beginning of the 20th century, the prevalence of allergic diseases has been constantly growing. Given that, currently sensitization to one or several common allergens approaches 40–50 % among the world population. Allergic (atopic) diseases develop due to interaction between individual genetic predisposition and exposure to environmental

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factors; this interaction has facilitated a rapid growth in this trend over the last five decades. Use of the twin method has revealed that the genetic contribution to allergic diseases is about 50 % with heritability estimates being equal to 33–95 % [1, 2]. Therefore, scientific research with its focus on allergic diseases that relies on up-to-date molecular-genetic methods for identifying individual genetic variability seems an important stage in implementing programs with their aim to early detect and mitigate risks of such diseases [3, 4].

Matrix metalloproteinases belong to the family of zinc-dependent endopeptidases responsible for tissue remodeling and degradation of various proteins in extracellular matrix. They are able to influence biologically active molecules and to regulate cellular and signal pathways both under normal physiological conditions and in case of a developing pathology. Gelatinase B (MMP-9) is a major participant in proteolytic extracellular matrix degradation as well as degradation of multiple non-matrix proteins. It modulates embryonic growth and development, angiogenesis, vascular diseases, inflammation, infectious processes, tumor growth, various aspects of the immune response, apoptosis, cell proliferation, differentiation and migration of immune cells; it releases some cytokines and growth factors [5, 6]. High polymorphism of matrix metalloproteinase genes and carriage of variable allele variants that determine enzymatic activity as well as external factors that can put hereditary information into effect (aromatic hydrocarbons, for example), can be considered potentially able to contribute significantly to pathological processes, allergic (atopic) diseases included [7, 8].

In this study, our aim was to identify features of immune regulation associated with Q279R MMP9 gene polymorphism (rs17576) and benzene contamination in biological media in children with allergic diseases.

Materials and methods. We examined schoolchildren who lived in the Urals region. The test group included 33 children (their average age was 12.0 ± 0.5 years; 15 boys and 18 girls) with diagnosed atopic dermatitis, allergic contact dermatitis, allergic rhinitis, and predominantly allergic asthma. The reference group was made of 40 relatively healthy children (their average age was 12.8 ± 0.45 years; 13 boys and 27 girls). Both groups were comparable as per sex, age, and ethnicity ($p < 0.05$).

All legal representatives of the examined children signed a voluntary informed consent to participate in the study. The study was carried out in accordance with the Declaration of Helsinki by the World Medical Association (revised in 2013) and approved by the Ethics Committee of the FBSI "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies".

Aromatic hydrocarbons (benzene) were identified in children's biological media by using gas chromatography on Kristal 5000 gas chromatographer with plasma ionization detector (Russia). Ratios of basic leukocyte populations were identified with Drew-3 hematological analyzer (USA). Leukocyte fractions as per membrane CD-markers were identified with FACSCalibur flow cytometer (Becton Dickinson, USA) on panels of tagged monoclonal antibodies; the device was controlled by the universal CellQuestPro software with not less than 10,000 events being registered in total. Levels of total IgE, interleukins (IL-1beta, IL-6, IL-4, IL-10), interferon-gamma (IFNgamma), and tumor necrosis factor (TNFalfa) were identified by performing ELISA tests with ELx808IU microplate reader (BioTek, USA) and commercial test-systems (Vektor-Best, Khema, Russia) according to the manufacturer's procedures.

The obtained data were analyzed by using Statistica 10.0 software package (StatSoft, USA). The results were given as the simple mean and the standard error of the mean ($M \pm m$) or quantity (%). Authenticity of intergroup differences was determined as per Student's t-test and the significance was taken as $p < 0.05$.

Biomaterials for further genetic analysis were taken from the oral mucosa. DNA was extracted with the adsorption method. Polymorphism of matrix metalloproteinase-9 *MMP9 Q279R* (rs17576) was identified by performing the real-time polymerase chain reaction with CFX96 detection system (Bio-Rad, USA) and SNP-screen kits (Syntol, Russia). All the obtained data were analyzed with 'Gene Expert' software and genotype frequencies were calculated as per the Hardy–Weinberg equilibrium. Authenticity of inter-group differences was determined with chi-square (χ^2) with its significance taken as $p < 0.05$. Data on allele frequencies were analyzed by using logistic regression with calculating odds ratio *OR* and 95 % confidence interval (95 % CI), as well as a relative risk *RR* (relative risk) and its 95 % confidence interval (95 % CI).

The study was performed in full conformity with the organizational standards and methodical guidelines.

Results. Chemical analysis established elevated benzene contamination in blood of the children from the test group. Benzene levels were by 2.7 times higher in them than in the reference group (0.000566 ± 0.00015 μg/cm^3 against 0.000213 ± 0.00011 μg/cm^3 accordingly; $p = 0.024$). We detected elevated specific sensitivity to benzene as per contents of IgG specific to benzene in 78.8 % of the children from the test group against the reference level (p = 0,000). This indicator was also by 1.8 times higher than in the reference group (0.378 ± 0.051 AU in the test group; 0.208 ± 0.036 AU in the reference group; the reference level is < 0.015 AU).

The study revealed changes in regulatory immune indicators in the children from the test group (Table 1). These changes were identified as per ratios of major immune-competent cell populations as the total leukocyte counts grew by 1.2 times; eosinophils, by 1.9 times; and the eosinophilic-lymphocytic index also grew by 2.1 times (p = 0.002–0.014). We identified an increase in CD3$^+$- and CD4$^+$-populations by 15 % and 11 % accordingly against the reference group (p = 0.032–0.043).

We detected elevated total sensitization as per total IgE contents in the children with allergic diseases, which was by 4.2 times higher than in the reference group ($p = 0.006$). Changes in markers of the cytokine immune regulation were identified as per levels of IL-4 and TNFalfa that were on average by 1.6 and 1.2 times accordingly (p = 0.013–0.03).

The results obtained by genetic analysis (Table 2) showed some peculiarities of how alleles and genotypes of *Q279R MMP9* gene polymorphism were distributed in the children with allergic diseases. Frequency of variant homozygote *GG* genotype and heterozygote *AG* genotype was by 1.7 higher in the test group than in the reference one ($p = 0.03$). The allele *G* of *Q279R MMP9* gene polymorphism can be considered a sensitivity marker and a risk factor of developing allergic diseases in children *(OR* = 2.34; 95 % CI = 1.17–4.65) whereas the allele *A* acts as a protective factor and is associated with minimization of risks that an allergic diseases would develop (*OR* = 0.43; 95 % CI = 0.22–0.85). Logistic regression analysis established the dominant model to be adequate ($p = 0.01$) and revealed a possible association between carrying *AG* and *GG* genotypes of *Q279R MMP9* gene polymorphism and developing allergic diseases (*OR* = 3.61; 95 % CI = 1.34–9.71). The distribution of allele and genotypes frequencies conformed to the Hardy–Weinberg equilibrium (χ^2 = 0.01–1.16; p = 0.28–0.9).

