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Contact Information:
82 Monastyrskaya Str.,
Perm, 614045, Russia
Tel/Fax: +7 (342) 237-25-34
E-mail: journal@fcrisk.ru
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AMBIENT AIR QUALITY AND HEALTH RISKS AS OBJECTIVE INDICATORS TO ESTIMATE EFFECTIVENESS OF AIR PROTECTION IN CITIES INCLUDED INTO THE 'CLEAN AIR' FEDERAL PROJECT

N.V. Zaitseva, I.V. May

Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 6140045, Russian Federation

It is important to estimate effectiveness and results achieved by measures implemented within the 'Clean Air' Federal project as regards public health in cities included into it.

The aim of this study was to analyze changes in levels of ambient air pollution and airborne health risks in cities included into the 'Clean Air' Federal project in dynamics over 2020–2022 and to estimate whether the measures aimed at reduction of emissions were adequate to risk rates and factors.

The study relied on analyzing the results of field observations over ambient air quality within social and hygienic monitoring. Monitoring covered priority chemicals that made 95 % contributions to impermissible health risks according to dispersion calculations. Risk assessment was performed as per standard algorithms and indicators. Adequacy of air protection and correctness of its orientation were estimated in Norilsk as an example city.

The study established that levels of harmful chemicals in ambient were higher than hygienic standards over the analyzed period in all the cities participating in the project. We did not detect any significant reduction in ambient air pollution; there were no positive trends in health risks rates either. In 2022, a risk of respiratory diseases under chronic exposure was ranked as high (hazard index or HI 10.5÷43) in Chelyabinsk, Mednogorsk, Norilsk, Krasnoyarsk, Lipetsk, and Chita; it was ranked as 'alerting' in Bratsk, Chita, Novokuznetsk, Magnitogorsk, and Omsk (HI 4.0÷5.8), A permissible risk was identified over the analyzed period only in Cherepovets (HI<3).

So far, reductions in emissions of pollutants declared by economic entities have not ensured absence of impermissible health risks in 11 out of 12 cities. Ungrounded orientation to a 20 % reduction in emissions of all the economic entities included in the experiment and failure to consider risk indicators when setting quotas for emissions can lead to absence of any substantial effects for public health in the analyzed cities. In some cases, this may even result in excessive spending on activities that do not have any significant influence on a sanitary-hygienic situation.

***Keywords**: Federal project, ambient air quality, health risk, air protection, setting quotas for emissions.*

The Federal Project 'Clean Air' was developed specifically to improve quality of the environment in several cities with high levels of ambient air pollution [1, 2]. These targets hardly seem arbitrary since negative effects of ambient air on medical and demographic indicators (population incidence and mortality) have been proven by multiple Russian and foreign researchers in their studies [3–9].

Initially, 12 cities were included into the project and the experiment on managing ambient air quality through a system of setting emission quotas. They were Bratsk, Krasnoyarsk, Lipetsk, Magnitogorsk, Mednogrsk, Nizhniy Tagil, Novokuznetsk, Norilsk, Omsk, Chelyabinsk, Cherepovets, and Chita. Twenty-nine new cities are going to be included in the experiment on September 01, 2023; they are

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

Irina V. May – Doctor of Biological Sciences, Professor, Deputy Director responsible for research work (e-mail: may@fcrisk.ru; tel.: +7 (342) 237-25-47; ORCID: https://orcid.org/0000-0003-0976-7016).

mostly located in Siberia and the Far East. Most programs and action plans are developed within the 'Clean Air' Project in a maximum integrated way. They cover all the possible activities aimed at reducing emissions such as industrial modernization, replacement of old boiler-houses, making private households switch from coal to environmentally friendly heating sources, development of transport infrastructure and introduction of public transport powered by liquefied natural gas.

However, the ultimate result of the project is substantial improvement of quality of life in cities included in the experiment [10, 11]. This target is not set directly within the project profile; however, it is public health protection and a growth in the human potential in the country that is in line with all the strategic directions in the country development [12–14].

The Federal Project envisages considerable financial investments; therefore, it is interesting to estimate productivity and effectiveness of all the funds spent by the federal budget on implementing the project activities. Optimization of such investments also seems quite relevant.

The RF Government Order[1] (Item 12 in the Rules…) stipulates that allocation of the budget transfers should yield the following results:

– reduction in ambient air pollution;

– reduction in aggregated emissions of pollutants into ambient air against the levels established in 2017;

– a growth in consumption of liquefied natural gas as a motor fuel.

Reduction in emissions and implementation of relevant technical, technological and organizational activities aimed at reducing aggregated emissions of pollutants are certainly the most important ultimate results of the project. However, the top priority is given to 'reduction in ambient air pollution' as its primary target. At the same time, this result has been transformed into 'Reduction in emissions of hazardous pollutants that produce the greatest negative effects on the environment and human health' in the Profile of the Federal Project. Accordingly, changes in levels of ambient air pollution are suggested to be described through emission volumes. Undoubtedly, levels of pollution in the lower atmosphere depend directly on masses of emitted chemicals. However, any relationships within the 'emission – ambient air pollution – public health' system are much more complex and require mandatory consideration since it is people who are to be provided with better environmental conditions on urbanized territories. Any activity implemented within the Federal Project is to be directed at satisfying people's need in a safe and healthy environment.

Obviously, several objective indicators can be applied to estimate effectiveness and productivity of air protection on a given territory including:

– concentrations of pollutants in ambient air identified by direct instrumental measurements at ecological and / or social-hygienic monitoring (SHM) posts, frequency and intensity of violations of hygienic standards;

– health risks assessed not only by dispersion calculations but also relying on field observation data;

– actual numbers of people asking for medical aid can be and should be considered when estimating effectiveness of implemented activities and financial investments [15–17].

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[1] Ob utverzhdenii Pravil predostavleniya i raspredeleniya inykh mezhbyudzhetnykh transfertov iz federal'nogo byudzheta byudzhetam sub"ektov Rossiiskoi Federatsii na realizatsiyu meropriyatii po snizheniyu sovokupnogo ob"ema vybrosov zagryaznyayushchikh veshchestv v atmosfernyi vozdukh, snizheniyu urovnya zagryazneniya atmosfernogo vozdukha v krupnykh promyshlennykh tsentrakh, obespechivayushchikh dostizhenie tselei, pokazatelei i rezul'tatov federal'nogo proekta «Chistyi vozdukh» natsional'nogo proekta «Ekologiya»: Postanovlenie Pravitel'stva Rossiiskoi Federatsii ot 05.12.2019 g. № 1600 [On Approval of the Rules for granting and distributing other inter-budgetary transfers from the federal budget to the budgets of the RF regions to be spent on implementation on activities aimed at reducing the total emissions of pollutants into ambient air, decreasing ambient air pollution in large industrial centers thereby providing achievement of goals, targets and results within the 'Clean Air' Federal Project of the 'Ecology' National Project: the RF Government Order issued on December 05, 2019 No. 1600]. Available at: http://static.government.ru/media/files/bgdJwTAcotUFNWAEh3nCNb7oUgh7f608.pdf (January 21, 2023) (in Russian).

The aim of this study was to analyze changes in levels of ambient air pollution and airborne health risks in cities included into the Federal Project 'Clean Air' in dynamics over 2020–2022 and to estimate whether the measures aimed at reduction of emissions were adequate to risk rates and factors.

Materials and methods. Ambient air quality in the analyzed cities was estimated over the period 2020–2022 relying on social-hygienic monitoring data. It is noteworthy that, opposed to Rosgidromet posts, social-hygienic monitoring posts are usually located in zones with the highest health risks thereby providing systemic observations of the greatest hazards and threats [18–20].

Instrumental research was accomplished in the selected cities by experts from the regional centers for hygiene and epidemiology. All the laboratory centers were certified to estimate ambient air quality. Air samples were taken as per comprehensive and / or non-comprehensive programs. Hygienic assessments and health risks assessment relied on data being adequate and sufficient for calculating average annual concentrations (not less than 300 single measurements or not less than 75 daily ones as per each chemical at each sampling point). The monitoring programs included all the chemicals making contributions to 95 % of unacceptable health risks; these chemicals were identified as priority ones and fixed as such by letters of the RF Chief Sanitary Inspector[2]. An average annual concentration in health risk assessment was taken at the top 95 % confidence limit. If a chemical concentration was identified below the limit of detection in more than 95 % of samples, it was excluded from health risk assessment.

Health risks were assessed in full conformity with algorithms and indicators stipulated in the Guide R 2.1.10.1920-04 Human Health Risk Assessment from Environmental Chemicals[3]. Health risks were classified in accordance with the Methodical Guidelines MR 2.1.10.0156-19 'Assessment of ambient air quality and public health risk analysis …'[4].

Air protection was analyzed by using calculated estimations of contributions made by specific chemicals and by economic entities as a whole to unacceptable health risks (the estimation relied on an aggregated database that contained parameters of emission sources in a given city).

A contribution made by a specific object (a facility, motor transport, or autonomous heat sources) to a risk rate was identified as weighted average of contributions made by a facility at specific points as per the formula:

$$\delta_j^k = \frac{\sum_i HQ_i^k \cdot \delta_{i,j}^k}{\sum_i HQ_i^k},$$

where δ_j^k is a contribution of the *j*-th facility to a hazard index at the *k*-th point;

HQ_i^k is a hazard quotient identified for the *i*-th chemical at the *k*-th point;

[2] Pis'mo Rospotrebnadzora ot 23.11.2020 № 02/23971-2020-23. Perechni prioritetnykh zagryaznyayushchikh veshchestv dlya territorii g. Bratsk, g. Nizhnii Tagil, g. Cherepovets [The Letter by Rospotrebnadzor dated November 23, 2020 No. 02/23971-2020-23. The lists of priority pollutants in Bratsk, Nizhniy Tagil, and Cherepovets] (in Russian); Pis'mo Rospotrebnadzora ot 11.12.2020 № 02/25401-2020-23. Perechni prioritetnykh zagryaznyayushchikh veshchestv dlya territorii eksperimenta (g. Noril'sk g. Lipetsk, g. Chelyabinsk, g. Krasnoyarsk) [The Letter by Rospotrebnadzor dated November 11, 2020 No. 02/25401-2020-23. The lists of priority pollutants on the experiment territories (Norilsk, Lipetsk, Chelyabinsk, and Krasnoyarsk)] (in Russian); Pis'mo Rospotrebnadzora ot 21.12.2020 № 02/26092-2020-23. Perechni prioritetnykh zagryaznyayushchikh veshchestv dlya territorii eksperimenta (g. Magnitogorsk, g. Omsk, g. Chita, g. Mednogorsk, g. Novokuznetsk) [The Letter by Rospotrebnadzor dated December 21, 2020 No. 02/26092-2020-23. The lists of priority pollutants on the experiment territories (Magnitogorsk, Omsk, Chita, Mednogorsk, and Novokuznetsk)] (in Russian).

[3] Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200037399 (January 21, 2023) (in Russian).

[4] MR 2.1.10.0156-19. 2.1.10. 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: Metodicheskie rekomendatsii [Methodical Guidelines MR 2.1.10.0156-19. 2.1.10. Assessment of ambient air quality and public health risk analysis in order to make well-grounded managerial decisions concerning provision of ambient air quality and sanitary-epidemiological wellbeing of the population: Methodical guidelines]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_415503/ (January 01, 2023) (in Russian).

$\delta^{k}_{i.j}$ is a contribution made by the *j*-th facility to ambient air pollution at the *k*-th point as per the *i*-th chemical.

Contributions made by specific objects to hazard indexes were calculated only in zones where unacceptable health risks were identified and separately for each critical organ or system. Assessment of these contributions gave grounds for identifying priority objects responsible for unacceptable public health risks in a given city.

Relevance and adequacy of air protection was assessed on the example of Norilsk. Parameters of implemented activities were taken in accordance with the Complex regional plan on reduction in pollutant emissions and the documents issued by economic entities to set quotas for emissions.

We compared suggested quotas and reduction in emission levels and contributions made by an economic entity to unacceptable health risks, both in total and as per specific chemicals in emissions.

Basic results. Instrumental research was established to be accomplished in conformity with the existing programs and in required volumes in all 12 cities.

Hygienic standards were violated actually in all the project cities and these violations were detected over the whole observation period as per one or several priority chemicals.

Thus, for example, average annual concentrations of six chemicals were higher than average annual maximum permissible ones (MPC) in Krasnoyarsk in 2022. Out of 14 priority chemicals (some types of priority dusts are measured as ‘particulate matter’ at the monitoring posts), elevated levels were identified for nitrogen oxide (3.32 average annual MPC), nitrogen dioxide (4.60 average annual MPC), particulate matter (1.21 average annual MPC), and benz(a)pyrene (up to 2.07 average annual MPC). Particulate matter $PM_{2.5}$ and PM_{10} were not included into the lists of priority chemicals[5] but they were still identified in high concentrations at monitoring posts, up to 1.47 average annual MPC and up to 2.11 average annual MPC accordingly. Benzene, a hazardous toxicant and carcinogen, was identified at monitoring posts in levels equal to 1 average annual MPC.

In Chelyabinsk, average annual concentrations of seven chemicals were higher than the existing hygienic standards over the same period: benzene (up to 4.81 average annual MPC), dimethyl benzene (up to 1.79 average annual MPC), prop-2-en-1-al (up to 5.61 average annual MPC), sulfuric acid (up to 26.4 average annual MPC), trichloroethylene (up to 1.43 average annual MPC), formaldehyde (up to 1.13 average annual MPC), and ethenylbenzene (up to 2.87 average annual MPC).

In Norilsk, nitrogen dioxide levels reached 1.5 average annual MPC; benzene, 5 average annual MPC; manganese, 5.6 average annual MPC; copper oxide, 15.4 average annual MPC.

In Omsk, average annual concentrations of two chemicals were higher than the hygienic standards in 2022, namely, benz(a)pyrene (1.8 average annual MPC) and benzene (3.3 average annual MPC).

The most favorable situation was in Cherepovets where the hygienic standards were violated only as per chromium compounds in 2022 according to social-hygienic monitoring data.

It is noteworthy that the sanitary-hygienic situation concerning ambient air quality did not change substantially over the analyzed period in the project cities. Any changes were either unstable or within statistical error. Table 1 provides average annual concentrations of priority chemicals in dynamics in Krasnoyarsk. There is a slight reduction in levels of some chemicals (manganese and formaldehyde). However, there is a growth in average annual levels of such chemicals as nitrogen oxide and dioxide, benz(a)pyrene, and fluorine compounds in ambient air in the city.

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[5] Due to the fact that these pollutants are not considered in emissions from industrial sources and, consequently, in aggregated calculations for the city as a whole.

A similar situation is observed in Norilsk (Table 2). Ground-level concentrations of nitrogen dioxide, particulate matter, and copper oxide grew over the analyzed period. These chemicals are known to make substantial contributions to negative effects on public health. There was no significant reduction in average annual concentrations of other chemicals.

Table 1

Average annual concentrations of priority pollutants in ambient air in Krasnoyarsk in 2020–2022 (according to SHM data), shares of *average annual MPC*

Chemical		av.an. MPC, mg/m^3	2020	2021	2022**
П*	Nitrogen (II) oxide	0.06	0.65	0.74	3.32 (2.62–4.36)
П	Nitrogen dioxide	0.04	0.74	1.0	4.60 (4.13–4.99)
П	Benz(a)pyrene	0.000001	1.72	1.94	2.07 (0.91–3.74)
П	Benzene	0.005	–***	0.99	1.00 (0.89–1.12)
П	Particulate matter	0.075	1.04	0.90	1.21(0.68–1.60)
П	Aluminum trioxide	0.005	0.08	–	0.07 (0.07)
П	Manganese and its compounds	0.0005	1.37	–	0.69 (0.69)
П	Nickel oxide	0.00005	1.63	–	–
П	Sulfur dioxide	–	–	–	–
П	Carbon (soot)	0.025	0.02	0.03	0.08 (0.08)
П	Formaldehyde	0.003	0.99	0.10	0.44 (0.10–0.80)
П	Gaseous fluorides	–	0.14	0.13	0.27 (0.20–0.41)
	PM_{10}	0.04	1.56	1.36	1.47 (0.92–2.25)
	$PM_{2.5}$	0.025	2.30	1.89	2.11 (1.30–2.46)

Note: * means a chemical is included into the priority list; ** means minimal and maximum values obtained at some SHM posts are given in brackets; *** means there are not enough measurements for calculating an average annual concentrations or a chemical levels was not measured at all.