We estimated genotype-associated changes in the immune regulation in the children with allergic diseases (the test group) depending on *Q279R MMP9* gene polymorphism (Table 3). The estimation revealed that a relative eosinophil count was authentically by 1.6 times higher, total IgE as a sensitization marker was by 2.8 times higher, and levels of cytokine mediators IL-4 and

Table 1

Immune profiles of the examined children with allergic diseases

Indicator	Reference level	Test group	Reference group	*p*
Leukocytes, 10^9/dm^3	3.9–6	6.36 ± 0.68	5.41 ± 0.37	0.014
Eosinophils, units	35–350	263.94 ± 78.81	142.18 ± 37.64	0.008
Eosinophils, %	0–3	4.03 ± 0.96	2.6 ± 0.64	0.014
$CD19^+$-lymphocytes, 10^9/dm^3	0.09–0.66	0.29 ± 0.04	0.27 ± 0.03	0.422
$CD19^+$-lymphocytes, %	6–25	12.46 ± 1.39	12.6 ± 1.15	0.874
$CD3^+$-lymphocytes, 10^9/dm^3	0.69–2.54	1.63 ± 0.17	1.42 ± 0.09	0.032
$CD3^+$-lymphocytes, %	55–84	66.7 ± 2.60	66.15 ± 1.983	0.737
$CD3^+CD4^+$-lymphocytes, 10^9/dm^3	0.41–1.59	0.89 ± 0.11	0.76 ± 0.06	0.043
$CD3^+CD4^+$-lymphocytes, %	31–60	36.15 ± 2.48	35.65 ± 2.13	0.760
Total IgE, IU/cm^3	0–99.9	181.39 ± 94.68	43.11 ± 20.82	0.006
IL-10, pg/cm^3	0–20	4.10 ± 1.77	3.16 ± 0.73	0.317
IL-1beta, pg/cm^3	0–11	3.23 ± 1.11	1.87 ± 1.19	0.060
IL-4, pg/cm^3	0–4	1.9 ± 0.36	1.20 ± 0.25	0.013
IL-6, pg/cm^3	0–10	2.50 ± 0.92	1.51 ± 0.41	0.890
INFgamma, pg/cm^3	0–10	3.44 ± 2.41	1.57 ± 0.32	0.126
TNFalfa, pg/cm^3	0–6	2.11 ± 0.26	1.7 ± 0.26	0.030

Note: *p* is the significance of intergroup differences between the test and reference group as per Student's t-test.

Table 2

Features of *MMP9 Q279R* gene polymorphism in the examined children with allergic diseases

Genotype, allele	Test group, %	Reference group, %	χ^2	*p*	*OR* (95 % CI)
Codominant model					
AA	27.3	57.5	6.71	0.03	0.28 (0.10–0.75)
AG	51.5	30.0			2.48 (0.95–6.48)
GG	21.2	12.5			1.88 (0.54–6.61)
Multiplicative model					
A	53.0	72.5	5.93	0.01	0.43 (0.22–0.85)
G	47.0	27.5			2.34 (1.17–4.65)
Dominant model					
AA	27.3	57.5	6.71	0.01	0.28 (0.10–0.75)
AG+GG	72.7	42.5			3.61 (1.34–9.71)
Recessive model					
AA+AG	78.8	87.5	1.0	0.32	0.53 (0.15–1.86)
GG	21.2	12.5			1.88 (0.54–6.61)

Note: *p* is the significance of intergroup differences; χ^2 is chi-square test; *OR* is odds ratio; 95 % CI is confidence interval.

Table 3

Immune regulation indicators in the examined children with allergic diseases associated with *MMP9 Q279R* genotypes

Indicator	Reference level	Genotype		*p*
		AG+GG	*AA*	
Leukocytes, $10^9/dm^3$	3.9–6	6.15 ± 0.62	6.93 ± 2.15	0.440
Eosinophils, units	35–350	292.54 ± 105.68	187.67 ± 70.99	0.098
Eosinophils, %	0–3	4.5 ± 1.26	2.78 ± 0.84	0.023
$CD19^+$-lymphocytes, $10^9/dm^3$	0.09–0.66	0.28 ± 0.04	0.33 ± 0.11	0.349
$CD19^+$- lymphocytes, %	6–25	11.79 ± 1.52	14.22 ± 3.41	0.153
$CD3^+$- lymphocytes, $10^9/dm^3$	0.69–2.54	1.65 ± 0.17	1.57 ± 0.50	0.750
$CD3^+$- lymphocytes, %	55–84	67.5 ± 2.28	64.56 ± 8.54	0.464
$CD3^+CD4^+$- lymphocytes, $10^9/dm^3$	0.41–1.59	0.90 ± 0.12	0.854 ± 0.30	0.769
$CD3^+CD4^+$- lymphocytes, %	31–60	36.63 ± 2.96	34.89 ± 5.56	0.550
Total IgE, IU/cm^3	0–99.9	219.96 ± 128.29	78.55 ± 45.36	0.042
IL-10, pg/cm^3	0–20	4.58 ± 2.46	2.88 ± 1.18	0.207
IL-1beta, pg/cm^3	0–11	3.71 ± 1.57	2.25 ± 1.59	0.117
IL-4, pg/cm^3	0–4	2.08 ± 0.47	1.44 ± 0.29	0.020
IL-6, pg/cm^3	0–10	2.37 ± 0.81	2.91 ± 3.37	0.723
INFgamma, pg/cm^3	0–10	4.05 ± 3.37	1.89 ± 1.26	0.229
TNFalfa, pg/cm^3	0–6	2.24 ± 0.30	1.69 ± 0.45	0.031

N o t e : *p* is the significance of intergroup differences between the test and reference group as per Student’s t-test.

TNFalfa were by 1.4 and 1.3 times higher accordingly in carriers of the variant allele *G* against those who carried the homozygote genotype *AA* ($p = 0.020–0.042$).

We comparatively analyzed indicators of the immune and allergic status in the children with allergic diseases associated with the allele *G* against those who carried the genotype *AA* of *Q279R MMP9* gene polymorphism. The analysis made it possible to verify tests (eosinophils, total IgE, IL-4 and TNFalfa) and a mechanism (extracellular matrix degradation) that underlies developing allergy associated with gene polymorphism of the matrix metalloproteinase *Q279R MMP9* (rs17576). Carriers of the allele G have by 2.1 times higher risks of allergy under elevated benzene contamination in biological media than those who carry the *AA*-genotype (*RR* = 2.08; 95 % CI = 1.13–3.83).

Discussion. Negative effects produced by aromatic hydrocarbons, benzene in particular, on immune reactivity indicators are associated with their immunotoxicity. Its intensity is determined by functional peculiarities of immune-competent cells and a stage in an immune response. Benzene contamination in biological media and further processes of its metabolism can aggravate allergy symptoms when, on one hand, oxidative stress is being activated and, on the other hand, Th-2-mediated processes are being stimulated through increased IgE and IL-4 production [9–11].

A mechanism that underlies developing allergic diseases is known to be associated with the disrupted immune regulation, imbalanced activation of allergen-specific Th2-clones, IgE synthesis by B-lymphocytes, infiltration and activation of eosinophils, basophils and mast cells in an inflammatory nidus migrating through capillary vessel walls and intersticium. All this makes high demands of extracellular matrix degradation determined by matrix metalloproteinases [12, 13]. *MMP9* is assumed to play a key role in tissue remodeling and recovery by degrading IV and V type collagen and elastin thereby facilitating cell migration. However, at present these enzymes are known to have much wider biological functions.

Matrix metalloproteinases play a key role in the development of immune cells, effector function, migration and receptor-ligand interactions; they influence immune responses through, among other things, regulating the signal pathways of cytokine receptors (TNFalfa, IL-6) associated with inflammatory processes. Experimental studies on mice with *MMP* deficiency show that in particular *MMP9* is secreted by inflammatory cells after a contact with an allergen and as a response to signal stimuli given by Th2-cytokines thereby facilitating recruitment of inflammatory cells through mobilization of anti-inflammatory cytokines, chemokines and growth factors [14, 15]. Thus, these mediators stimulate inflammatory cells to penetrate the airway lumen from tissues in patients with asthma. *MMP* also supports hyperactivity of the airways and extracellular matrix remodeling by influencing contraction of smooth muscles, fibroblast invasion and submucous collagen accumulation. *MMP*-induced regulation of cellular signals transmission through proteolytic detachment and activation of key growth factors such as TGFbeta stimulates proliferation of airway cells and modulates matrix production thereby supporting fibrosis development. In addition, *MMP9* has a key role in infiltration of eosinophils through the basal membrane into airway walls in patients with asthma and in subsequent induction of hyper-reactivity. *MMP9* levels identified in exhaled air condensate of children with asthma are elevated and correlate with a weaker lung function and other inflammation markers such as IL-4 / IL-10 [16, 17].

Some studies show that *MMP9*, given its authentically higher expression and levels in blood plasma, also makes a specific contribution to maintaining allergic inflammation with structural fibrosis-like changes and intensive cellular infiltration in patients with allergic rhinitis and atopic dermatitis [18, 19].

The *MMP9* gene is localized in the 20q11.2-q13.1 chromosome and consists of 13 exons. *Q279R MMP9* gene polymorphism (rs17576) is located in the 6th exon in the collagen-binding domain of the enzyme and is associated with adenine *A* being replaced with guanine *G* in the position 836 (*836 A/G*). As a result, the uncharged amino acid glutamine *Q* is replaced with the positively charged amino acid arginine *R* (*p.Gln279Arg*). This polymorphism potentially has some effects on biological properties of an end protein product since it is able to change a three-dimensional structure of it. This increases affinity to substrate and effectiveness of binding and in case of any changes in *MMP9* enzymatic activity can reinforce developing pathologic processes associated with elevated functional activity of the enzyme [20, 21]. Some research works show that the variant *279R* (*G* allele) makes *MMP9* more active and is associated with growing risks of cardiovascular diseases, asthma and chronic obstructive pulmonary disease [22, 23].

Allergic diseases are becoming one of the most widely spread chronic pathologies in the contemporary society. Their timely diagnostics and selection of adequate treatment strategies is a serious challenge for public healthcare in the 21st century since insufficient therapy leads to a significant decline in work ability thereby influencing people's health and quality of life. It is necessary to perform comprehensive studies with their focus on additional and alternative approaches that will allow implementing patient-specific treatment strategies. Such strategies should rely on profound examinations of specific pathogenetic components and individual sensitivity of the body based on molecular-genetic methods for predicting and monitoring disease development [3, 24–26].

Conclusion. The examination of children with diagnosed allergic diseases revealed some disorders of immune regulatory indicators against elevated benzene contamination in biological media. They were associated with changes in ratios of basic leukocytic fractions, elevated eosinophilic-lymphocytic indexes, growing hypersensitivity and elevated levels of cytokine immune mediators that indicate there has been a Th2-directed shift in immune homeostasis. We showed associations between *MMP9 Q279R* gene polymorphism and a risk of developing allergic diseases; the allele *G* can be considered a sensitivity marker in children with allergic diseases (*OR* = 2.34; 95 % CI = 1.17–4.65) and the *G* allele carriers have by 2.1 times higher risks of allergy than those who carry the *AA* genotype (*RR* = 2.08; 95 % CI = 1.13–3.83). Therefore, *Q279R MMP9* gene polymorphism (rs17576) in children with allergic diseases is accompanied with an imbalanced cytokine profile and its allele *G* is associated with a risk (*RR* = 2.1) of allergy. It can be considered a sensitivity marker and used to solve tasks related to early diagnostics and prevention of atopic diseases in children under benzene contamination in biological media.

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Research article

ASSESSING RISKS OF FUNCTIONAL DISORDERS OF HEPATOBILIARY SYSTEM IN WORKERS EMPLOYED AT BUTYL RUBBER PRODUCTION ALLOWING FOR ANALYSIS OF THE *OGG1* GENE POLYMORPHIC VARIANT rs1052133

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Contemporary petrochemical productions maintain strict control over contents of adverse chemicals in workplace air. Despite that, the chemical factor remains one of the major harmful occupational factors and can produce adverse effects on workers' health by increasing, among other things, risks of general somatic diseases. Given that, prevention of chronic non-communicable diseases in workers employed at chemical productions remains a vital challenge for occupational medicine. A way to tackle it is to timely detect risk groups relying on, among other things, analysis of workers' genetic peculiarities.

This article presents a study with 140 volunteers participating in it; they had basic occupations required at contemporary butyl rubber production. It was conducted within a periodical medical examination that involved using up-to-date hygienic, clinical-laboratory and genetic methods. The study included hygienic assessment of the chemical factor at the analyzed production, examination of hematologic and biochemical blood indicators, identification of workers' genetic status as per the rs1052133 polymorphic variant of the OGG1 gene and the severity of DNA breaks.

The study revealed adverse effects produced by the chemical factors on health of workers with basic occupations based on deviations in biochemical blood indicators obtained by tests that included indicator enzyme identification, and DNA damage. Following the study results, a risk group was created as per the state of the hepatobiliary system. To preserve workers' health, it is necessary to implement certain preventive measures that include providing safe working conditions as regards the chemical factor, timely detection of risk groups and rehabilitation activities.

Keywords: *health, workers, blood, liver, polymorphic variant, OGG1 gene, DNA breaks, preventive medicine.*

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Many chemicals that are used in production can disrupt such biological processes in a cell as synthesis and modification of biomolecules, cell breathing, metabolic transformations and cellular signal transmission [1–4]. Xenobiotics entering the human body often induce formation of reactive oxygen species that stimulate lipid peroxidation primarily by impairing morphofunctional properties of cells with active metabolism, hepatocytes being among them [5–8]. Toxic effects produced by chemicals even in low concentrations induce cytotoxic and cholestatic lesions in the liver [8–19].