Table 2

Average annual concentrations of priority pollutants in ambient air in Norilsk in 2020–2022 (according to SHM data), shares of *average annual MPC*

Chemical		av.an. MPC, mg/m^3	2020	2021	2022**
П*	Nitrogen dioxide	0.04	0.28	0.98	1.48 (1.43–1.54)
П	Benz(a)pyrene	0.000001	–***	–	–
П	Benzene	0.005	–	–	4.93 (4.55–5.97)
П	Particulate matter	0.075	0.29	0.30	0.46 (0.46)
П	Dihydrosulfide	0.002	0.13	0.28	0.09 (0.05–0.17)
П	Manganese and its compounds	0.0005	–	–	5.58 (4.16–6.68)
П	Copper oxide	0.00002	9.10	18.02	15.43 (5.65–32.9)
П	Nickel oxide	0.00005	0.67	1.70	–
	Sulfur dioxide	0.05****	49.4	40.7	–
	Lead and its inorganic compounds	0.00015	0.28	0.32	0.30 (0.27–0.33)
П	Chromium (per Cr+6)	0.000008	–	–	0.13 (0.08–0.16)
	PM_{10}	0.04	0.53	0.52	0.20 (0.16–0.26)
	$PM_{2.5}$	0.025	0.76	0.71	0.08 (0.04–0.11)

Note: * means a chemical is included into the priority list; ** means minimal and maximum values obtained at some SHM posts are given in brackets; *** means there are not enough measurements for calculating an average annual concentrations or a chemical levels was not measured at all; **** means average daily MPC.

Table 3

Dynamics of chronic non-carcinogenic risks (for the respiratory organs) and total carcinogenic risk for people in 12 cities included into the 'Clean Air' Federal Project

City	Chronic non-carcinogenic risks of respiratory diseases (HI)*			Total carcinogenic risk (Rcr)		
	year			year		
	2020	2021	2022	2020	2021	2022
Chelyabinsk	33.5	16.5	42.6	9.1E-03	6.5E-03	5.5E-03
Mednogorsk	10.5	8.2	13.5	6.8E-05	6.7E-05	7.8E-05
Norilsk	30.9	43.6	29.0	2.8E-07	3.3E-07	1.23E-4
Bratsk	16.3	6.3	4.5	2.1E-04	2.5E-04	8.4E-05
Chita	12.2	13.1	34.3	3.2E-06	9.2E-05	1.4E-04
Nizhniy Tagil	5.0	12.5	5.0	2.8E-04	2.5E-04	4.6E-04
Krasnoyarsk	5.11	3.7	10.5	3.2E-04	2.4E-05	2.9E-05
Novokuznetsk	8.4	4.9	5.1	1.7E-04	1.1E-04	6.9E-05
Lipetsk	2.4	17.5	17.9	7.7E-06	1.0E-05	3.2E-05
Magnitogorsk	5.6	6.3	4.0	5.4E-06	5.8-04	1.1E-05
Omsk	4.7	5.6	5.8	1.1E-04	1.3E-04	1.3E-04
Cherepovets	1.2	2.7	1.6	3.7E-07	1.2E-06	3.6E-07

Note:

- High risk, HI > 6.0; Rcr > 1.1E-0.3
- Alerting risk: 6.0 ≥ HI > 3.0; 1.1E-03 ≥ Rcr > 1.0E-04
- Low, permissible risk 3.0 ≥ HI > 1.0; 1.0E-04 ≥ Rcr > 1.0E-06
- Target negligible risk HI < 1.0; Rcr ≤ 1.0E-06

Obviously, ambient air quality did not improve in the analyzed cities and, accordingly, exposure levels did not change either. As a result, health risks also changed only slightly. Table 3 provides the total dynamics of carcinogenic health risks and a chronic non-carcinogenic risk of respiratory diseases for people in the analyzed cities. The respiratory system is most frequently affected under exposure to ambient air pollution.

It is noteworthy that risks had not been assessed in the cities prior to 2020 relying on instrumental measurements and the data in dynamics are available only for the period 2020–2022.

Absence of any substantial changes in ambient air quality does not always correlate with data on emissions of pollutants into ambient air and data on implemented air protection activities. Thus, according to the State Report 'On the ecological situation and environmental protection in the Russian Federation in 2020'[6], Chelyabinsk authorities declared that emissions were reduced by 13 % (18.2 thousand tons) in 2020 only. As opposed to 2017, emissions went down by 17 %; this figure is very close to the target indicator fixed in the Federal Project where emissions are expected to fall by 20 %. However, public health risks in the city not only have remained high but have grown in 2022 against both 2020 and 2021 as regards chronic non-carcinogenic risks.

Industrial enterprises in Lipetsk also declare reductions in emissions. According to statistical reports, emissions went down by almost 10 thousand tons in 2022 against 2021 (mostly, due to reduced emissions of nitrogen oxide and carbon oxide). Nevertheless, this reduction has not secured any substantial improvement since risks of respiratory diseases remained high (mostly due to absence of any considerable reduction in emissions of highly toxic and carcinogenic chromium compounds). Attention should also be paid to a growth in a potential carcinogenic health risk; this growth,

[6] O sostoyanii i ob okhrane okruzhayushchei sredy Rossiiskoi Federatsii v 2020 godu: Gosudarstvennyi doklad [On the ecological situation and environmental protection in the Russian Federation in 2020: the State Report]. *Ministry of Natural Resources and Environment of the Russian Federation*. Available at: https://2020.ecology-gosdoklad.ru/ (January 21, 2023) (in Russian).

Table 4

Predicted results of air protection activities implemented by the Zapolyarnyi branch of the OJSC MMC Norilsk Nickel

Emission component	Emissions, tons/year, 2019	Emissions in 2024, tons/year (20 % reduction)	Sufficiency as per health risk indicators	Residual risk (for this chemical emitted by this enterprise)
Nickel sulfate	1.12	0.90	Sufficient	Acceptable
Lead and its compounds	12.31	9.85	Insufficient	Unacceptable, alerting
Copper oxide	487.50	390.00	Insufficient	Unacceptable, high
Nickel oxide	238.19	190.55	Insufficient	Unacceptable, high
Sulfuric acid	13,454.23	10,763.39	Insufficient	Unacceptable
Benzene	3.47	2.78	Excessive; the enterprise does not contribute to unacceptable risks	Acceptable
Nitrogen (II) oxide	2001.65	1601.32	Sufficient	Acceptable
Nitrogen dioxide	12,731.81	10,185.45	Sufficient	Acceptable
Sulfur dioxide	1,802,181.58	1,441,745.26	Insufficient	Unacceptable, alerting
Carbon oxide	20,121.67	16,097.34	Excessive, the enterprise is not the major source of the chemical	Acceptable
Total dusts (particulate mater)	8473.33	6778.67	Insufficient	Unacceptable, alerting

though small, is rather stable. It is still considered low and permissible but it grew by almost four times over three years of observation.

In Norilsk, data provided by economic entities (the report forms No. 2-tp Air) give evidence that emissions decerased by more than 216 thousand tons between 2019 and 2022. However, a chronic non-carcinogenic risk of respiratory diseases remained practically the same in 2022 as in 2020 and carcinogenic risks even grew.

To analyze the situation, we made an attempt to predict what results would be achieved by implementation of air protection activities by the Zapolyarnyi branch of the OJSC MMC Norilsk Nickel (the major economic entity and a source of ambient air pollution). The prediction was made considering the necessity to provide hygienic safety for population which was taken as absence of impermissible public health risks. We considered the tasks that economic entities had to tackle during the experiment on setting emission quotas and estimated possible outcomes of 20 % reduction in emissions of hazardous chemicals. The generalized results are provided in Table 4.

Obviously, insufficient reduction in emission of copper and nickel oxides leads to persistently high public health risks (respiratory diseases, blood diseases, and some systemic disorders). At the same time, quotas for emissions of carbon oxide and benzene as well as reduction in their levels are excessive and do not have any substantial influence on health risk rates.

The situation in Norilsk seems typical for all the cities included into the Federal Project.

Obviously, 20 % reduction in total emission volumes will not secure complete absence of unacceptable health risks even if all the project targets have been achieved.

Given all that, it seems that it is Rospotrebnadzor that can and should take on the responsibility for providing complete sanitary-epidemiological wellbeing of the population. This can be achieved by fixing health risk indicators in regulatory documents as regards the whole system for managing quality of the environment [21].

The assessment was accomplished considering a solution to an optimization task when an optimization indicator is minimum reduction in emissions able to ensure that the existing hygienic standards are met at any calculation point in a residential area within a city and carcinogenic and non-carcinogenic health risks remain within their acceptable ranges.

Conclusions. In general, this study has established that:

- economic entities in the cities included into the Federal Project 'Clean Air' declare such reduction in emissions that is still unable to secure absence of unacceptable airborne health risks for people in 11 out of 12 cities.

According to social-hygienic monitoring data, hygienic safety of the population is provided only in Cherepovets where health risks remain low, permissible and do not require any additional activities. Still, systemic control of the environmental situation is mandatory;

- the authorities responsible for regulating the experiment on setting emission quotas focus on the total 20 % reduction in emissions from all the economic entities included into the experiment and do not properly consider health risk rates when setting these quotas. This may lead to absence of any substantial effects on public health in the project cities and in some cases even create excessive financial costs spent by economic entities on some activities that do not have any substantial influence on the sanitary-hygienic situation;

- it is advisable to make reconnaissance estimates whether air protection activities are relevant and adequate using permissible health risk levels as estimation criteria and to assess residual health risks after implementation of both isolated activities and the whole set of air protection measures stipulated by the Complex plans on reduction in emissions; their timely improvement and / or adjustment is also advisable;

- achievement of permissible risk levels should obviously be evidenced by epidemiological data on a given territory and results of profound biomedical research aimed at creating solid evidence base of either absence or persistence of public health harm after emissions have been reduced to a target level fixed by ecological standards;

- comprehensive analysis of integrated data within the ‘dispersion calculation – results of instrumental (field) measurements of ambient air quality – health risk – actual health harm’ system gives solid grounds for making optimal managerial decisions primarily aimed at protecting public health in the cities participating in the Federal Project ‘Clean Air’ and accomplishing the experiment on setting emission quotas;

- analysis of the Complex plans revealed the necessity to develop medical and preventive measures and to include them into plans of compensatory activities when it was temporarily impossible to reduce health risks down to their permissible levels due to some technical and / or organizational limitations. Health protection within medical and preventive programs including those funded by economic entities as sources of health risks seems to be able to have significant economic and social effects due to declining social anxiety, less intensive environmental tensions and a better image of authorities and businesses as socially responsible structures.

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Research article

MORTALITY AMONG ADULTS IN THE ARCTIC MACRO-REGION: DYNAMICS, STRUCTURE AND FEATURES

B.A. Revich[1], T.L. Khar'kova[1,2], E.A. Kvasha[2]

[1]Institute of Economic Forecasting of the Russian Academy of Sciences, 47 Nakhimovskii Ave., Moscow, 117418, Russian Federation
[2]Vishnevsky Institute of Demography of National Research University 'Higher School of Economics', 3 Bolshoi Trekhsvyatitelskii lane, Moscow, 109028, Russian Federation

An expected increase in the number of workplaces in the Arctic macro-region will require a lot of available local workforce. However, currently the working age population is declining markedly there. Between 2014 and 2020, the population decreased everywhere, except the Yamal Nenets Autonomous Area; the decrease was the most apparent in the Arkhangelsk region, Murmansk region, and the Komi Republic. Fluctuations in the share of the working age population in the Arkhangelsk region and the Nenets Autonomous Area practically have the same dynamic as in the country as a whole (the decline equals 2–3 %). The greatest declines (5–7 %) were observed in the Magadan region, Chukotka, the Murmansk region and the Komi Republic.

Mortality rates in the Arctic macro-region tend to be higher than the national average rate due to uncomfortable Arctic climate, long distances between the settlements and basic healthcare facilities, and some other reasons. A current decline in mortality among the working age population has not reached its national average level yet. The major causes of elevated mortality in the Arctic macro-region include ischemic heart disease (in Chukotka, the Arkhangelsk region and the Murmansk region); stroke (in Karelia and the Komi Republic); external causes including accidental alcohol poisoning (in Karelia, the Komi Republic, and the Arkhangelsk region).

To protect health of the working age population, it is necessary to develop and implement regional programs aimed at reducing mortality due to the major causes, which differ from one region to another. These programs should consider the experience gained in the other Arctic regions. It is also necessary to implement more effective healthcare management systems. This includes development of specific models for various population groups with specific working conditions and lifestyle; development of private-public partnerships; making healthcare more available.

Keywords: *Arctic, demography, public health, mortality, working age population, prevention, working conditions and lifestyle, macro-region, natural and climatic conditions.*

The development of the Arctic macro-region is a major task within the overall country development. Therefore, demographists, healthcare professionals, economists, sociologists and experts in other spheres have addressed the outstanding task of preservation of population size in these territories [1–4]. The population outflow from the Arctic is greater than that from other Russian regions. Active migration from these territories began in the last decade of the 20th century. Between 2000 and 2018, the population declined from 11.6 to 9.9 million people, or almost by 15 % [5].

According to the RF President Order issued in 2014[1], the Arctic zone in Russia includes the whole territory of the Murmansk

Boris A. Revich – Doctor of Medical Sciences, Professor, Chief Researcher and Head of the Laboratory for Environment Quality Prediction and Population Health (e-mail: brevich@yandex.ru; tel.: +7 (499) 129-36-33; ORCID: https://orcid.org/0000-0002-7528-6643).

Tat'yana L. Khar'kova – Candidate of Economic Sciences, Senior Researcher at the Laboratory for Environment Quality Prediction and Population Health (e-mail: tatkharkova@mail.ru; tel.: +7 (910) 479-69-84; ORCID: https://orcid.org/0000-0001-7460-1966).

Ekaterina A. Kvasha – Candidate of Economic Sciences, Senior Researcher (e-mail: keapost@gmail.ru; tel.: +7 (916) 811-19-52; ORCID: https://orcid.org/0000-0002-2003-5484).

[1] O sukhoputnykh territoriyakh Arkticheskoi zony Rossiiskoi Federatsii: Ukaz Prezidenta RF ot 2 maya 2014 g. № 296 [On land territories located in the Arctic zone of the Russian Federation: the RF President Order issued on May 2, 2014 No. 296]. *GARANT: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/70547984 (December 18, 2022) (in Russian).

region, the Nenets Autonomous Area, the Yamal Nenets Autonomous Area and Chukotka as well as some parts of the Arkhangelsk region (58 %), the Komi Republic (9 %), Yakutia (7 %) and Karelia (6.5 %). Since 2014, some other territories in Yakutia and Karelia have been added to the Arctic zone. To estimate mortality rates among the working age population in the Arctic zone, we used a concept of 'macro-region', that is, a geographical area that comprises different regions with similar natural and climatic conditions due to their close proximity to the Arctic Circle. The data on mortality rates are generally more reliable on the regional rather than on the municipal level, because the regional authorities regularly review and validate these data according to the issued death certificates. This is why we used the regional level data on mortality among the Arctic populations. Besides, specialized healthcare, including high-tech one, is available mostly in the regional capitals in the Arctic zone and we should consider this fact when interpreting data on mortality rates and other health indicators in the Arctic.

The demographic processes in the Russian Arctic zone have been described in detail in the monograph coauthored by several authors and edited by Professor V.V. Fauzer [3]. Still, mortality rates among the working age population as well as some territory-specific differences have not been sufficiently investigated. Some studies established that mortality rates in small industrial towns (Apatity, Kandalaksha, Kirovsk, and Monchegorsk) in the Murmansk region were higher than those in Murmansk city [6]. Very interesting results were reported in a study that compared mortality rates in two cities with highly uncomfortable climate, Yakutsk (population 340,000) and Nizhnevartovsk (population 250,000). Mortality due to cardiovascular diseases in the age group between 20 and 44 years was higher in Nizhnevartovsk than that in Yakutsk. The authors explained this finding by a higher share of indigenous people in Yakutsk because the latter are genetically better adapted to cold. Another factor that contributed to such difference could be traditional diets of Yakut people. However, mortality rates due to cerebrovascular diseases (stroke) in the older age groups were higher in Yakutsk [7].

Some quite unexpected results were reported in a study that addressed the high mortality rates due to external causes. It was based on data collected in 2011–2016 and provided by the Unified Interdepartmental Information and Statistics System (UIISS). Arctic territories covered by this study included Yakutia and the Magadan region where total mortality and mortality due to circulatory system diseases (CSD) went down whereas mortality due to external causes did not decline. Moreover, mortality due to external causes surpassed the CSD-related mortality by 5.8 % in 2016 for the first time and reached 227 cases per 100,000. Thus, the external causes became the main contributor to the total mortality [8], which has never been observed in any other territory.

Spatial heterogeneity in mortality rates is quite common for the Arctic macro-region; it can occur even within the same region [9, 10]. However, there were no studies focusing on the whole Arctic territory. Meanwhile, spatial differences in mortality rates in Russia remain a major topic for the demography studies which predict the socioeconomic situation in the country[2] [11, 12]. Without such analysis, it is impossible to identify the most problematic areas with the greatest mortality rates among the working age population, and to develop future projections of the demographic indicators.

It is in the Arctic that health of the working age population becomes a crucial issue since the target is to create 180,000 workplaces in the macro-region by 2030. These workplaces cannot be filled only by the shift workers. Besides, the shift workers have a lot of specific health problems as a separate group of the working age population. Specific psychology tests applied to this group concluded

[2] Shchur A.E. Mortality differentiation by regions, cities of different population sizes and rural areas in Russia: the abstract of the thesis … for the Candidate of Social Science degree. Moscow, 2022, 24 p. (in Russian); Shkol'nikov V.M. Geograficheskie faktory prodolzhitel'nosti zhizni [Geographical factors of life expectancy at birth]. *Izvestiya AN SSSR. Seriya geograficheskaya*, 1987, no. 3, pp. 35–44 (in Russian).

that the shift workers in the Arctic should be intellectually developed, able to give subjective health self-assessments and take care of themselves [13]. It is not evident that it will be possible to find such specifically eligible shift workers since their satisfaction with work in shifts has been declining–over recent years. High wages is their only incentive [14] but in future, when oil and gas production will go down, this advantage will also disappear.

Materials and methods. Mortality rates among population, including working age people (aged 15–64 years) were taken from the annual reports issued by the Russian Statistical Service (Rosstat). In particular, Table C51 'Distribution of deceased citizens as per sex, age, and causes of death' summarizes the data on average population numbers in 2000–2021. Age-specific mortality rates were calculated for several major causes of death and for total mortality. To remove any influence of the differences in age structures among the regions, regional mortality rates were compared based on calculated standardized mortality rates according to the International Classification of Diseases, the 10th Revision (ICD-10). The European standard population (1976) was taken as the reference population in this study. The temporal trends in mortality rates were estimated by linear regression analysis using IBM SPSS Statistics 21 software. The differences between the analyzed trends were considered statistically significant at $p \le 0.05$. There were substantial fluctuations in the cause-specific mortality rates in the Nenets Autonomous Area, Yamal Nenets Autonomous Area, Chukotka and Magadan region. For this reason, and considering small population sizes in these regions, we did not report the results for these regions in Tables 1 and 2.

Table 1

Statistically significant ($p < 0.05$) descending trends in cause-specific mortality among the population in the Arctic macro-region and Russia as a whole in 2000–2021

Region	Men		Women	
All-cause mortality				
	Trend	95 % CI	Trend	95 % CI
Nenets Autonomous Area	-65.2	-82.6 ÷ -47.7	-42.7	-52.2 ÷ -33.2
Yamal Nenets Autonomous Area	-31.5	-41.7 ÷ -21.3	-21.8	-27.1 ÷ -16.4
Chukotka	-66.0	-94.4 ÷ -37.5	n/a	
Karelia	-58.7	-74.6÷ -42.7	-26.2	-34.1 ÷ -18.2
Komi Republic	-56.2	-70.1 ÷ -42.4	-27.2	-34.5 ÷ -19.8
Arkhangelsk region	-59.8	-72.7 ÷ -46.8	-26.1	-32.2 ÷ -19.9
Murmansk region	-54.5	-68.7 ÷ -40.2	-22.7	-30.2 ÷ -15.2
Magadan region	-71.0	-81.0 ÷ -61.1	-27.5	-33.1 ÷ -21.9
Yakutia	-46.2	-54.5 ÷ - 38.0	-25.1	-30.6 ÷ -19.7
Russian Federation	-45.3	-54.6 ÷ - 36.1	-17.8	-23.2 ÷ -12.4
Circulatory system diseases				
Nenets Autonomous Area	-49.3	-63.1 ÷ -35.6	-41.9	-47.9 ÷ -35.9
Yamal Nenets Autonomous Area	-19.4	-25.6 ÷ -13.1	-19.5	-21.7 ÷ -17.3
Chukotka	n/a		n/a	
Karelia	-40.4	-46.8 ÷ -34.0	-24.9	-28.6 ÷ -21.2
Komi Republic	-34.4	-41.4 ÷ -27.4	-21.3	-24.6 ÷ -18.1
Arkhangelsk region	-36.8	-41.9 ÷ -31.6	-19.8	-22.7 ÷ -17.0
Murmansk region	-37.4	-43.8 ÷ -31.1	-20.4	-23.1 ÷ -17.6
Magadan region	-40.7	-47.7 ÷ -33.7	-24.9	-28.5 ÷ -21.2
Yakutia	-22.6	-28.1 ÷ -17.0	-16.4	-19.4 ÷ -13.3
Russian Federation	-31.7	-35.7 ÷ -27.6	-19.7	-21.9 ÷ -17.6
including				
Ischemic heart disease				
Nenets Autonomous Area	-35.6	-46.3 ÷ -24.8	-18.7	-22.1 ÷ -15.3
Yamal Nenets Autonomous Area	n/a		n/a	

End of the Table 1

Region	Men		Women	
Chukotka	n/a		n/a	
Karelia	-20.6	-24.6 ÷ -16.6	-9.4	-11.5 ÷ -7.3
Komi Republic	-15.4	-19.4 ÷ -11.5	-9.4	-11.5 ÷ -7.3
Arkhangelsk region	-15.6	-17.6 ÷ -13.5	-3.4	-4.9 ÷ -1.9
Murmansk region	-17.5	-20.6 ÷ -14.4	-5.3	-6.5 ÷ -4.1
Magadan region	-16.8	-23.1 ÷ -10.5	-6.6	-8.9 ÷ -4.3
Yakutia	-8.1	-11.3 ÷ -4.9	-4.0	-6.0 ÷ -1.9
Russian Federation	-15.0	-17.1 ÷ -12.8	-7.0	-8.1 ÷ -5.9
Strokes				
Nenets Autonomous Area	n/a		-13.5	-16.1 ÷ -10.9
Yamal Nenets Autonomous Area	n/a		-9.1	-11.1 ÷ -7.1
Chukotka	n/a		n/a	
Karelia	-19.1	-21.6 ÷ -16.6	-15.7	-17.2 ÷ -14.2
Komi Republic	-17.9	-20.6 ÷ -15.1	-14.5	-16.4 ÷ -12.6
Arkhangelsk region	-17.6	-21.2 ÷ -14.0	-14.4	-17.4 ÷ -11.5
Murmansk region	-16.5	-19.7 ÷ -13.3	-12.3	-14.6 ÷ -10.0
Magadan region	n/a		-6.4	-8.2 ÷ -4.6
Yakutia	-4.6	-5.7 ÷ -3.5	-4.1	-4.8 ÷ -3.3
Russian Federation	-12.5	-13.9 ÷ -11.1	-9.9	-10.9 ÷ -8.9
External causes				
Nenets Autonomous Area	-20.0	-24.4 ÷ -15.5	-4.8	-6.2 ÷ -3.3
Yamal Nenets Autonomous Area	-8.9	-10.3 ÷ -7.4	-2.8	-3.3 ÷ -2.3
Chukotka	-11.1	-15.8 ÷ -6.5	n/a	
Karelia	-19.4	-22.4 ÷ -16.4	-5.0	-5.6 ÷ -4.4
Komi Republic	-17.0	-19.3 ÷ -14.7	-4.6	-5.5 ÷ -3.8
Arkhangelsk region	-16.6	-19.0 ÷ -14.1	-3.8	-4.4 ÷ -3.2
Murmansk region	-9.7	-12.0 ÷ -7.4	-2.0	-2.6 ÷ -1.3
Magadan region	-12.7	-15.3 ÷ -10.2	-3.9	-4.8 ÷ -3.0
Yakutia	-13.1	-13.9 ÷ -12.2	-2.7	-3.0 ÷ -2.4
Russian Federation	-13.1	-14.5 ÷ -11.7	-3.1	-3.4 ÷ -2.8

Table 2

Statistically significant ($p < 0.05$) descending trends in case-specific mortality among the working age population (aged 15–64 years) in the Arctic macro-region and Russia as a whole in 2000–2021

Region	Men		Women	
All-cause mortality				
	Trend	95 % CI	Trend	95 % CI
Nenets Autonomous Area	-61.6	-70.1 ÷ -53.1	-16.4	-20.6 ÷ -12.2
Yamal Nenets Autonomous Area	-28.7	-33.0 ÷ -24.5	-8.8	-10.5 ÷ -7.1
Chukotka	-35.1	-47.5 ÷ -22.7	n/a	
Karelia	-58.2	-71.7 ÷ -44.7	-14.9	-20.0 ÷ -9.8
Komi Republic	-51.4	-62.2 ÷ -40.6	-15.6	-20.3 ÷ -10.9
Arkhangelsk region	-59.2	-70.5 ÷ -47.8	-14.6	-18.4 ÷ -10.9
Murmansk region	-47.9	-59.5 ÷ -36.2	-12.3	-16.1 ÷ -8.4
Magadan region	-42.5	-48.8 ÷ -36.1	-11.1	-15.2 ÷ -7.1
Yakutia	-39.4	-44.1 ÷ -34.7	-12.8	-14.9 ÷ -10.7
Russian Federation	-40.8	-47.4 ÷ -34.1	-9.4	-12.2 ÷ -6.6
Circulatory system diseases				
Nenets Autonomous Area	-33.1	-37.9 ÷ -28.2	-11.9	-14.5 ÷ -9.2
Yamal Nenets Autonomous Area	-12.1	-13.6 ÷ -10.5	-5.0	-5.7 ÷ -4.3

End of the Table 2

Region	Men		Women	
Chukotka	n/a		n/a	
Karelia	-22.4	-27.1 ÷ -17.8	-7.4	-9.5 ÷ -5.3
Komi Republic	-20.0	-24.6 ÷ -15.3	-8.7	-10.7 ÷ -6.7
Arkhangelsk region	-24.8	-29.5 ÷ -20.1	-8.0	-9.7 ÷ -6.3
Murmansk region	-26.2	-31.6 ÷ -20.7	-9.3	-11.0 ÷ -7.6
Magadan region	-18.3	-22.2 ÷ -14.4	-6.8	-8.9 ÷ -4.8
Yakutia	-14.0	-16.6 ÷ -11.5	-6.5	-7.5 ÷ -5.4
Russian Federation	-16.2	-18.8 ÷ -13.6	-5.8	-6.7 ÷ -4.9
External causes				
Nenets Autonomous Area	-26.5	-33.0 ÷ -20.1	-5.7	-7.1 ÷ -4.3
Yamal Nenets Autonomous Area	-11.3	-12.9 ÷ -9.8	-2.7	-3.2 ÷ -2.2
Chukotka	n/a		n/a	
Karelia	-26.6	-30.6 ÷ -22.5	-6.4	-7.3 ÷ -5.6
Komi Republic	-22.7	-25.8 ÷ -19.6	-6.1	-7.2 ÷ -5.0
Arkhangelsk region	-23.0	-26.5 ÷ -19.5	-4.9	-5.7 ÷ -4.1
Murmansk region	-14.4	-17.8 ÷ -11.0	-2.2	-3.0 ÷ -1.4
Magadan region	-13.8	-16.7 ÷ -10.9	-3.9	-4.9 ÷ -2.9
Yakutia	-17.0	-18.0 ÷ -15.9	-3.2	-3.5 ÷ -2.9
Russian Federation	-17.5	-19.4 ÷ -15.6	-3.7	-4.1 ÷ -3.3

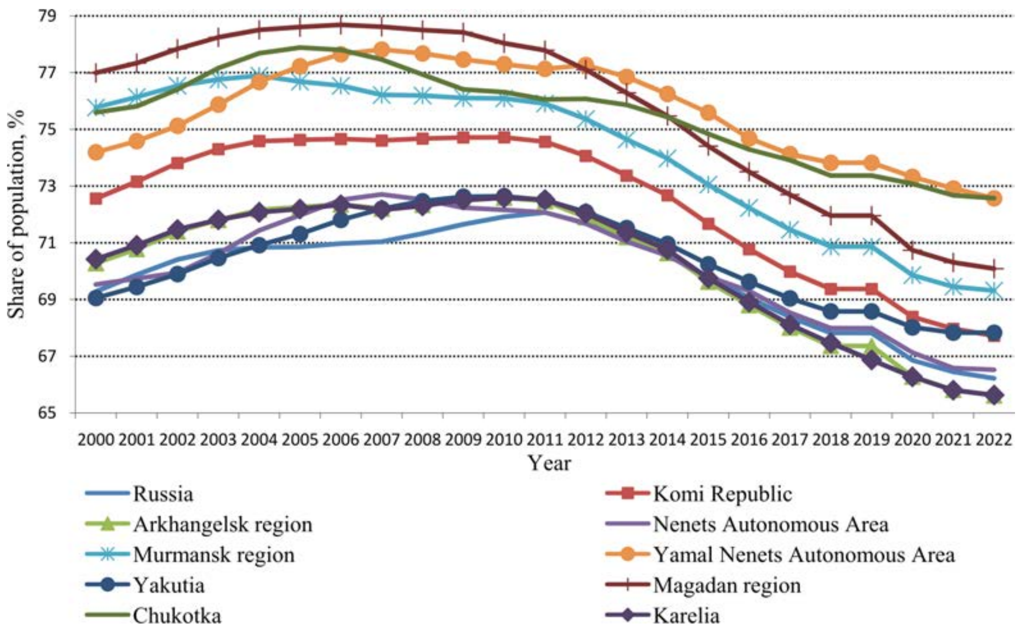

Figure 1. The shares of people aged 15–64 years (the working age population) in the total population in the regions included in the Arctic macro-region and in Russia over 2000–2022

Results. The working age population is declining steadily in the Arctic macro-region. This obviously leads to deficit in local workforce. Population outflows occurred in all Arctic regions between 2014 and 2020, except the Yamal Nenets Autonomous Area. The greatest reductions in population occurred in the Magadan region (6.8 %), the Komi Republic (5.9 %) and the Arkhangelsk region excluding the Nenets Autonomous Area (4.9 %). The average population loss in all other Arctic regions was 4 % [15]. Figure 1 shows the dynamics of the working age population in the Arctic regions. The trends in the Arkhangelsk region and the Nenets Autonomous Area are similar to those observed in the national level (the decline by 2–3 %) but the declines are greater in the Magadan region, Chukotka and the Komi Republic (5–7 %). Despite this substantial decline in the share of the working age population, this share remains the highest in these regions and exceeds 73 %. In future, the established trend is likely to continue depending on the socioeconomic situation in each Arctic region.