Oxidation of biomolecules in the liver stimulates a growing number of neutrophils in blood, which, together with other non-parenchyma liver cells, are significant sources of pro-oxidant chemical compounds able to damage DNA in other cells [8, 20]. At present, there are data available in research literature on changes in DNA molecules in leucocytes in the form of breaks, chromosome aberrations, a growing share of altered bases, micronuclei and sister-chromatid changes in workers exposed to toxicants including those employed at petrochemical productions [21–23].

Lower reparation activity due to occurring polymorphisms in a specialized group of genes is a risk factor [24, 25]. The human *OGG1* gene (chromosome 3, short arm p25.3, 9749944-9788246 b.p., plus-chain) codes 8-oxoguanin-DNA-glycosylase (the molecular mass is 38782 Da, 345 amino acids). This protein participates in reparation of double-stranded DNA molecules by cutting residual 8-oxoguanine from the DNA nucleotide chain and reducing 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine [26][1]. Products of the *OGG1* gene participate in pathogenesis of many diseases, including hepatobiliary ones such as bile duct cancer, non-alcoholic fatty liver disease (NAFLD), cirrhosis, gallbladder cancer, and hepatocellular carcinoma[2].

In case the allele C is replaced with the allele G in rs1052133 polymorphism of the *OGG1* gene, we can observe changes in the protein sequence 326 as the amino-acid residue serine is replaced with the residue cysteine and this might be associated with developing hepatocellular carcinoma [27]. Patients with chronic hepatitis C and hepatocellular carcinoma much more frequently had GG genotype (Cys/Cys) and the allele G in the *OGG1* gene against the reference group together with 8-oxoguanin detected in urine [28]. An assumed reduction in reparation activity can be primarily associated with dimerization of the homozygote recessive variant (GG) of the OGG1 enzyme and its resistance to stimulation by the APE1 protein (AP-endonuclease 1) that also participates in reparation [29]. According to some other data, the occurrence of the dominant homozygote genotype CC is associated with developing lung cancer and head and neck cancer in smokers; this is probably due to a highly active OGG1 enzyme in this genotype [30].

Over the last years, DNA-comet assay has become a significant instrument for examining human health. A project called hCOMET was started by the European Comet Assay Validation Group and supported by the European Cooperation in Science and Technology in 2016. Within its implementation, it was suggested to develop

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the ComNet international database (since 2011) with information on investigations of human DNA-comets obtained from 1999 to 2019 [31].

It is quite relevant to examine molecular-genetic lesions and their reparation in workers exposed to harmful chemicals at their workplaces. Research in the area makes it possible to identify significant DNA damage at the earliest stages in development of a toxic process.

In this study, our aim was to examine a relationship between rs1052133 polymorphism of the *OGG1* gene and the state of the hepatobiliary system in workers employed at butyl rubber production.

Materials and methods. The study involved voluntary participation by 107 male workers who had a profound medical examination. They were employed at an enterprise that manufactured butyl rubber. Their age varied between 21 and 66 years. The reference group was made up of 33 engineers and technicians who were not exposed to chemical factors at their workplaces; they were of the same age as the test group. Each participating worker gave his voluntary informed consent to it.

Workers who were not exposed to chemicals at their workplaces and were included into the reference group did not differ from the workers in the test group as per their average age and work records ($p > 0.05$). Both groups did not have any statistically significant differences as per age and work records when compared with Student's t-test for independent samplings ($p > 0.05$). The test group of 107 workers included those with the following occupations: 31.78 % were operators; 34.58 %, repairmen responsible for technological equipment (TE repairmen for short); 33.64 %, repairmen responsible for control and automatic equipment (C&AE repairmen for short).

Sanitary-hygienic examinations were accomplished by using conventional procedures in accordance with the existing regulations. Clinical tests of biochemical blood indicators were performed by using standardized and unified laboratory diagnostic procedures on Humalyzer-900 Plus autoanalyzer[3]. Total blood count, including hemoglobin concentration, was performed with DREW-3 hematology analyzer (Drew Scientific, USA). Erythrocyte sedimentation rate (ESR) was identified as per Panchenkov method.

Leucocytes were extracted from workers' blood by extraction in ficoll gradient (1.077 g/cm^3, PanEko, Russia). Leucocyte micro-slides were prepared from 100 µl of a freshly extracted cellular suspense in 1 % low-melting agarose. The micro-slides were submerged into a cooled lytic salt solution (pH = 10) and kept there for 1.5–2 hours (+2 – +8 °C). Then, they were incubated for 20–25 minutes in a cooled alkaline buffer solution for further electrophoresis (pH > 13) of single cell DNA (the procedure was performed under 0.9–1 W/cm). As soon as electrophoresis completed, the microslides were fixed in ethanol, and then dried, dyed with ethidium bromide and their photos were made with Zeiss Axio Imager.D2 microscope (magnification 100x) and AxioCam MRc5 digital camera. Not less than 150 'comets' were photographed in each sample. Relative DNA contents in 'comet' tails (%) were identified with ImageJ 1.48 software (Wayne Rasband).

We used DNA in blood leucocytes extracted with Extract DNA Blood kit (Evro-

[3] Kishkun A.A. Rukovodstvo po laboratornym metodam diagnostiki [The guide on laboratory diagnostic procedures]. Moscow, GEOTAR-Media, 2007, 798 p. (in Russian).

gen, Russian Federation) to analyze rs1052133 polymorphism of the *OGG1* gene. A pair of primers (forward and reverse) and a pair of fluorescent probes that were different as per one nucleotide were created to identify G and C alleles of rs1052133 single nucleotide polymorphism in DNA sequence of the *OGG1* reparation gene.

Amplification and detection were accomplished with the Rotor-Gene Q real-time cycler (Qiagen). Optimal reaction conditions providing high fluorescence of an accumulated product were selected specifically to amplify a section in each gene and detect probe fluorescence. The resulting curves of fluorescence accumulation were analyzed with Rotor-Gene 6000 Series Software.

The research data were statistically analyzed with SPSS Statistics 25.0 software package.

Results and discussion. Butyl rubber is a most significant product manufactured with petrochemical synthesis. It is used in varied branches. Production technology for butyl rubber manufacture involves use of chemicals with general toxic, irritating and hepatotropic effects.

Workers employed at butyl rubber production are exposed to multiple adverse occupational factors, chemicals being the leading one. The chemical factor occurs due to chemicals in workplace air that produce general toxic, irritating, narcotic and hepatotropic effects. These chemicals enter workers' bodies from workplace air through inhalation and skin contacts. Major chemicals occurring in workplace air at butyl rubber production include olefins (butadiene, ethylene, isobutylene, etc.) and alkanes (methane, propane, butane, and pentane), methyl chloride, and aromatic hydrocarbons (benzene and toluene).