Dynamics and specific features of the all-cause mortality. During the first two decades of the 21st century, the trends in mortality identified in the Arctic macro-region were generally similar to those identified in Russia as a whole but the rates were different (Figure 2). In the Yamal Nenets Autonomous area, the standardized mortality rate (SMR) for all-cause mortality among men was 10 % lower than the national average. The same rate was greater by ≥ 20 % than the national average, in Chukotka, the Magadan region, the Komi Republic, and Karelia. The same was true for the Nenets Autonomous Area, the Arkhangelsk region and the Murmansk region where the SMR for all-cause mortality among men was by 10–15 % higher than the national average and it was close to the national average only in Yakutia (where it was 3 % higher). Notably, the spread in estimated mortality rates in the analyzed territories somewhat diminished over the last two decades.

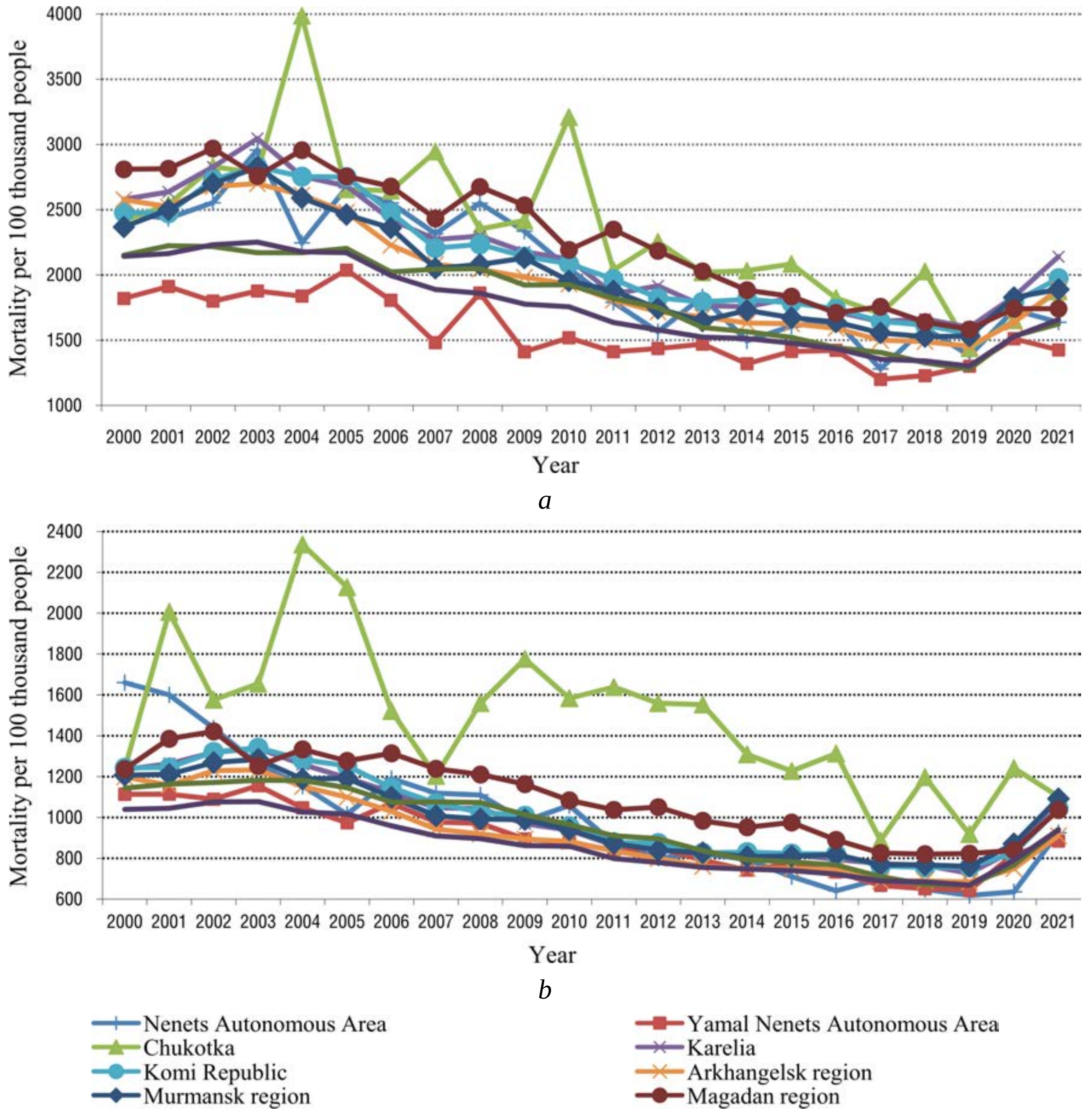

Figure 2. Dynamics of standardized mortality rates for all-cause mortality among men and women in the Arctic macro-region and in Russia as a whole per 100,000 people in 2000–2021: *a*) men, *b*) women

The situation with female population is somewhat different. The SMR for all-cause mortality among women in the Yamal Nenets Autonomous Area is the nearest to the national average, being 3.3 % higher than that. The greatest differences between the regional SMR and the national average were identified in Chukotka (where it was 50 % higher) and in the Magadan region (where it was 33 % higher). This indicator was also higher in the Nenets Autonomous Area, Karelia, the Komi Republic, and Yakutia; the difference varied between 10 and 15 % on average. This difference was 5.5 % higher in the Arkhangelsk region. Interestingly, the SMR for all-cause mortality among women in the Arkhangelsk region was even lower than the national average in 2020 and 2021, by 5.3 and 3.1 % accordingly. The diversity between the regional mortality rates among women was smaller than that for men. By 2021, the regional mortality rates among women generally converged, and the spread became smaller.

In general, mortality among men tends to be higher than that among women, both in Russia as a whole and in the Arctic macro-region. The SMR for all-cause mortality among men is almost two times higher than that among women. This difference is smaller only in Chukotka (it is 50 % there). This is caused primarily by relatively higher mortality among women in this region compared to the other regions.

Table 1 summarizes the trends in all-cause and cause-specific mortality in the Arctic macro-region and in the country as a whole in 2000–2021. Non-significant trends are marked ‘n/a’ (mostly in the Nenets Autonomous Area and Chukotka). A decline in all-cause mortality rates among men in the Arctic is generally more pronounced than the national average trend. The only exception is the Yamal Nenets Autonomous Area where this decline was 33 % smaller than the national average. The decline in all-cause mortality among women in the Arctic was greater than the national average, especially in the Nenets Autonomous Area and Chukotka. All-cause mortality among men decreased faster than that among women, both in Russia as a whole and in the Arctic macro-region.

In general, the trends in mortality of the working age population (people aged 15–64 years) in the Arctic macro-region were similar to those identified for the country as a whole, with certain exceptions (Figure 3). Thus, the SMR among people aged 15–64 among both men and women in the Yamal Nenets Autonomous Area in 2000–2021 was almost 25 % lower than the national average rate, whereas in Chukotka and Karelia, the SMR among men of the working age was more than 30 % higher than that among women.

The difference between the regional SMR and the national average rate was the greatest in Chukotka (where it was 50 % higher) and the Magadan region (where it was almost 33 % higher) both for women and men. Mortality among men and women of the working age was also higher than the national average in the other Arctic regions as well.

The declining trends in mortality among men aged 15–64 were a bit lower in the Yamal Nenets Autonomous Area and Chukotka than the national average. The same trends in the Magadan region and Yakutia were quite similar to the national average. On the contrary, mortality among the working age population in the other regions went down faster than the national average. Mortality rates among women of the working age declined faster in all Arctic regions than the national average, the Yamal Nenets Autonomous Area being the only exception.

In general, the structure of cause-specific mortality among the working age population in the Arctic is similar to that identified in the country: circulatory system diseases (CSD), neoplasms and external causes account for about 80 %. It is noteworthy that the share of these three major groups of causes in the Arctic macro-region is higher than that in Russia as a whole, primarily, among men. The share of the external causes in the Arctic is more than two times higher than that in Russia as a whole, whereas the share of CSD is significantly lower than the national average.

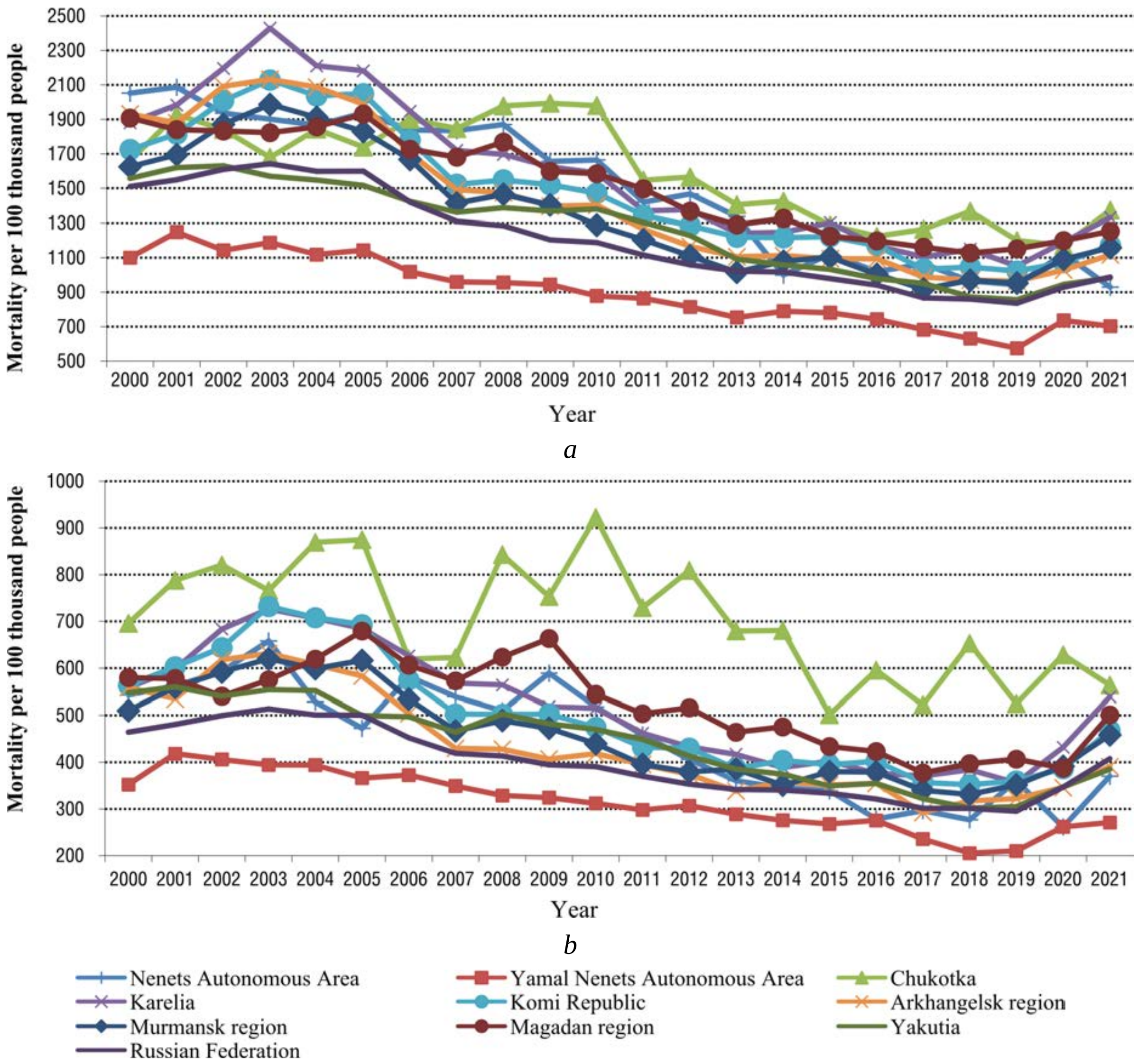

Figure 3. Dynamics of standardized mortality rates for all-cause mortality among men and women aged 15–64 years in the Arctic macro-region and in Russia as a whole per 100,000 people, in 2000–2021: *a*) men, *b*) women

Dynamics and specific features of cause-specific mortality. During the period 2000–2021, mortality among men due to CSD in the Arctic macro-region was generally higher than the national average rate, with the exception of the Yamal Nenets Autonomous Area. For example, this mortality rate was more than 30 % higher in the Murmansk region and Chukotka; more than 20 % higher in the Arkhangelsk region, Magadan region and Karelia; 15 % higher in the Komi Republic and the Nenets Autonomous Area. The SMR for the circulatory system diseases in Yakutia was almost the same as the national average rate, and this indicator in the Yamal Nenets Autonomous Area was 10 % lower than the national average rate.

Regarding the trends in CSD mortality rate, it went down in each analyzed region until 2019. After that, it increased again in 2020 and 2021 during the developing COVID-19 pandemic. Thus, the highest statistically significant decreases in CSD-caused mortality compared with the national average level during the analyzed period – were identified for both men and women in the Nenets Autonomous Area, Karelia, and the Magadan region. The decreases in CSD mortality rate were slightly greater than that for the national average rate in the Komi Republic, Arkhangelsk region, and the Murmansk region. The same decrease among men was 50 % smaller than the national average in the Yamal Nenets Autonomous Area and SCD mortality rate for women in this region was equal to the national average. The CSD-caused mortality among men and women in Yakutia went down slower than in Russia on average.

Ischemic heart disease and strokes account for about 80 % of all CSD-related deaths among men and women both in Russia as a whole and in the Arctic macro-region. In 2000–2021, the SMR due to ischemic heart disease in all analyzed regions was higher than the national average rate, except in Yakutia for men and women, in the Yamal Nenets Autonomous area for men and in Karelia and the Komi Republic for women. The SMR due to ischemic heart disease was lower in these four regions than the national average rate. At the same time, the SMR due to ischemic heart disease among men in the Arkhangelsk region, Murmansk region, and Chukotka was almost 30 % higher than the national average rate. The greatest deviations from the national average in the SMR due to ischemic heart disease for women were observed in Chukotka (by 50 %) and the Nenets Autonomous Area (by 33 %). It should be noted that the statistical reports contain large uncertainties when it comes down to registering deaths due to this cause; in addition, there are certain problems related to healthcare availability in these regions [16].

The inter-regional differences in mortality caused by strokes in the Arctic are very similar for men and women. The SMR due to strokes in Karelia, the Komi Republic and the Murmansk region was almost 50 % higher than the national average rate. Conversely, this indicator in Yakutia, the Yamal Nenets Autonomous Area and Chukotka was significantly lower than the national average rate.

We observed a common tendency in the mortality caused by ischemic heart disease and strokes among men and women in the Arctic regions: the higher SMR in a specific region, the more apparent the declining trend was.

The inter-regional differences in mortality caused by neoplasms among men and women in the Arctic macro-region were generally less pronounced. However, the SMR due to neoplasms among men and women was almost 33 % higher in the Magadan region compared to the national average rate. The same indicator among men in Karelia and the Komi Republic was 20 % higher than the national average rate. The smallest deviations in this indicator from the national average rates were observed among men in Yakutia and among women in the Arkhangelsk region.

High mortality due to external causes in Russia is known to be a major reason for the differences in life expectancy at birth between Russia and the developed countries. Mortality due to external causes was higher than the national average in nearly all Arctic regions. The greatest deviations in this indicator from the national average rate were recorded in Chukotka (70 % for men and 160 % for women) and in the Nenets Autonomous Area (50 % for both sexes). On the contrary, the SMR due to external causes among men and women was 10 % lower than the national average in the Yamal Nenets Autonomous Area and the Murmansk region. This indicator in the other Arctic regions was 20–40 % higher than the national average rate.

Suicides, homicides and injuries accounted for approximately 50 % of all deaths due to external causes among men and for 40 % of those among women, both in Russia as a whole and in the Arctic macro-region. This share was considerably higher in the Murmansk region (70.4 % among men and 66.9 % among women) in 2000–2021. During the analyzed period, the SMR due to suicides, homicides and injuries was higher in all Arctic regions (except the Yamal Nenets Autonomous Area) than the national average rate. The highest SMRs among men were identified in Chukotka (70 % higher than the national average), in the Nenets Autonomous Area and the Magadan region (50 % higher). The highest SMRs among women were identified in Chukotka (160 % higher than the national average), the Magadan (60 % higher) and Murmansk regions (50 % higher).