Working conditions of operators at butyl rubber production were ranked as hazardous, overall hazard category 3.2, as per average shift concentrations of harmful chemicals, in particular, single maximum concentrations of methyl chloride and aromatic hydrocarbons. Workplace air was polluted most intensely when some gas-involving hazardous works were performed (technological sampling, cleaning and maintaining the equipment). Chemical analysis of workplace air at workplaces of TE repairmen established that, as a rule, levels of saturated hydrocarbon did not exceed MPC during routine repairs. Levels of unsaturated hydrocarbons reached 2 MPC (35–39 % of the analyzed samples) and levels of methyl chloride were up to 3 MPC (80 % of the analyzed samples). When machinery overhaul was performed and cases of devices and pipelines were opened, levels of methyl chloride reached their peak in workplace air at TE repairmen's workplaces, up to 4 MPC. Therefore, the chemical factor was reasonably estimated at these workplaces as corresponding to hazard categories 3.2–3.3. Workplace air at C&AE repairmen's workplaces did not contain any chemicals in concentrations exceeding MPC and this made it possible to rank their working conditions as permissible ones (class 2) as per the chemical factor.

Harmful chemicals in workplace air determined the significance of hematological and biochemical blood indicators that should be analyzed in workers exposed to them. Table 1 provides the results.

Obviously, the results obtained by analyzing hematological and biochemical indicators clearly indicated there were apparent negative changes in liver indicator enzymes in TE repairmen and operators in comparison with the reference group (Table 1). The most apparent change was an increase in alanine aminotransferase activity, which was by 1.5 times higher in TE repairmen and operators than in the reference group ($p < 0.05$). We did not identify any statistically significant

Table 1

Hematological and biochemical blood indicators in workers employed at petrochemical production

Indicator	Operators	TE repairmen	C&AE repairmen	Reference group
Leucocytes, $\times 10^9$/l	7.41 ± 2.36	7.47 ± 1.43*	6.47 ± 1.34	6.46 ± 1.22
Erythrocytes, $\times 10^{12}$/l	5.03 ± 0.34	5.18 ± 0.52	5.12 ± 0.45	4.98 ± 0.39
Hemoglobin, g/l	138.79 ± 8.83	144.43 ± 7.41	140.28 ± 3.82	141.48 ± 5.67
Thrombocytes, $\times 10^9$/l	227.38 ± 36.19	225.70 ± 39.10	212.33 ± 32.93	218.36 ± 33.17
ESR, mm/hours	6.94 ± 3.83	7.30 ± 2.71	6.06 ± 1.12	6.67 ± 1.43
AST, U/l	25.24 ± 3.26	25.84 ± 3.11*	22.78 ± 1.76	23.85 ± 2.96
ALT, U/l	28.88 ± 4.26*	29.68 ± 3.33*	18.94 ± 2.53	19.76 ± 2.50
De Ritis ratio	0.89 ± 0.16*	0.88 ± 0.14*	1.21 ± 0.08	1.21 ± 0.07
γGTP, U/l	25.49 ± 1.50	26.08 ± 1.57*	24.70 ± 1.61	24.53 ± 1.59
AP, U/l	89.65 ± 5.49*	90.86 ± 3.03*	73.33 ± 3.35	72.21 ± 4.57
Cholesterol, mmol/l	4.86 ± 0.63*	5.22 ± 0.44*	4.35 ± 0.63	4.06 ± 0.40
Glucose, mmol/l	4.89 ± 0.35	5.02 ± 0.65	5.06 ± 0.34	4.91 ± 0.40
Total bilirubin, μmol/l	11.24 ± 5.32	11.62 ± 6.05	11.03 ± 3.32	10.81 ± 3.81

Note: * means statistically significant difference from the reference group ($p < 0.05$). Abbreviations: ESR is erythrocyte sedimentation rate, AST is aspartate aminotransferase, ALT is alanine aminotransferase, γGTP is γ- glutamyl transpeptidase, AP is alkaline phosphatase.

differences between hematological and biochemical indicators of C&AE repairmen and workers from the reference group ($p > 0.05$). Besides, we detected a statistically significant increase in average DNA levels in a ‘comet’ tail that was by 1.1–1.4 times higher in all the occupational groups of workers exposed to hepatotropic chemicals than in the reference group; this indicates clearly that these workers face increased chemicals burdens (Table 2). Median DNA levels in a ‘comet’ tail were by 1.2–1.4 times higher in TE repairmen and operators against the reference group ($p < 0.05$). In addition, average occurrence of ‘comets’ with lesions detected in more than 5 % of DNA was by 2.2 times higher in TE repairmen than the same indicator in the reference group ($p < 0.05$).

We used results obtained by amplifying the *OGG1* gene section to calculate odds ratios for impaired reparation in case a specific genotype of the rs1052133 polymorphism was identified in workers with the basic analyzed occupations. We established that the recessive G allele in the rs1052133 polymorphic variant was a risk factor for operators (OR = 4.464; 95 % CI: 1.564–12.744), TE repairmen (OR = 5.134; 95 % CI: 1.820–14.481) and C&AE repairmen (OR = 3.906; 95 % CI: 1.391–10.969) against the reference group (Table 3).

Risks of weaker DNA reparation might be associated, first of all, with dimerization of the homozygote recessive variant (GG) of the *OGG1* enzyme as it was described in literature [29].

Table 2

DNA breaks in leucocytes in peripheral blood of workers employed at petrochemical production

Group	Average DNA levels in 'comet' tails ± standard deviations, %	Median DNA levels in 'comet' tails (interquartile interval 25–75), %	Average occurrence of 'comets' with lesions detected in more than 5 % of DNA in a tail ± standard error of mean, %
TE repairmen	4.43 ± 1.31*	3.69 (2.92–3.98)*	27.30 ± 3.80*
Operators	4.09 ± 0.73*	3.45 (3.10–3.86)*	15.59 ± 3.43
C&AE repairmen	3.45 ± 0.80*	2.85 (2.46–3.25)	14.96 ± 2.08
Reference	3.28 ± 0.50	2.73 (2.38–2.94)	12.36 ± 5.16

Note: * means statistically significant difference from the reference group as per Kruskal – Wallis test ($p < 0.05$).

Table 3

Risks of impacts exerted by the G allele of the rs1052133 polymorphic variant of the *OGG1* gene on DNA reparation in workers exposed to hepatotropic chemicals

Occupation	Odds ratio	95 % CI
TE repairmen	5.134	1.820–14.481
Operators	4.464	1.564–12.744
C&AE repairmen	3.906	1.391–10.969

Therefore, in case the allele G occurs in the rs1052133 polymorphism of the *OGG1* gene, workers who have negative changes in activity of indicator enzymes and DNA lesions can be considered a risk group and should be provided with medical screening in dynamics.

Conclusions:

1. The chemical factor occurs at the analyzed workplaces due to harmful chemicals with predominantly general toxic and hepatotropic effects. Working conditions estimated as per this factor are ranked as hazardous, hazard category 3.2, at operators' workplaces and as hazardous, hazard category 3.3, at TE repairmen's workplaces.