The highest SMRs due to accidental alcohol poisoning among men were observed in the Komi Republic (120 % higher than the national average), in the Archangelsk region (twice as higher), and in Karelia (80 % higher). The SMR due to accidental alcohol poisoning among women was 3 times higher in the Komi Republic than the national average; 2.7 times higher in Chukotka; and 2.3 times higher in Karelia. At the same time, the SMR due to this cause was lower than the national average for men and women in the Yamal Nenets Autonomous Area and Yakutia.

In 2000–2021, mortality from respiratory diseases in the Magadan region was 70 % higher for men and 110 % higher for women than the respective national averages (by 1.7 times among men and by 2.1 times among women). The same indicators in Chukotka were 60 % higher for men and 180 % higher for women than the respective national averages. Respiratory disease mortality among men in the Komi Republic was 20 % higher than the national average rate, and the same increase was observed among women in the Yamal Nenets Autonomous Area. Until 2019, the SMR due to respiratory diseases had been declining both in Russia as a whole and in the Arctic macro-region after that, it increased again in 2020–2021, most likely, due to the COVID-19 pandemic.

Mortality from gastrointestinal diseases among men was higher than the national average in one-half of the analyzed regions. The SMR due to this cause among women was higher than the national average in all the analyzed regions. The highest rates were identified in Chukotka (80 % higher), the Magadan region and the Komi Republic (50 % higher).

Spatial differences in mortality among the working age population. Inter-regional comparisons were used to identify the areas with the greatest problems and the principal causes contributing to elevated mortality rates among the working age population. The most favorable situation was observed in the Yamal Nenets Autonomous Area, a relatively wealthy and economically developed region with a larger proportion of the young population and modern cities (Nadym, Noviy Urengoy, Tarkosale, shift settlements). The Arkhangelsk region and Yakutia also fared well in this respect, with its well-developed public healthcare system, high-tech medical equipment, and the Regional Center for Cardiovascular Pathologies. These regions have large differences in the age-specific population structure. Thus, in the Yamal Nenets Autonomous Area, people aged 65 years and older accounted for 4.7 % in the total regional population in 2021 whereas this share was 9.3 % in Yakutia and 16.7 % in the Arkhangelsk region. On the other hand, the regions with the highest mortality rates include Chukotka, the Nenets Autonomous Area, the Komi Republic, Karelia, the Murmansk region, and the Magadan region. Better medical services offered by the centers for cardiovascular pathologies in these regions may lead to a decrease in the mortality caused by strokes and other cardiovascular diseases.

A very interesting situation is with mortality rates due to neoplasms. In the Arkhangelsk region and Yakutia, it is only slightly different from the average country level (probably, due to successful functioning of the university oncology department and oncological hospitals). This mortality rate is somewhat higher than on average in the country in all the other regions. It is in the Arkhangelsk region and Yakutia, with their long histories of successful public healthcare, and in the two Autonomous Areas (the Yamal Nenets and the Nenets) that the lowest mortality rates are registered among the working age population of the Arctic macro-region. But if these two Autonomous Areas and Yakutia have the highest human development index (HDI[3]) and are in the first quartile among all the RF regions, then in the Arkhangelsk region this index is relatively low due to the low income level. Probably, the low mortality rates have been achieved in this region due to the effective regional public healthcare system, the

[3] Indeks chelovecheskogo razvitiya v Rossii: regional'nye razlichiya [The Human Development Index in Russia: regional differences]: the analytical report. *The Analytical Center of the RF Government*, 2021. Available at: https://ac.gov.ru/uploads/2-Publications/analitika/2022/_2021_long.pdf (December 22, 2022) (in Russian).

whole medical society, and the Northern Medical Institute.

Mortality rates due to the external causes are rather difficult to interpret. This mortality rate is the lowest in the Yamal Nenets Autonomous Area and the Murmansk region; the highest mortality from the external causes was observed in Chukotka, the Nenets Autonomous Area, and the Magadan region. Unfortunately, such a common cause of death as accidental alcohol poisoning in the autonomous republics and autonomous areas of the Arctic macro-region is twice as higher as the national average. Mortality rate due to this cause is lower than the national average only in the Yamal Nenets Autonomous Area and Yakutia.

Discussion. What actions can be taken to reduce additional mortality among the working age population in the Arctic macro-region? An extremely important article that outlines the most vital tasks in the organization of public healthcare on this territory [17] addresses *'multi-aspect peculiarities'* of the Arctic and postulates the necessity to implement specific improvements in public healthcare for different population groups with specific working conditions and lifestyles. Such actions can be tentatively divided into several groups.

First, we should improve healthcare provided for the working age population and people living in industrial cities. Corporate healthcare is the most developed in the Arctic macro-region, especially in the oil and gas, mining and metallurgy companies. Comparative analysis of mortality among men in Norilsk and Monchegorsk in 2010–2017 provided a valuable example of the results of an effective modernization of the regional healthcare system. In Norilsk, an interregional hospital with 1000 beds was built and equipped with the most up-to-date high-tech medical equipment with the financial support from Nornickel LLC. Several specialized healthcare programs were implemented for effective treatment of specific diseases, healthcare and prevention became more available and some other measures were taken. Nothing like that has been implemented in Monchegorsk. As a result, all-cause mortality went down in Norilsk, mortality rates due to circulatory system diseases and some other causes there became approximately 10 % lower than those in Monchegorsk[4].

Approximately 10,000 people work for Gazprom Dobycha Nadym LLC in the Yamal Nenets Autonomous Area. This company has created a powerful network of occupational and preventive medicine. This network now includes 32 treatment and prevention facilities that use a three-stage rehabilitation system with sanatorium-resort therapy. As a result, the incidence rates among shift workers went down considerably [18]. Local residents can also use the services offered by these treatment and prevention facilities if needed.

The second group of actions involves improvements of publicly funded healthcare programs aimed at prevention of lethal diseases. Such programs are already being implemented, and their effectiveness is monitored by the federal and regional authorities. For example, several regional cancer prevention programs contain certain quantitative targets with respect to expected reductions in mortality rates and increases in early cancer detection rates to be achieved by 2024. These projects also have other goals such as establishment of outpatient healthcare centers, primary oncological consulting rooms, and implementation of other measures aimed at reducing cancer incidence and mortality. Unfortunately, there are not enough projects preventing deaths from the external causes that contribute significantly to the mortality structure.

The third group of actions considers development of public-private partnerships in the public health sector. Such partnerships make health management much more effective, facilitate rapid implementation of business plans and make medical aid more available.

[4] Bryleva M.S. Sotsial'no-gigienicheskoe issledovanie smertnosti naseleniya promyshlennykh monogorodov Arktiki [Social and hygienic examination of mortality among population in industrial monotowns in the Arctic]: the abstract of the thesis … for the Candidate of Biological Sciences degree. Moscow, 2021, pp. 17–18 (in Russian).

Fourth, the availability of healthcare services should increase greatly everywhere in the Arctic macro-region. This can be achieved by wider implementation of air ambulance services, mobile medical teams, and optimization of transportation routes using the traffic graph technique [19]. The fifth task is to attract highly qualified healthcare professionals to the region. Sixth, priority development targets should include distant medical technologies and medical services tailored for the specific and the most susceptible population groups such as indigenous populations, seamen, shift workers, and some others. Corporate healthcare has a lot to offer in the sphere. For example, the Center for Corporate Public Healthcare was established in cooperation with the Tomsk Medical University. This Center actively develops new methods of remote telemedicine, medical evacuation, and provision of emergency medical aid to the hard-to-reach Arctic settlements, shift camps, offshore oil platforms, ships and the like.

Elevated mortality caused by the exposure to low air temperatures is a specific problem in the Arctic macro-region. In Yakutia, deaths due to the exposure to extreme cold was the second largest contributor to deaths from all external causes before 2015. In the Arctic, more people die from freezing injuries (the ICD-10 code X31 "Exposure to excessive natural cold") than from traffic accidents.

Improvement of public healthcare influences the overall health status of the Arctic residents more than that in any other region of the RF. Still, the state of public health depends upon many other factors, primarily socioeconomic ones. Quantitative health risk assessment of these factors (income, poverty, unemployment, bad housing, natural and climate conditions, toxic environmental pollution, behavioral and personal aspects) have been addressed in many studies, including our previous research in the Arctic macro-region [20]. The health effects produced by a reduction in the number of hospital beds in the rural areas in Yakutia were considered in [21].

Excessive mortality among the working age population is both a social and economic problem. Different statistical techniques have been applied to estimate economic losses caused by elevated population morbidity and mortality. Here are some examples. Based on the *Rospotrebnadzor* Guidelines (MR 5.1.0095-14)[5], the total economic loss due to the excessive morbidity and mortality in the Komi Republic in 2014–2019 was estimated as 1.5 % of the regional GDP. This loss was mainly attributed to circulatory system diseases, neoplasms and exposure to harmful environmental factors [22]. A similar estimate was produced in a study of economic costs of primary hypertension. The total economic loss due to this disease was estimated as 870 billion Rubles or 1 % of the Russian GDP. Substantial losses are also associated with mortality caused by ischemic heart disease, strokes, and chronic obstructive pulmonary disease (COPD) [23]. Economic losses associated with all causes of death in the country were equal to 1 trillion Rubles or 3 % of the Russian GDP [24].

The number of years of potential life lost (YPLL) also serves as an estimation of the premature deaths. High mortality rates among people under 65 years of age indicate that the public management is not very effective in the socioeconomic sector. The assessments provided for specific territories showed how huge economic losses really were. For example, a study in Vologda region reported the loss of 64,875 billion Rubles due to the premature deaths among the working age population, or

[5] MR 5.1.0095-14. Raschet fakticheskikh i predotvrashchennykh v rezul'tate kontrol'no-nadzornoi deyatel'nosti ekonomicheskikh poter' ot smertnosti, zabolevaemosti i invalidizatsii naseleniya, assotsiirovannykh s negativnym vozdeistviem faktorov sredy obitaniya: metodicheskie rekomendatsii; utv. rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii A.Yu. Popovoi 23 oktyabrya 2014 g. [Calculation of actual economic losses and those prevented due to control and surveillance activities in case such losses are caused by mortality, incidence and disability among population associated with exposure to harmful environmental factors: methodical guidelines; approved by A.Yu. Popova, the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector, on October 23, 2014]. Moscow, the Federal Center for Hygiene and Epidemiology of Rospotrebnadzor, 2015, 60 p. (in Russian).

about 20 % of the gross regional product in 2018 [25].

Economists also use the public utility and actuarial method to estimate economic losses caused by excessive population mortality. This method is used to estimate both short-term and long-term economic losses. For example, the excess mortality during the severe heat wave in Moscow in 2010 was estimated as 11,000 additional deaths, which was equivalent to 7–12.3 billion rubles or 1.23–1.57 % of Moscow GRP [26]. In 2018, when the Wet-Bulb Globe Temperature (WBGT) index rose above 24 °C, the worldwide losses in labor productivity grew up to 5 % (Watts et al., 2020) [27].

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Research article

PARAMETERS FOR HEALTH RISK ASSESSMENT ASSOCIATED WITH CHRONIC EXPOSURE TO HYDROGEN SULPHIDE IN AMBIENT AIR

P.Z. Shur[1], K.V. Chetverkina[1,2], A.A. Khasanova[1]

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

High levels of chemical pollution in ambient air due to industrial emissions can facilitate development of functional disorders in various organs and systems. They are a significant component to be considered when assessing health risks under exposure to combined multi-factorial pollution. However, the issue of methodical approaches to assessing possible effects on health under exposure to combinations of chemicals has not been studied enough as regards public health risk assessment. Given that, we suggest a trend to develop the methodology that involves revising and substantiating indicators applied in health risk assessment. This should be done as new research data on influence exerted by chemicals on health (including exposure to levels higher than reference ones) become available.

We have substantiated a system of quantitative indicators (including additional reference concentrations) for health risk assessment under chronic exposure to hydrogen sulphide in ambient air (including its elevated levels). Points of departure and modifying factors were established by analyzing studies on effects produced on health by hydrogen sulphide. On their basis, we developed parameters for non-carcinogenic health risk assessment.

The reference concentration equaled 0.002 mg/m^3 (the critical systems were respiratory organs and the nervous system). The additional reference concentration for risk assessment under elevated exposure to hydrogen sulphide was substantiated as equal to 0.07 mg/m^3 (impaired development being the critical system in the case).

The suggested system of quantitative indicators enhances and specifies parameters for health risk assessment. This makes it possible to perform more adequate assessment of health risks under combined exposure to chemicals in ambient air including those contained in levels higher than reference ones.

The suggested system of quantitative indicators was tested properly; as a result, the system was established to give an opportunity to obtain more comprehensive and accurate results of health risk assessment under combined exposure to chemical pollutants.

Keywords: *health risk assessment, hydrogen sulphide, chronic exposure, the system of quantitative indicators, reference concentration, respiratory system, nervous system, development.*

Environmental pollution and its influence on public health hold a significant place in the state policy in Russia; every year, issues arising in this sphere are given more attention and finding solutions to them is recognized as vital [1]. The methodology for assessing health risks caused by chemical pollution in the environment is a most significant instrument to explore this relationship. The health risk assessment methodology is applied both in fundamental research and in practical activities. However, the issue of methodical approaches to assessing possible effects on health under exposure to combinations of chemicals has not

Pavel Z. Shur – Doctor of Medical Sciences, Chief Researcher – Academic Secretary (e-mail: shur@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0001-5171-3105).

Kristina V. Chetverkina – Candidate of Medical Sciences, Senior Researcher – Head of Environmental Risks Analysis Laboratory (e-mail: chetverkina@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-1548-228X).

Anna A. Khasanova – Researcher at the Health Risk Analysis Department (e-mail: KhasanovaAA@inbox.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0001-7438-0358).

been studied enough as regards public health risk assessment. Given that, we suggest an obvious trend to develop the methodology that involves revising and substantiating indicators applied in health risk assessment. This should be done as new research data on influence exerted by chemicals on health (including exposure to levels higher than reference ones) become available.

It is common knowledge that at present health risk assessment that addresses risks caused by chemicals with threshold effects relies on reference levels. These levels have been developed based on NOAELs or LOAELs and systems and organs that are authentically the first to react to these levels are accepted as critical ones [2]. At the same time, under exposure to a concentration higher than these levels, a pathological process can additionally involve organs or their systems, which have not been considered in risk assessment, including that with its focus on combined exposures. As a result, occurring risk levels are underestimated.

Some documents confirm health outcomes under various levels of exposure to concentrations higher than reference ones. For example, the Agency for Toxic Substances and Disease Registry, when revising its toxicological profiles, provides detailed information about the results obtained by epidemiological and toxicological studies. It deliberately highlights statistically authentic results for various organs and systems that could be affected by an analyzed chemical.

Elevated levels of chemicals, hydrogen sulphide included, were established in ambient air in some cities in Russia within implementation of the 'Clean Air' Federal project of the 'Ecology' National project. These levels occur due to industrial emissions into ambient air in Lipetsk, Magnitogorsk, Nizhniy Tagil, Novokuznetsk, Bratsk, Norilsk, Omsk, Chelyabinsk and Chita and are higher than permissible ones[1] [3].

Respiratory organs are known to be the basic target under long-term exposure to hydrogen sulphide[2] [4–6] but the chemical is established to affect the nervous system and the development processes as well. However, at present the latter effects are not considered when risk assessment is performed under combined exposure to several chemicals.

Given that, **the aim of this study** was to identify a reference concentration more precisely and to substantiate a system of quantitative indicators (including additional reference concentrations) for health risk assessment under chronic exposure to hydrogen sulphide in ambient air (including its elevated levels).

To achieve this, the following tasks were formulated and solved:

To analyze research data on influence exerted on health by hydrogen sulphide to establish points of departure and modifying factors for further development of parameters for non-carcinogenic health risk assessment.

To specify the reference level of hydrogen sulphide and relevant critical organs and systems under chronic inhalation exposure to it.