2. Health disorders were identified in operators and TE repairmen including a significant increase in activity of such indicator enzymes as aspartate and alanine aminotransferase, gamma-glutamyl transpeptidase, and alkaline phosphatase against the reference group. These disorders indicate developing impairments of the hepatobiliary system under exposure to harmful chemicals.

3. We examined DNA extracted from leucocytes in workers' peripheral blood and identified increased levels of DNA breaks and damage; this can be applied as a biomarker of negative effects produced by harmful chemicals at petrochemical production.

4. We performed genetic examinations with their focus on the rs1052133 polymorphism of the *OGG1* gene; as a result, workers who have the recessive allele G in their genotype are considered a risk group as regards the hepatobiliary system (OR = 4.474; 95 % CI: 1.848–10.835).

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Review

MOLECULAR AND GENETIC ASPECTS OF HEALTH RISKS AND THEIR ASSOCIATION WITH ADVERSE ENVIRONMENTAL CONDITIONS AND DIETS (SYSTEMIC REVIEW)

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At present, it is vital to examine adverse effects produced on gene expression by negative environmental factors and nutrients. In this study, our aim was to generalize data available in literature on an association between health risks and polymorphisms of genes that participated in xenobiotic detoxification and allergic status, food intolerance included, in adults and children. We also considered influence exerted by various components in diets on gene expression.

Available research data indicate that GSTP1 and SOD gene polymorphisms have their effects on a decline in detoxification and antioxidant functions and early development of allergic, occupational and oncological diseases under exposure to harmful chemicals. Micronutrients in diets that can protect from adverse effects produced by chemicals can act not only as substrates but also as detoxification enzyme inducers. Great quantities of biologically active compounds in the Mediterranean diet are assumed to be able to modulate functional activity of certain genes. Such nutrients as polyphenols, flavonoids, catechins, glucosinolates, anthocyanins, stilbenes, carotinoids, polyamines spermidine and spermine produce anti-genotoxic and anti-carcinogenic effects.

Use of combined nutrigenetic and phenotypic data seems a promising trend in effective modeling of a healthy diet.

The research data outlined in this review indicate there is solid evidence that health risks can depend on a genotype, phenotype and quality of the environment. These risks also differ depending on a diet. Modeling a healthy diet based on available knowledge on nutritional genetic and nutritional genomics is a promising trend within non-carcinogenic health risk management, including risks of oncological diseases caused by exposure to adverse environmental factors.

Keywords: *gene polymorphism, detoxification, environment, phenotype, genotype, nutritional genetics, nutritional genomics, nutrients, biologically active compounds.*

Contemporary molecular genetic research is developing rapidly attracting experts of various specialties since it provides them with an opportunity to conduct profound studies with their focus on health effects of environmental and lifestyle factors on the human body in terms of genetic predisposition to a disease. There are multiple genetic features determining individual responses to environmental exposures. Molecular genetic studies are particularly relevant in the regions with highly developed industries posing risks of adverse health outcomes, especially in children. High prevalence of pediatric allergies,

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including food intolerance, in such regions is of special concern and requires serious efforts aimed at identifying causes of metabolic disorders [1–4]. There are also studies that concentrate on developing new approaches to prevention of diseases and preservation of proper homeostasis allowing for genetic peculiarities[1]. However, very few studies investigate issues concerning combined effect of ordinary nutrients and adverse environmental factors on gene, proteome, and metabolome expression. In this review, we examine publications on human responses to food quality and certain environmental factors.

In this review, **our aim** is to summarize literary data on the relationship between health risks and polymorphisms of genes that participate in xenobiotic detoxification and allergic status, food intolerance included, and on effects produced by diets on gene expression in adults and children.

Materials and methods. We performed a search for original research articles on the topic in SNPedia, PubMed, Web of Science, eLIBRARY, and Google Scholar search engines using the following keywords: gene polymorphism; gene expression; ST, SOD, NAT genes; transposable element; allergies; xenobiotics; heavy metals; food; food intolerance; epigenomics; epigenetics; nutritional genomics, and nutritional genetics. Of 396 search results, 73 papers were selected for this review.

Results and discussion. It is relevant to examine body responses to environmental exposures, especially in industrially developed areas where industrial pollutants pose serious health risks, bearing in mind that these responses depend, inter alia, on personal heredity. A high prevalence of environmental diseases in such areas, especially among children, raises concern and requires special efforts aimed at identifying causes of metabolic disorders [1, 5].

Consequently, the analysis of published data on associations between gene polymorphisms and environmental factors and a risk of health disorders posed by exposure to environmental toxicants is of current interest in hygiene. It facilitates effective management of detected risks with nutrients being among helpful instruments. It is also important to understand the effect produced by polymorphism of the genes responsible for detoxification and development of environmental diseases on changes in the immune response. Some studies have demonstrated that interactions between genes and the environment can influence early formation of the immune system pattern and subsequent development of such allergic diseases as bronchial asthma and atopic dermatitis [6–8]. Besides, there exists convincing evidence that infants' exposure to adverse environmental factors plays a key role in gene activation or suppression by changing DNA methylation. Gene expression changes thus determining a phenotype and risks of a disease [9].

Various environmental toxicants are known to be able to stimulate production of reactive oxygen species inducing inflammation and sensitization and causing damage to cells of the respiratory epithelium [10, 11]. Susceptibility to inflammation is deemed to be characterized by inherited deficiency of detoxification efficiency. Glutathione-S-transferases (GST) coded by the GSTP1, GSTT1, and GSTM1 genes are among the enzymes able to conjugate many hydrophobic and electrophilic compounds with reduced glutathione and to detoxify a wide range of toxins and carcinogens [12–14].

Gene polymorphism can lead to partial or complete loss of glutathione-S-transferase

[1] Volobuev V.V., Polunovskii V.V., Tsvetkovich A.V., Seledtsova L.A. Pat. RF RU 2691145. Sposob formirovaniya individual'nykh dieticheskikh rekomendatsii na osnove DNK anali-za: patent na izobretenie [Patent Number RU 2691145 C2. A method of developing individual dietary recommendations based on DNA test results], 2019.

activity. The SNP database contains descriptions of 13 single nucleotide polymorphisms (reference sequences) of the most frequently investigated GSTP1 gene, four of the GSTM1 gene, and three of the GSTT1 gene[2]. Since GSTP1 is the dominating GST that participates in xenobiotic detoxification and is expressed intensively in the respiratory epithelium of the lung [15, 16], it is postulated that the altered GSTP1 activity in the bronchial tissue can affect xenobiotic detoxification causing inflammation and oxidative stress [17]. Some studies showed that the GSTP1 Val105/Val105 genotype was less frequent in patients with a history of atopy while GSTP1 Ile105/Ile105 was more frequently found in those with severe bronchial obstruction/hyperresponsiveness than in healthy people [18]. However, it was later reported that carrying the GSTP1 Val105/Val105 slow activity genotype was associated with airway inflammation in asthma [19]. The studies examining South African schoolchildren revealed a significant protective effect against atopy in children with one or two copies of the allele G of glutathione-S-transferase GSTP1 rs 1695 [20].