To substantiate the system of quantitative indicators (including additional reference concentrations) for health risk assessment under chronic exposure to hydrogen sulphide in ambient air in levels higher than its reference concentration.

Materials and methods. We analyzed previously accomplished studies available in

[1] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2019 godu: Gosudarstvennyi doklad [On sanitary-epidemiological welfare of the population in Russian Federation in 2019: The State report]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2020, 300 p. (in Russian); O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2020 godu: Gosudarstvennyi doklad [On sanitary-epidemiological welfare of the population in Russian Federation in 2020: The State report]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2021, 256 p. (in Russian).

[2] 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 (November 22, 2022) (in Russian).

ATSDR and U.S. EPA databases as well as those stored in the Internet databases with open access and then selected key ones that contained the most relevant data.

The study designs were estimated in order to identify modifying factors and points of departure. They were later applied to establish parameters for health risk assessment under chronic inhalation exposure; the parameters were determined as a value of a selected point of departure to a value of the total (complex) uncertainty factor ratio [7].

The lowest value among the obtained ones was taken as a reference concentration; an organ (or a system) for which a reference indicator was established was considered a critical one.

The suggested methodical approaches were applied to develop a system of quantitative indicators (additional RfC) that characterized safe elevated exposure levels as regards specific disorders (excluding those that are critical for RfC).

Results and discussion. Having estimated the available research data, we established that studies addressing impacts exerted by hydrogen sulphide in ambient air were the most convincing as per validity and completeness of the relevant materials. These studies addressed influence, first of all, on the respiratory organs and nervous system as well as on a developing body (development abnormalities) exerted by the chemical within the range of levels comparable to actual pollution [8–12].

Effects produced by hydrogen sulphide on the respiratory organs were described the most comprehensively and precisely by Brenneman with colleagues [13] and by the CIIT [14]. Our analysis of the study designs made it possible to identify elements common for both studies; according to them, the key modifying factors and their values were determined.

The studies were established to involve animal experiments and this allowed selecting a modifying factor that considered interspecific extrapolation; its value equaled 10. Correctness of selecting this factor is validated by the fact that rats are obligate as per nose breathing and therefore more susceptible to the nasal cavity damages. In addition, rats' nasal cavity (50 %) has more olfactory epithelium than human one (5–10 %). This epithelium covers a complicated net of ethmoturbinates and this considerably increases a surface area where chemicals can be absorbed and slows down airflow thereby creating much more favorable conditions for effective deposition of inhaled toxicants [15, 16].

The experiments did not consider sensitive sub-populations; therefore, the next modifying factor was selected to allow for a specific population if intraspecific extrapolation was needed. Its value also equaled 10. Since only sub-chronic exposures were examined, the next modifying factor considered duration of chronic exposure and was taken as equal to 3. The total (complex) uncertainty factor for each study that addressed negative influence exerted by hydrogen sulphide on the respiratory organs was equal to 300.

At the same time, we can see certain differences in the experiment results. Thus, the study by the CIIT established a NOAEL equal to 43 mg/m^3. An initial NOAEL value (14 mg/m^3) was established by Brenneman with colleagues based on methodical approaches developed by the U.S. EPA; it was then recalculated into hydrogen sulphide level equivalent for the human body ($NOAEL_{HEC}$) considering weekly uninterrupted exposure and anatomic and physiological peculiarities related to development of an observed health disorder. The ultimate value equaled 0.64 mg/m^3. We established certain differences at the structural level of detected histological changes in the respiratory organs. Thus, Brenneman with colleagues identified certain disorders at the cellular level such as basal cell hyperplasia in the nasal cavity mucosa whereas the study by CIIT established some changes in tissues such as signs of inflammation in the nasal cavity mucosa.

Therefore, the study conducted by Brenneman with colleagues yielded more scientifically valid and precise results as regards establishing parameters that could provide absence of non-carcinogenic health risks.

Table 1 provides NOAELs for hydrogen sulphide effect on the respiratory system and relevant parameters for health risk assessment calculated on their basis.

The lowest no effect dose (NOAEL) that did not cause any health disorders of the respiratory organs such as basal cell hyperplasia in the nasal cavity mucosa was equal to 0.002 mg/m^3.

We examined research articles that addressed effects produced by hydrogen sulphide on the nervous system and spotted out two key studies, by Morgan with colleagues [19] and Dorman with colleagues [20]. Since both study designs were quite similar, we identified key modifying factors that provided their identical description. Both studies involved animal experiments with sub-chronic exposure to hydrogen sulphide and this made it possible to assuredly identify an interspecific extrapolation factor equal to 10, an intraspecific extrapolation factor equal to 10, and a factor to allow for duration of exposure equal to 3. The total (complex) modifying factor was equal to 300.

Certain differences between two studies were identified in the established points of departure and histopathology. The no effect doses applied in the studies were NOAEL equal to 420 mg/m^3 (Morgan with colleagues) and $NOAEL_{HEC}$ equal to 0.64 mg/m^3 (Dorman with colleagues). A point of departure recalculated as per a dose equivalent for the human body seems more eligible for establishing parameters for health risk assessment.

The experiments by Morgan with colleagues established behavioral and neurological effects such as weaker paw grip, hypotonia, loss of weight, necrosis and / or cavitation and / or bilateral symmetrical cerebral malacia of the parietal cortex, and impaired functioning of somatosensory neurons. All this indicates there are rather severe tissue damages in the nervous system. At the same time, changes established in the experiments by Dorman with colleagues indicated not so severe damages in the nervous system; they were only cell ones since certain signs of damage to olfactory neurons were identified.

Both studies give evidence of adverse effects on the nervous system but at different levels and with different severity of the established disorders. Table 2 provides parameters for health risk assessment under chronic inhalation exposure to hydrogen sulphide as per studies on its adverse effects on the nervous system.

Having analyzed the results of the aforementioned studies, we established the lowest exposure level (0.002 mg/m^3); any exposure to

Table 1

Calculation of parameters for health risk assessment under chronic inhalation exposure to hydrogen sulphide as per results of studies on its adverse effects on the respiratory organs

Parameter / study	Brenneman et al.	CIIT
Test group	Sprague – Dawley rats	$B_6C_3F_1$ mice
Duration of exposure	6 hours a day during 10 weeks	6 hours a day, 5 days a week, during 90 days
Adverse effect	Basal cell hyperplasia in the nasal cavity mucosa	Inflammation of the nasal cavity mucosa
Point of departure (POD)	NOAEL = 10 ppm (14 mg/m^3) $NOAEL_{ADJ}$ = 14 mg/m^3 × 6/24 h × 7/7 days/week = 3,5 mg/m^3 [17] $NOAEL_{HEC}$ = 3.5 mg/m^3 × 0.184 = 0.64 mg/m^3 [18]	NOAEL = 43 mg/m^3
Total (complex) uncertainty factor	MF = 300 10 – intraspecific extrapolation; 10 – interspecific extrapolation; 3 – a factor allowing for duration of exposure	MF = 300 10 – intraspecific extrapolation; 10 – interspecific extrapolation; 3 – a factor allowing for duration of exposure
Calculation	$NOAEL_{HEC}$ / MF = 0.64 mg/m^3 / 300 = 0.002 mg/m^3	NOAEL / MF = 43 mg/m^3 / 300 = 0.143 mg/m^3
Value	0.002 мг/м^3	0.143 mg/m^3

Table 2

Calculation of parameters for health risk assessment under chronic inhalation exposure to hydrogen sulphide as per results of studies on its adverse effects on the nervous organs

Parameter / study	Morgan et al.	Dorman et al.
Test group	Fischer 344 rats	Fischer-344 rats, Sprague – Dawley rats and $B_6C_3F_1$ mice
Duration of exposure	6 hours a day, 5 days a week, 12 weeks	6 hours a day, 5 days a week, not less than 90 days
Adverse effect	Weaker paw grip, hypotonia, slight weight loss, necrosis and / or cavitation and / or bilateral symmetric cerebral malacia of the parietal cortex, functional disorders of somatosensory neurons	Multifocal two-side symmetric loss of olfactory neurons
Point of departure (POD)	NOAEL = 420 mg/m^3	$NOAEL_{HEC}$ = 0.64 mg/m^3
Total (complex) uncertainty factor	MF = 300 10 – intraspecific extrapolation; 10 – interspecific extrapolation; 3 – a factor allowing for duration of exposure	MF = 300 10 – intraspecific extrapolation; 10 – interspecific extrapolation; 3 – a factor allowing for duration of exposure
Calculation	NOAEL / MF = 420 mg/m^3 / 300 = 1.4 mg/m^3	$NOAEL_{HEC}$ / MF = 0.64 mg/m^3 / 300 = 0.002 mg/m^3
Value	1.4 mg/m^3	0.002 mg/m^3

a higher concentration may result in adverse effects on the nervous system and such negative health outcomes as multifocal two-sided symmetric loss of olfactory neurons under chronic inhalation exposure to hydrogen sulphide.

Studies accomplished by Hayden with colleagues [21] and Skrajny with colleagues [22] provide the most convincing description of influence exerted by hydrogen sulphide on occurring development abnormalities. These studies involved animal experiments with sub-chronic inhalation exposure to hydrogen sulphide.

We established that both experiments used LOAELs as their points of departure but the values were different and equaled to 28 mg/m^3 (Hayden et al.) and 110 mg/m^3 (Skrajny et al.).

The studies also reported different adverse effects produced on development by hydrogen sulphide. Thus, Hayden and others detected an authentic subtle increase in time of ear detachment and hair development; Skrajny with colleagues established an authentic elevated level of serotonin and lower level of epinephrine in the cerebellum and frontal cortex on the 14th and 21st day postnatal. All this leads to the nervous system not developing properly in a fetus.

However, the beginning of a pregnancy and the beginning of poisoning is a vital moment in an experiment aiming to examine adverse effects produced on a developing fetus. Poisoning started on the 1st day of a pregnancy in the experiment performed by Hayden with colleagues whereas its start was only on the 5th day of a confirmed pregnancy in the experiment performed by Skrajny and others. This was a key difference in establishing a value of a modifying factor that allowed for duration of an exposure.

Having analyzed these studies, we established LOAELs for effects produced by hydrogen sulphide on occurring development abnormalities and parameters for health risk assessment calculated on their basis (Table 3).

The analysis revealed that LOAEL equal to 0.07 mg/m^3 was the lowest safe dose and any dose higher than it would induce development abnormalities (an increase in time of ear detachment and slower hair development in animals).

Table 3

Calculation of parameters for health risk assessment under chronic inhalation exposure to hydrogen sulphide as per results of studies on its adverse effects on a developing body (development abnormalities)

Parameter / study	Hayden et al.	Skrajny et al.
Adverse effect	An authentic slight increase in time of ear detachment and hair development	An authentic increase in a level of serotonin and a decrease in a level of norepinephrine in the cerebellum and frontal cortex on the 14th and 21st day postnatal
Point of departure (POD)	LOAEL = 28 mg/m^3	LOAEL = 110 mg/m^3
Total (complex) uncertainty factor	MF = 400 10 – intraspecific extrapolation; 10 – interspecific extrapolation; 4 – a factor allowing for duration of exposure	MF = 700 10 – intraspecific extrapolation; 10 – interspecific extrapolation; 7 – a factor allowing for duration of exposure
Calculation	LOAEL / MF = 28 mg/m^3 / 400 = 0.07 mg/m^3	LOAEL / MF = 110 mg/m^3 / 700 = 0.15 mg/m^3
Value	0.07 mg/m^3	0.15 mg/m^3

Therefore, our analysis of all the aforementioned studies allowed us to establish relevant parameters for assessing non-carcinogenic health risks under chronic inhalation exposure to hydrogen sulphide. The lowest identified dose, which is equal to 0.002 mg/m^3, is taken as the reference concentration. Any dose higher than this concentration can create risks for not only the respiratory organs (such as basal cell hypoplasia in the nasal cavity mucosa) but also for the nervous system with multifocal two-sided symmetrical loss of olfactory neurons. This fact not only confirms the existing RfC for hydrogen sulphide but also indicates that a list of critical organs and systems susceptible to the chemical should be expanded. The research data indicate that the airways and the nervous system are the most susceptible (critical) organs / systems under exposure to hydrogen sulphide.

We also established an additional RfC for hydrogen sulphide (0.07 mg/m^3). Any dose higher than it can produce adverse effects on a developing body and this should be considered when conducting health risk assessment under combined exposure to chemicals that can influence development in concentrations being higher than the basic reference one.

We comparatively assessed non-carcinogenic health risks associated with chronic inhalation exposure to hydrogen sulphide (0.028 mg/m^3) and several other chemicals (nitrogen dioxide, 0.03 mg/m^3; chloromethane, 0.03 mg/m^3; methanol, 3 mg/m^3) that produce adverse effects on critical organs and systems similar to those susceptible to hydrogen sulphide. The assessment revealed certain differences in a risk level (HI) under combined exposure (Table 4).

We established additional unacceptable non-carcinogenic risks for the nervous system (*HI* = 14.33) and intrauterine development (*HI* = 1.15) under the same levels of exposure to chemicals when the developed system of quantitative indicators was applied to test it. These testing results indicate that the suggested system of quantitative indicators makes it possible to obtain more comprehensive and accurate results of health risk assessment under combined exposure to chemical pollutants including elevated levels of exposure (higher than reference ones).

Conclusion. A suggested system of quantitative indicators for risk assessment includes a reference concentration and additional indicators under elevated levels of exposure. It is advisable to use it when assessing health risks associated with multicomponent chemical pollution in ambient air under chronic inhalation exposure.

Table 4

Comparative analysis of the results obtained by health risk assessment under combined chronic inhalation exposure to chemicals in accordance with the parameters stipulated in the Guide R 2.1.10.1920-04[3] and the developed system of quantitative indicators

Critical organ / system	In accordance with the parameters in R 2.1.10.1920-04			In accordance with the developed system of quantitative indicators		
	Chemical	HQ	HI	Chemical	HQ	HI
Respiratory organs	Nitrogen dioxide	0.75	14.75	Nitrogen dioxide	0.75	14.75
	Hydrogen sulphide	14.00		Hydrogen sulphide	14.00	
Nervous system	Chloromethane	0.33	–	Hydrogen sulphide	14.00	14.33
				Chloromethane	0.33	
Development	Methanol	0.75	–	Hydrogen sulphide	0.40	1.15
				Methanol	0.75	

To substantiate its use, this study describes the system of quantitative indicators for assessing health risks associated with chronic inhalation exposure to hydrogen sulphide. It includes the reference concentration, which is equal to the valid one and is 0.002 mg/m^3. Following the study, the list of critical organs and systems was enhanced for this reference concentration since any exposure to hydrogen sulphide in a dose higher than this level can produce adverse effects both on the respiratory organs and nervous system.

The additional reference concentration for risk assessment under elevated exposure to hydrogen sulphide was substantiated as equal 0.07 mg/m^3 with its critical effects produced on intrauterine development (development abnormalities).

The substantiated system of quantitative indicators enhances and specifies parameters for health risk assessment. This makes it possible to perform more adequate assessment of health risks under combined exposure to chemicals in ambient air.

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Research article

ON NEW METHODS FOR MEASURING AND IDENTIFYING DUST MICROPARTICLES IN AMBIENT AIR

A.N. Kokoulin[1], I.V. May[2], S.Yu. Zagorodnov[2], A.A. Yuzhakov[1]

[1]Perm National Research Polytechnic University, 29 Komsomolskii Ave., Perm, 614990, Russian Federation
[2] Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 6140045, Russian Federation

Established health hazards posed by dust microparticles require automated and mobile devices for their assessment. Such devices should provide an opportunity to analyze component and disperse structures of the solid component in ambient air pollution operatively and in real time. In future, they will replace labor-consuming sampling and separate identification of fraction structure and chemical composition of dusts.

The aim of this study was to develop and test new methodical, procedural and instrumental approaches to monitoring of solid particles in ambient air. We suggest a hardware and software complex that implements a two-stage scheme for identifying solid particles sampled in ambient air according to the from-coarse-to-fine principle. The first stage involves identifying the total concentration of solid particles by laser diffraction. Microphotographs are taken with iMicro Q2 mini portable microscope with magnification x800. The microscope lens is connected to a camera, which is linked to nVidia Jetson Nano micro PC. The micro PC classifies particles, identifies their contours by using a neural network and deals with image segmentation. The second stage relies on using computer vision that makes it possible to automate routine recognition of particle images created by the microscope in order to calculate levels of different substances in a sample. All the data are analyzed by the second neural network that performs preset calculations in accordance with mathematical logic (model). The network is trained using a library that contains attributed microphotographs of dusts with different qualitative and disperse structures.

The algorithm has been tested with some promising results. Identified disperse structures and chemical composition of dusts turn out to be quite similar to those identified by conventional approaches and measurement methods. The method has been shown to offer wide opportunities to identify dust composition and structure, to create dust pollution profiles, and to estimate a contribution made by a specific source to overall pollution.

The study results ensure more correct and precise health risk assessment under exposure to dusts in ambient air.

Keywords: *dust pollution, concentration of solid particles, dust fraction structure and chemical composition, ambient air, image recognition, computer vision.*

Airborne dusts are a risk factor of additional population mortality and incidence and therefore it is important to perform systemic monitoring of their levels in ambient air. Harmful effects of dusts have been proven by multiple Russian and foreign studies; in particular, there is evidence that the smallest $PM_{2.5}$ are able to penetrate through the blood-air barrier and get into the circulatory system [1]. The available estimates indicate that if PM_{10} levels grow by 10 μg/m^3, daily all-cause mortality also increases by 0.2–0.6 %. Under chronic exposure to $PM_{2.5}$, each rise in $PM_{2.5}$ levels by 10 μg/m^3 makes long-term risks of cardiopulmonary mortality grow by 6–13 % [2–4]. B.A. Revich (2018) focused on ambient

Andrey N. Kokoulin – Candidate of Technical Sciences, Associate Professor, Department of Automation and Telemechanics (e-mail: a.n.kokoulin@at.pstu.ru; tel.: +7 (342) 239-18-16; ORCID: https://orcid.org/0000-0002-1095-4508).

Irina V. May – Doctor of Biological Sciences, Professor, Deputy Director responsible for research work (e-mail: may@fcrisk.ru; tel.: +7 (342) 237-25-47; ORCID: https://orcid.org/0000-0003-0976-7016).

Sergey Yu. Zagorodnov – Senior Researcher (e-mail: zagorodnov@fcrisk.ru; tel.: +7 (342) 237-18-04; ORCID: https://orcid.org/0000-0002-6357-1949).

Alexander A. Yuzhakov – Doctor of Technical Sciences, Professor, Head of the Department of Automation and Telemechanics (e-mail: uz@at.pstu.ru; tel.: +7 (342) 239-18-16).

air pollution with fine particles in his study and showed that additional mortality in 219 cities in Russia equaled 67.9 thousand cases per year under exposure to PM_{10} and 88.2 thousand cases per year under exposure to $PM_{2.5}$ [5].

Accumulative data indicate that exposure to particulate matter $PM_{2.5}$ as a major air pollution source is toxic for skin and affects it considerably. These particles are especially harmful for the epidermis structure and functioning [6, 7].

Peters, Choi, and Mihye describe negative effects of $PM_{2.5}$ on cognitive functions and a risk of early-onset dementia [8–10]. Even relatively low $PM_{2.5}$ levels can be a significant harmful environmental factor able to influence how the brain structure develops in childhood [11]. The negative role of dust particles in spread of communicable diseases, COVID-19 in particular, has also been proven [12, 13]. All this indicates that it is extremely vital to monitor levels of fine-dispersed dusts and to manage ambient air pollution in cities in Russia. This can be done, among other things, by setting standards for emissions of economic entities.

Thus, according to Rosgidromet[1], daily measurements of PM_{10} levels in such cities as Moscow, Sochi, Krasnoyarsk, Irkutsk, Angarsk, Gusinoozersk, Nakhodka, Ulan-Ude, Chita, and others indicate that the existing hygienic standards are violated practically everywhere. Average annual concentrations of PM_{10} equaled 1.6 average annual MPC in Gusinoosersk; 1.3 average annual MPC in Selenginsk; 1.1 average annual MPC in Chita and Shelekhov. High levels of pollution with PM_{10} were established in Baikalsk in 2021 where they reached 23.3 average annual MPC. $PM_{2.5}$ are measured in seven cities (11 posts). Average annual concentrations reach 1.8 average annual MPC (Ulan-Ude). The maximum daily concentration was identified in Selenginsk where it reached 8.2 average daily MPC. $PM_{2.5}$ levels higher than permissible daily ones were detected in all the cities where this pollutant was measured except from Angarsk. Data collected by Rosgidromet state monitoring system are confirmed by data collected at social-hygienic monitoring posts and regional monitoring posts.

However, management of ambient air quality as regards pollution with fine-dispersed dust fractions is not always effective. This is mostly due to the fact that industrial enterprises very rarely declare fine-dispersed dusts in the structure of emitted gas and dust mixtures. At present, fine-dispersed dusts are covered by only three out of 118 procedures recommended to be used by economic entities when they make inventories of their emission sources[2]. Guided by the Ministry documents, economic entities do not disclose the disperse structure of the solid component in their emissions. As a result, hazardous dust fractions ‘drop out’ from ecological standardization. It is impossible to identify sources that create excessive $PM_{2.5}$ and PM_{10} levels in ambient air and this pollution remains beyond the state regulation.

Absence of emission management is apparent according to the following situation: economic entities themselves declare reductions in emissions of solid components. In 2010, approximately 2.4 million tons of dusts were emitted in the country; the figure went down to 1.6 million tons in 2010 (by more than 30 %). Still, average annual PM concentrations measured at monitoring posts changed only slightly over the same period and equaled to 116 $\mu g/m^3$ in 2010 and 109 $\mu g/m^3$ in 2020 (only a 6 % reduction).

Another difficulty in managing dust emissions is that dust is a commonly spread pollutant. Consequently, it is very difficult to identify a contribution made by a specific eco-

[1] Sostoyanie zagryazneniya atmosfery v gorodakh na territorii Rossii za 2021 g.: ezhegodnik [The levels of ambient air pollution in cities in Russia over 2021: annual data collection]. Saint Petersburg, 2022 (in Russian).

[2] Perechen' metodik rascheta vybrosov vrednykh (zagryaznyayushchikh) veshchestv v atmosfernyi vozdukh statsionarnymi istochnikami [The list of procedures for calculating emissions of harmful chemicals (pollutants) into ambient air from stationary sources]. *GARANT: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/402674938/ (January 15, 2023) (in Russian).

nomic entity and a level of dust pollution created by it. At the same time, Rospotrebnadzor experts face the challenge to identify both a source and pollution created by it both during control and surveillance activities involving laboratory tests of ambient air and when analyzing social-hygienic monitoring data. Mandatory identification of sources that emit fine-dispersed dusts is fixed as a task to be solved by the WHO document [14].

Fine-dispersed particles are measured in ambient air by using several procedures: gravimetry[3], laser diffraction[4], nephelometric analysis and some others. Dust analyzers of DustTrak type (models 8530, 8533) are widely used in measuring; they are based on laser nephelometry. Some procedures involve using ATMAC devices that rely on piezoelectric measurement of a piezoelement frequency when aerosol particles are being deposited on its surface.

At the same time, it is still a vital task to perform simultaneous and interrelated measurements of fraction and chemical structure of dusts.

In this study, our aim was to develop and test new methodical, procedural and instrumental approaches to monitoring of solid particles, fine-dispersed included, within monitoring of ambient air quality.

Materials and methods. We have developed a two-stage scheme aimed at making monitoring of dust more effective. The scheme makes it possible to recognize airborne solid particles as per the coarse-to-fine principle [15, 16].

The first stage in the scheme involves rapid identification of the total PM levels using the SDS011 sensor, which employs laser diffraction to estimate PM_{10} and PM_{25} concentrations [17]. The best location for the second stage when more precise measurements are performed can be selected by identifying peak concentrations with this sensor.

The second stage in the scheme relies on using ‘computer vision’ that automates routine operations of object image recognition for calculating a percentage of a specific chemical in a sample. Airflow is sucked into the device by the sensor ventilator and dust particles are deposited partially on the slide while moving along the channel. Images of deposited particles made by the microscope contain all the relevant features of an analyzed scene in its numeric form. These data are analyzed by an imitation of a biological neural network performing a preset collection of calculations according to mathematical logic (model) [18, 19]. Microphotographs are taken with iMicro Q2 mini portable microscope with magnification x800. The microscope lens is connected to a camera, which is linked to nVidia Jetson Nano micro PC. The micro PC classifies analyzed particles and identifies their contours by using a neural network, that is, deals with image segmentation. As a result, a mass of each particle in a frame is calculated and relevant ROI (regions of interests) are cut from the initial frame. They contain images of all the identified particles that have also been classified as per a chemical they represent.

Monitoring data can be analyzed by using both cloud technologies applied, among other things, for the Internet of Things (IoT) and decentralized computations performed, for example, as per the EDGE-devices principle [18, 19].

Cloud technologies are able to provide centralized solutions to issues of segmentation and classification due to using centralized data processing by powerful servers available at a cloud platform. The servers process data flows generated by various devices with artificial intelligence methods and visualize them; the procedure requires a reliable channel for data transfer.

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[3] CSN EN 12341. Ambient air – Standard gravimetric measurement method for the determination of the PM_{10} or $PM_{2.5}$ mass concentration of suspended particulate matter.

[4] MUK 4.1.3242-14. Izmerenie massovoi kontsentratsii melkodispersnykh chastits PM_{10} i $PM_{2.5}$ v atmosfernom vozdukhe s ispol'zovaniem metoda lazernoi difraktsii [Methodical guidelines MUK 4.1.3242-14. Measurement of mass concentrations of fine-dispersed particles PM_{10} and $PM_{2.5}$ in ambient air by suing laser diffraction]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200132738 (January 15, 2023) (in Russian).

On the other hand, a portable EDGE-device can implement multi-stage data processing within the coarse-to-fine paradigm in itself to solve monitoring tasks:

– continuous measurements of airborne $PM_{2.5}/PM_{10}$ levels (without morphology estimates and recognition of dust particles);

– morphological and component analysis of particles using optical object recognition and comparison of recognized particles to dust profiles of enterprises.

Use of this scheme ensures that resource-consuming operations connected with optical image processing (Computer Vision) are accomplished only at those points where it is necessary and the results are extrapolated onto neighboring points. As a result, pollution maps are compared operatively in stream processing.

Particles are classified and their sized are identified using a neural network model trained on hundreds of examples attributed by experts. The network structure is one-directional (without feedbacks) and multi-layered. The network is trained by conventional backpropagation.

In this study, the neural network was trained using libraries with microphotos of dusts emitted at various productions and from various technological devices. Images on each microphoto were described with chemical, fraction and morphological structure of dusts. They were labeled with locations and contours of objects the neural network had to recognize.

Labels were made with Coco-annotator, web-based image annotation tool designed for versatility and to efficiently label images to create training data for image localization. The tool makes it possible to save the labeling results in CoCo format.

It is rather difficult to use microphotos in a portable device due to the necessity to focus a camera and to process several photos to get a qualitative image of one scene. In this study, we resorted to focus stacking to achieve greater depth of image sharpness by combining several images made from different focus lengths into one image with greater sharpness and definition of both front and background objects. The method uses several photos of the same scene obtained with different focus lengths and combines them into one definite image [18].

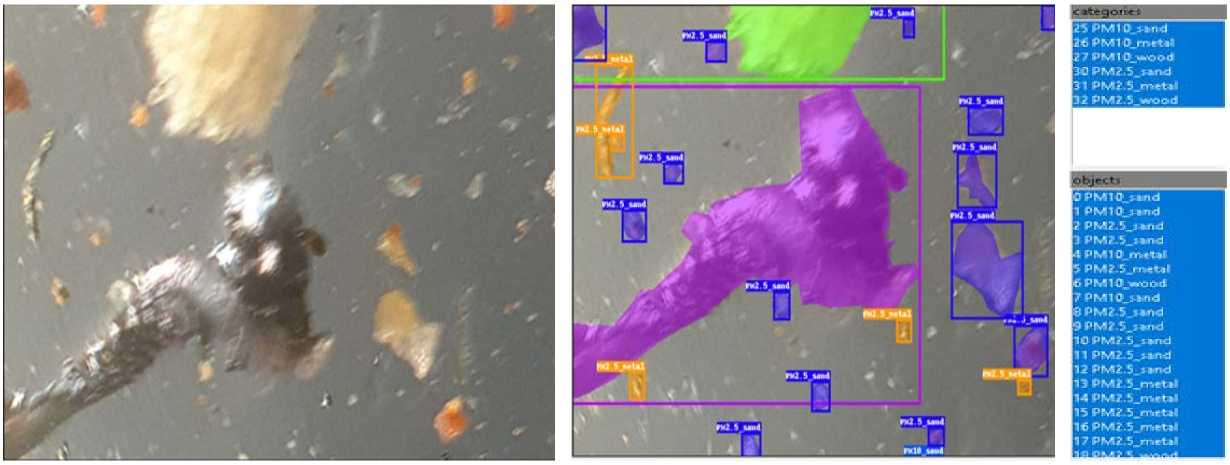
Hardware and software was tested by analyzing actual ambient air pollution in a zone influenced by a mining facility. The database with microphotos that was employed to train the neural network was created in 2020–2021.

Dust pollutions were simultaneously analyzed with conventional techniques in order to verify the results yielded by the new approaches. The disperse structure of analyzed dusts was identified with the Microtrac S3500 6 laser particle size analyzer. The component (chemical) structure of dust emissions was identified using a high-resolution scanning electronic microscope with thermal emission and S-3400N X-ray fluorescence device (HITACHI, Japan); air samples were also analyzed with X-ray diffraction (XRD) analysis using XRD-700 diffractometer (Shimadzu, Japan). X-ray photos were processed with XRD 6000/7000 Ver. 5.21 software. The phase structure of the analyzed samples was identified using ICDD PDF-4+ 2012 database at the Center for Collective Use of the Perm National Research Polytechnic University.

Basic results. The study established that dust pollution near an industrial facility with intensive emissions of solid particles into ambient air had a complex component and dispersed structure. Dusts, which were identified as 'particulate matter' at Rosgidromet monitoring posts, contained salts and oxides of iron, silicon, magnesium, manganese, aluminum, and some other metals. Similar results were obtained by using the new suggested approaches.

The employed neural network brought some good results concerning image recognitions. The method made it possible to identify contours of separate particles and each particle was identified and classified. As a result, a table with data on identified chemicals was created for each sample; percentage of each chemical was calculated as per the number of its particles; statistical characteristics were also obtained (Figure 1).

Table 1 provides both data obtained by conventional procedures and the new suggested method.



a *b* *c*

Figure 1. Initial image of dust particles (*a*) and results of particle recognition (*b*, *c*)

Table 1

Identified quantitative and qualitative indicators of the dust component

Chemical	Level in the analyzed sample, mg/m^3		Validity of differences	
	Conventional procedures	Image recognition	t-test	P (the level of significance)
Fe_2O_3	19.49 ± 0.2	17.25 ± 0.29	6.36	0.00
SiO_2	31.04 ± 12.98	27.16 ± 13.68	0.21	0.84
Al_2O_3	22.22 ± 2.75	21.03 ± 4.58	0.22	0.82
NaCl	1.3 ± 3.85	1.9 ± 1.53	0.14	0.88
CaO	20.12 ± 2.67	18.86 ± 3.74	0.27	0.78
MgO	4.21 ± 0.75	4.01 ± 1.26	0.14	0.89
KCl	1.62 ± 0.19	0.85 ± 0.97	0.78	0.44
$MnSO_4$	0.05 ± 3.62	0.00 ± 0.00	0.01	0.99
$AlCl_3$	0.85 ± 1.58	0.1 ± 0.04	0.47	0.64
Others	2.38 ± 0.63	4.89 ± 6.68	0.37	0.71
PM_{10}	74.28 ± 15.3	72.75 ± 12.92	0.08	0.94
$PM_{2.5}$	21.3 ± 1.16	19.49 ± 4.54	0.39	0.70

Obviously, similarity between the measurements can be considered quite satisfactory. The qualitative database with microphotos of dust samples created at previous stages in this research has made a substantial contribution to the quality of the results [20–22].