Another study confirmed the role played by genes controlling the anti-oxidant system in the development of childhood allergic disease following the exposure to traffic-related pollution in infants (0–1 years). High levels of inhalation exposure to nitrogen oxide made children with the Ile105Val/Val105Val GSTP1 genotypes more susceptible to allergens [21].

Bad habits, such as smoking, can increase the toxic burden. Some researchers gave convincing evidence of the relationship between specific detoxification-gene variants, ability to detoxify components in cigarette smoke in smoking mothers, and the orofacial cleft risk [22]. However, in population studies, small samples represent a serious challenge for estimating the association between gene polymorphisms, environment and sensitization to common allergens.

Poor detoxification of environmental carcinogens can affect susceptibility to these chemicals and induce neoplasms; the latter can, in its turn, depend on the activity of certain genes. For example, experts observed an obvious interaction between the GSTT1-null and the N-acetyl-transferase 2 (NAT2) slow activity genotype that influenced susceptibility to colorectal cancer was observed [23]. The CYP1A1 gene polymorphism (G allele of rs1048943 and C allele of rs4646903) can increase risks of colorectal cancer by raising the levels of activated metabolites with high carcinogenic potential [24]. N-acetyltransferases (NAT) catalyze the enzymatic acetylation of aromatic amines, mainly xenobiotics. The most abundant data in literature are available in studies that investigate the role played by histone acetylation providing gene transcription activity in the development of lung and colorectal cancer [25, 26]. Smokers with the GSTM1-null genotype are at a higher risk of bladder cancer [27].

The GSTP1 Ala114/Val114 gene polymorphism can promote earlier development of occupational diseases related to airborne chemical hazards, allergen-induced asthma, and respiratory hypersensitivity, whereas the GSTP1 Ile105/Val105 genotype protects from them [28]. Literature sources provide data on the association between GSTP1 Ile105Val and Ala114Val polymorphism and ophthalmic pathology in workers employed in metallurgy and exposed to chemical factors and high temperatures [29]. The GSTP1 Ile105Val genotype was shown to be a potential risk factor causing both inflammatory and dystrophic changes in the eye [30].

Superoxide dismutases (SOD) also play a significant role in detoxification. They are a widely spread group of antioxidant metallo-

[2] SNPs3D. Available at: http://www.snps3d.org (October 02, 2022).

proteinases, which consists altogether of three genetically different isoforms coded by the SOD1, SOD2, and SOD3 genes, the activity of which is apparent in the cytoplasm, mitochondria, and extracellular space, respectively. The best-studied SOD1 gene affects lipid metabolism by inhibiting activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [31, 32]. Single nucleotide polymorphisms (SNPs) in workers exposed to occupational hazards can change gene activity, moderate the protein function, and have some other effects at the molecular level. This may lead to less effective detoxification and antioxidant protection and earlier development of occupational diseases and neoplasms. Thus, the results of studying the relationship between occupational lead exposure and brain tumors give certain evidence of the fact that lead can induce multiform glioblastoma and meningioma through the mechanisms of oxidative damage [33, 34].

Genetic characteristics of the SOD2 haplotypes (TAA, TCA, TCG, and CCG) influence the risk of lung tumors in the conjunction with the smoking status and a number of cigarettes per day; at the same time, people with the TCG haplotype have a lower risk of lung adenocarcinoma, which confirms the hypothesis about the effect produced by a genetic profile on detoxification [35].

Susceptibility to noise-induced hearing loss also can depend on the genetic background. A Chinese case – control study established a noise protection factor in rs2070424 of the SOD1 gene in A allele carriers compared with those with the G allele [36].

At least 45 % of the human genome is comprised of transposable elements (TEs), which have been recently shown to participate in cellular functions and gene remodeling. TEs induce irreversible genetic lesions making the host's genome develop multiple protective mechanisms that involve a wide range of inhibition ways to minimize their effects. Regulation of epigenetic modifications and DNA-associated histone proteins using small RNA is aimed to suppress TEs expression. Suppression of TEs proliferation and related harmful mutations is considered to be the main function of cytosine methylation. Genome demethylation activates TEs expression while DNA hypomethylation is most closely connected with carcinogenesis as a potential TEs deregulation [37, 38]. Environmental pollution, including that with heavy metals, influences interactions between TEs and genome by enhancing adverse effects of the contaminants [39].

Metabolic processes in the body reflect a complex process of interaction with the environment. The overwhelming majority of nutrients enters the body through the gastrointestinal tract; it is, therefore, of interest to investigate changes in gene expression in response to the intake of various food components and in relation with adverse health effects of environmental factors. Such studies typically aim to search for ways to prevent many diseases. Xenobiotic metabolism is associated with detoxification enzymes. Chemoprotective micronutrients can act not only as substrates but also as inductors of these enzymes. For example, it is assumed that abundant biologically active compounds in the Mediterranean diet can modulate functional activity of GSTM1, GSTT1 GSTP1, and NAT2 genes [40]. Nutriogenetic studies often investigate GSTP1, GSTM1, GSTT1, and NAT2 isozymes. Deletion GSTM1 and GSTT1 polymorphisms were shown to result in a complete loss of enzymatic activity whereas other SNPs such as the GSTP1 p.Ile105Val (c.313A>G) (rs1695) reduced enzymatic activity of the NAT2 (590G>A-rs1799930) [40–42].

Induction of glutathione-S-transferase is intensified by isothiocyanates (sulforaphane) contained in cruciferous vegetables; these substances become involved into biotransformation of phase II detoxification, thus promoting clearance of carcinogens and preventing DNA alterations [43]. Some studies found a relationship between the excessive intake of cruciferous vegetables and colorectal adenomas. The established link between low activity of GSTP1 GG (A313G) and/or GSTA1 TT (C69T), accumulation of glucosinolates, secondary metabolites found in Brassicaceae and related families, and cancer is obviously associated with expression of the 1-CYP1A2 phase gene induced by indole-3-carbinole (glucosinolate derivative) and accumulation of genotoxic products. Long-term stimulation with excessive quantities of cruciferous vegetables does not help neutralize slow activity by glutathione-S transferases [44]. Flavonoids conjugate with glucuronide and sulfate and are excreted with urine and bile. As a result, polymorphisms of the UDP-glucuronosyltransferase and sulfotransferase can facilitate changes in the phytochemical clearance and flavonoid effectiveness. Genetic polymorphisms in the enzymes that metabolite phytochemical substances can partially explain different levels of risk of certain diseases; they should also be considered within the context of other aspects of human genetics [44, 45]. Rocket (Eruca vesicaria subsp. sativa) that contains carotenoids, vitamin C, dietary fiber, polyphenols and glucosinolates, is distinguished among cruciferous vegetables as the plant having a pronounced antigenotoxic effect [46].

The potential of dietary interference in inflammation becomes obvious through changes in methylation of the GSTP1 gene and LINE-1 (a transposable element in the gene sequence that changes their transcription activity; it belongs to retrotransposon family in the human genome) [47] as well as telomere length ratio. The DNA methylation mechanism underlies antioxidant and anti-inflammatory effects of the functional components of foods (catechins, flavonoids, anthocyanins, stilbenes, and carotenoids) [48]. Dietary polyamines spermidine and spermine produce many physiological effects similar to those produced by antioxidant and anti-inflammatory agents [49]. Fermented rice bran inclusion in the diet can also help reduce DNA damage by reactive oxygen species (ROS) and associated inflammation at earlier stages of a disease [50]. Data have been accumulated on the impact of flavonoids, such as genistein, quercetin, and epigallocatechin gallate, on biological systems in the human body. They induce Nrf2/ARE nuclear receptors that regulate expression of antioxidant enzymes and phase II detoxification enzymes coded by such genes as GST (P, T, M) SULT, NAT, NQO1, UGT, and GPX [51]. Various studies have proven that dietary polyphenols (epigallocatechin in green tea, turmeric acid in cinnamon, resveratrol in grapes, and curcumin in curcuma) can induce dramatic changes in epigenome of tumor cells and be used for cancer prevention [52]. Many nutrients, being ligands of various transcription factors, can affect the immune response and inflammatory reactions, e.g. phytoestrogens in fruits and vegetables that exert their influence on relevant receptors thereby producing anti-inflammatory, antioxidant and anti-tumor effects [53]. It is assumed that the protective effect produced by soya against prostate cancer is associated with epigenetic modifications (demethylation) of DNA in tumor suppressor genes by the CpG island promoter [54]. Dietary intake of a wide range of antioxidants can be inversely associated with the risk of stomach cancer altered by genetic variants rs 1871042 in the GSTP1 gene [55]. Without normal functioning of GSTT1, increased consumption of dried meat during pregnancy accounted for a higher risk of brain tumors in children [56].

Diallyl disulfide (DADS) in garlic was shown to activate genes regulating normal cell division, similar to sulforaphane in cruciferous vegetables [57]. Basic mechanisms of effects produced by DADS in prevention and/or treatment of diseases include inhibition of inflammation, oxidative stress, and cellular apoptosis. In addition, DADS can produce neuroprotective effects and protect endothelium of the heart and other organs from cell or tissue damage caused by toxicants [58]. Garlic oil was established to have a protective effect against hepatocarcinogenesis induced by nitrosodiethylamine (NDEA). The activity of nuclear transcription factors of the peroxisome proliferator-activated receptor and anti-inflammatory effects can be, at least partially, associated with modulation of enzymes involved in liver phase I detoxification, including cytochrome P450 (CYP2E1, CYP1A2 and CYP1A1), and phase II enzymes, including glutathione-S-transferases (GST) [59, 60].

There are also data on the impact exerted by polyunsaturated fatty acids on lipid metabolism and thermal genesis [61–64].

Gut microbiota plays a significant role in metabolic processes in the body and correction of allergies as immune system disorders. Microorganisms help metabolize bioactive compounds contained in foods (ellagic acid and ellagitannins are metabolized into urolithins), thus modulating their bioavailability. In addition, gut bacteria produce numerous bioactive low molecular weight compounds able to play a role in epigenetic processes (e.g., folic acid, butyrate, biotin, and acetate) and are responsible for absorption and excretion of such elements as zinc, selenium, iodine, and cobalt [65, 66]. Experiments on rats and human cell line THP-1 demonstrated the effect of inducing expression of IL-8, TNFα and IL-10 cytokine genes by exposure to three probiotic strains *Lactobacillus rhamnosus K32, Bifidobacterium longum GT15,* and *Enterococcus faecium L-3* and their supernatants. In particular, in human cell culture, enterococci and products of lactobacillus metabolism stimulated only TNFα expression. It, in its turn, activated Nf-kB, a major transcription factor able to control expression of genes responsible for the immune response, apoptosis and cellular cycle. Live *Bifidobacterium longum* GT15 caused simultaneous expression of genes responsible for formation of IL-8, TNFα, and IL-10 cytokines while live *Enterococcus faecium L-3* increased mRNA that coded TNFα [67]. Gut microbiota can influence the activity of histone deacetylase (HDAC) through microbe metabolites. This enzyme is involved in various pathologies and diseases, from cancer and colitis to cardiovascular diseases and neural degeneration. HDAC is inhibited by butyric acid produced by human gut bacteria following the consumption of dietary fibers contained in berries, fruits, vegetables, and legumes [68].

Jagoe with colleagues [69–71] reported individual variations of gene expression associated with obesity and blood pressure after food deprivation and consumption in the same person. A complex adaptive program is usually activated during fasting; it induces transcriptional changes that facilitate protein degradation and suppress glucose oxidation in muscles [70], giving the opportunity to determine a molecular phenotype in the context with a disease. Moreover, a transcriptional response to food intake is stable [69].

The data presented in this review provide convincing evidence that human health risks can depend on the genotype, phenotype, and environmental factors. Besides, an individual diet may also modify risks [71]. If we understand how environmental factors and nutrients influence the genome, DNA hypomethylation, histone acetylation, and other little-studied metabolic processes in the body, we can manage these complex processes more effectively.

It is worth noting, however, that human studies are usually limited by the search for marker genes and the analysis of gene associations in population groups with this or that disorder, chronic diseases or in people who stick to a specific diet with exactly known contents of certain nutrients. Such studies are considered to be unable to provide unambiguous results since statistical correlations become less significant when pooling data obtained on different populations and ethnic groups. Responses to dietary interferences may vary considerably between people even within the same genetic sub-group [42, 72].

Achievements in precision nutrition have given the opportunity to identify genetic mutations that can increase the risk of certain diseases, including cancer, in case of specific nutrient deficiencies. These mutations can potentially serve as new or unconventional biomarkers for predicting diseases and preclinical signs [73]; they can be also used in individual diet modeling to reduce adverse environmental effects, increase body detoxification capabilities and resistance. A promising trend in this sphere is an attempt to combine nutriogenetic and phenotypic data, biomarkers included, to create a new nutrition model.

Conclusions. The data presented in this review provide convincing evidence that human health risks can depend on the genotype, phenotype, and environmental factors. Besides, an individual diet may also modify risks. Nutrition modelling based on expertise in genomics, epigenomics, nutritional genetics, and nutritional genomics is a promising trend in managing risks of environmental, occupational, and other non-communicable diseases, including cancer.

A complex approach to assessing personified clinical and phenotypic characteristics, such as individual food preferences, food intolerance and allergies, cultural and social factors, lifestyle and environmental factors is required for creating individual and group nutrition models aimed at disease prevention.

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