The suggested approach to measuring dusts within social-hygienic monitoring has an obvious advantage. It is a possibility to identify dispersed and component structure of dusts quite operatively as opposed to conventional procedures when it takes several days or even several weeks to identify and quantify chemical structure of a dust component in ambient air pollution.

The obtained data can give grounds for identifying a contribution made by a specific economic entity to ambient air pollution and, which is no less significant, to public health risks. It is vital to identify such contributions correctly, both for an economic entity itself (an emission source) and a surveillance authority. The approach was tested when assessing a sanitary-hygienic situation near an industrial site of a large facility dealing with potassium salts mining. Inventory of emission sources located at the facility involved component analysis of solid particles in dusts, creation of a ‘dust emission profile’ and taking microphotos of particles.

We analyzed the chemical structure of emissions and dust components in them at the boundary of the sanitary protection zone (SPZ) (1 km away from the boundary of the industrial site) and established significant differences between taken air samples (Table 2). Marker chemicals typical for the facility occurred in dusts sampled at the boundary of the sanitary protection zone and described emission sources. However, contributions of these chemicals into the total emission mass were approximately 5.9 % (4.6–7.2 %) in the analyzed samples. Even though some chemicals occurred in levels reaching 4.2 single maximum MPC at the boundary, which meant the hygienic standards were violated, the economic entity was able to prove that it did not create excessive levels of pollution and did not have those chemicals in emissions, which accounted for the bulk of dusts identified at the boundary of the sanitary protection zone.

The obtained results were also confirmed by comparing microphotos of particles taken at the emission sources of the facility and at the boundary of the sanitary protection zone (Figure 2). Most particles emitted by the facility had a similar crystal-like shape typical for salts. Solid particles identified at the boundary of the sanitary protection zone had fundamentally different shapes.

On the one hand, these results ensure no incorrect administrative measures will be taken as regards the economic entity; on the other hand, they pose a new challenge, which is to identify actual sources of ambient air pollution.

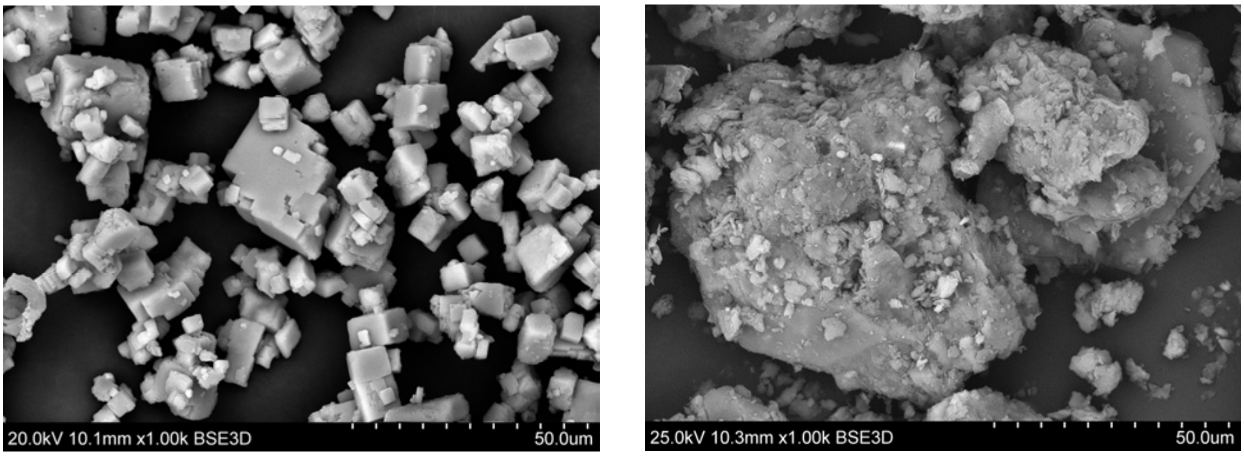
Another positive aspect of creating dust profiles is a possibility to correctly assess public health risks. Dusts that are declared by economic entities in their emission inventories as 'particulate matter' or, for example, as a 'dust containing less than 20 % of SiO_2' often contain salts and/or oxides of heavy metals. Such chemicals cause much greater health hazards than simple particulate matter. Some examples are provided in Table 3. Thus, emissions of processing machinery are declared by an economic entity as 'particulate matter' (*RfC*[5] = 0.075 mg/m^3); in reality, they are a complex mixture of iron, magnesium, and aluminum salts and oxides. It is noteworthy that reference levels of each component are lower than those of unidentified particulate matter. In addition, fine-dispersed PM_{10} and $PM_{2.5}$ are identified in emissions and they are much more harmful for health than the total solid particles.

Table 2

Contributions made by emission sources at the mining facility to dust pollution in ambient air at the boundary of the sanitary protection zone

Chemical	Dust profile, mg/m^3		A contribution made by the facility at the SPZ boundary, %		Background pollution	
	Emissions from the facility	At the measuring point	To a chemical concentration, %	To total dust pollution	mg/m^3	% of the total
KCl	0.0383	0.0383	100.0	1.47	0	0
NaCl	0.0296	0.0296	100.0	1.13	0	0
$AlCl_3$	0.002	0.006	33.3	0.08	0.0040	0.15
$MgSO_4$	0.0029	0.0118	24.6	0.11	0.0089	0.34
SiO_2	0.0284	0.7762	3.66	1.09	0.7478	28.64
MgO	0.0015	0.0535	2.80	0.06	0.0520	1.99
Al_2O_3	0.009	0.4133	2.18	0.34	0.4043	15.48
CaO	0.0014	0.1989	0.70	0.05	0.1975	7.56
Fe_2O_3	0.0033	0.9589	0.34	0.13	0.9556	36.60
$MnSO_4$	0.000	0.0135	0.00	0.00	0.0135	0.52
Others	0.0377	0.111	33.96	1.44	0.0733	2.81
Total:	**0.1541**	**2.6110**		**5.90**	**2.4569**	**94.10**

[5] *RfC*, reference concentration is an average daily concentration of a chemical; it is established considering all the available research data and, probably, does not create unacceptable health risks for sensitive population groups (the Guide R 2.1.10.1920-04).



a *b*

Figure 2. Dust particle shapes: *a*) in emissions from the facility (magnification x1000); *b*) in ambient air at the SPZ boundary (magnification x1000)

Table 3

Example coding and an inventory list of chemicals as per data of calculations and research results

Emission source	Coded by a facility		Dust components identified by research		
	Chemical	*RfC*, mg/m^3	Identified structure	*RfC*, mg/m^3	% in dusts
Mechanical processing of metals (cutting)	Particulate matter	0.075	Iron sulfate	0.007–	30.58
			Silicon dioxide	0.05	20.07
			Iron oxide	0.04–	19.67
			Magnesium oxide	0.05–	16.81
			Aluminum oxide	0.005	3.23
			Others (not identified)	0.075	9.43
			PM_{10}	0.05	33.0
			$PM_{2.5}$	0.015	7.1
Mechanical processing of metals (drilling)	Particulate matter	0.075	Iron oxide	0.04–	97.35
			Mn and its compounds	0.00005	1.15
			Chromium (recalculated as per chromium (VI) oxide)	0.000008	0.99
			Other chemicals	0.15/0.075	0.40
			Aluminum oxide	0.005	0.11
			PM_{10}	0.04	13.00
			$PM_{2.5}$	0.015	7.00
Turnery	Particulate matter	0.075	Iron oxide	0.04	98.19
			Mn and its compounds	0.00005	1.07
			Others (not identified)	0.075	0.41
			Chromium	0.000008	0.33
			PM_{10}	0.05	12.00
			$PM_{2.5}$	0.015	0.40

Both toxic elements in the dust structure and a considerable share of fine-dispersed particles allow assuming that potential health risks in a zone influenced by the analyzed facility might be substantially underestimated with the existing system of chemical identification.

The last assertion was also confirmed by analyzing a sanitary-hygienic situation in a zone influenced by emissions from a primary aluminum production at a production facility in Krasnoyarsk. Emissions were coded as inorganic dusts (the reference concentration taken as per TSP is 0.075 mg/m^3) whereas aluminum oxide (Al_2O_3) was identified as a primary chemical in the dust fraction accounting for 84–87 % (the safe reference concentration equals 0.005 mg/m^3).

A ground-level dust concentration measured at the boundary of the sanitary protection zone around the analyzed facility equaled 0.035 mg/m^3. The data provided by the facility indicate that health risks are acceptable for people in the closest residential area since the hazard quotient (*HQ*[6]) is below 0.47, its permissible levels being 1.0. The facility is not given any tasks to reduce emissions or take any other actions aimed at minimizing public health risks.

Considering that 85 % of dust particles are aluminum oxide with its ground-level concentration being approximately 0.030 mg/m^3, health risks reach 7.0 HQ and this rate is 'high'[7]. A zone under harmful influence of the facility grows substantially in such a situation. Harmful exposure ($HQ > 1.0$) affects people living in 10 residential buildings, children attending pre-school children facilities and owners of more than 60 land spots with country houses. The situation is considered unfavorable and requires immediate measures to be taken to reduce aluminum oxide emissions.

Discussion. Dust pollution poses serious threats and health hazards. Managerial decisions concerning its level are usually made based on hygienic assessments including health risk assessment and characteristics. Given that, it is especially vital to correctly estimate dispersed and component structures of airborne dusts.

The major goal of social and hygienic monitoring is to establish reasons for violations of environmental safety and cause-effect relations within the 'environment – health' system. This activity requires advanced techniques for dust quantification and differentiation.

The suggested approaches based on such advanced techniques can be useful for continuous observations performed by Rospotrebnadzor experts. It is important that the method and its hardware support make it possible to identify and establish quantitative characteristics of both component and dispersed structure of dusts. This provides a solution to a whole set of tasks that may arise within monitoring activities or control of ambient air quality. Thus, actual levels of hazardous chemicals can be identified in ambient air (including heavy metal compounds etc.); contributions made by specific sources to pollution can be provided with solid evidence; population exposures can be estimated more precisely and adequately etc.

Undoubtedly, implementation of the suggested approaches largely depends on availability of libraries that contain attributed (annotated) microphotos. Such libraries (databases) ensure proper training of a neural network and subsequent recognition of sampled dust particles. At the same time, dust emissions are being examined widely enough at the moment [23–25]. This makes it possible to constantly accumulate and expand available data. In addition, many parties seem to have considerable interest in creation of such libraries. Surveillance authorities can rely on operative identification of marker chemicals to prove objectivity of administrative measures taken in a situation when some violations have been detected. Economic entities can both perform self-assessment within production control and defend their interests in difficult situations in case violations of hygienic standards have been detected during control and surveillance activities.

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[6] *HQ*, hazard quotient is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected (reference concentration) (the Guide R.2.1.10.1920-04).

[7] MR 2.1.10.0156-19. 2.1.10. Gigiena. Kommunal'naya gigiena. Sostoyanie zdorov'ya naseleniya v svyazi s sostoyaniem okruzhayushchei sredy i usloviyami prozhivaniya naseleniya. 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 [The Methodical guidelines 2.1.10.0156-19. 2.1.10. Hygiene. Communal hygiene. Public health associated with quality of the environment and living conditions. Assessment of ambient air quality and public health risk analysis in order to make well-grounded managerial decisions concerning provision of ambient air quality and sanitary-epidemiological wellbeing of the population]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_415503/ (January 15, 2023) (in Russian).

Conclusions. In this study, we have described a new method to identify and quantify chemical and fraction structures of airborne dusts. The method is based on sampling and taking microphotos of dusts and subsequent analysis of the solid component in them using a neural network that has been trained relying on previously collected data about dust structure and their dispersed composition. Several technical decisions are suggested for image processing in order to raise an image quality and achieve more adequate results.

The suggested approaches can be useful for continuous observations performed by Rospotrebnadzor experts.

The method requires further adjusting and testing on a wider range of various types and levels of dust pollution. At the same time, the study results indicate the method offers wide opportunities that make it possible to:

– rapidly identify component and dispersed structure of dusts in ambient air;

– create a dust pollution profile;

– comparatively analyze and estimate contributions made by different sources to ambient air pollution;

– rely on obtained results to ensure more correct and precise health risk assessment.

Implementation of the suggested approaches largely depends on availability of libraries that contain attributed microphotos of dusts with various qualitative and disperse structure. Creation of such databases (libraries), data renewal and acquisition in them and their use in solving practical tasks can be a promising trend in further development of social and hygienic monitoring.

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Competing interests. The authors declare no competing interests.

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Research article

SUBSTANTIATION OF THE OPTIMUM SCREEN BRIGHTNESS PARAMETERS OF THE INTERACTIVE PANEL TO REDUCE THE RISK OF GENERAL AND VISUAL FATIGUE OF SCHOOLCHILDREN

M.V. Ayzyatova, I.E. Aleksandrova, I.P. Lashneva, A.M. Kurgansky

National Medical Research Center for Children's Health, 2 Lomonosovskii Ave., bldg 1, Moscow, 119991, Russian Federation

The digital transformation of modern education contributes to the active introduction of interactive panels (IP) into the educational process, replacing traditional chalkboards. Minimizing possible risk factors when using an IP also requires considering the visual characteristics of its screen. At present, there are no results of such studies in the scientific literature. The purpose of this work was to substantiate the optimal range of IP screen brightness when it is used in the classroom to prevent general and visual fatigue of schoolchildren. We analyzed research articles describing studies in visual hygiene, lighting engineering, display technologies, etc.

Our study involved measuring brightness and pulsation coefficient of a working IP screen. Ranges of IP screen brightness that could produce harmful effects on children's health have been empirically established. With the help of a specially designed questionnaire, complaints of students attending the 4th grade of secondary schools were studied to identify general and visual fatigue, as well as factors caused by the IP and negatively affecting the respondents' well-being. The relative risk values are calculated, namely a probability that these complaints would occur in schoolchildren, depending on parameters of IP screen brightness. The optimal range of IP screen brightness is justified for a working mode that significantly reduces the probability of students complaining about general and visual fatigue. Monitoring and correction of IP screen brightness mode during classes will reduce the risks of students' health disorders. It is necessary to continue research to substantiate the optimal visual characteristics of the IP screen based on investigating indicators describing the functional state of the child's body.

***Keywords**: prevention, interactive panel, risk factors, screen brightness, fatigue.*

The digital transformation of modern education contributes to the active introduction of electronic devices in the educational process, starting from the elementary school. Traditional chalkboards are being replaced with digital models everywhere. The latest generation of electronic boards is an interactive panel (IP), which is an independent device: a touch display that runs on its own software.

The use of interactive panels in the classroom in the modern school is becoming a routine practice. Given that the panel is an electronic means of collective use (for the whole class team), an increasing number of schoolchildren come across it in the learning process.

The use of IP implies the presence of undeniable advantages in the visualization of information over traditional teaching aids. Along with this, modern scientific research shows the impact resulting from use of electronic technologies on the formation of visual

Marina V. Ayzyatova – Junior Researcher at the Laboratory for Complex Problems of Hygiene of Children and Adolescents (e-mail: 9855123020@mail.ru; tel.: +7 (495) 917-10-60; ORCID: https://orcid.org/0000-0003-0381-3253).

Irina E. Aleksandrova – Doctor of Medical Sciences, Head of the Laboratory for Complex Problems of Hygiene of Children and Adolescents (e-mail: accialex@yandex.ru; tel.: +7 (495) 917-10-60; ORCID: http://orcid.org/0000-0002-8664-1866).

Irina P. Lashneva – Candidate of Medical Sciences, Assistant-Researcher of the Laboratory for Complex Problems of Hygiene of Children and Adolescents (e-mail: iplash@mail.ru; tel.: +7 (495) 917-48-31; ORCID: https://orcid.org/0000-0003-4115-2847).

Alexander M. Kurgansky – Candidate of Medical Sciences, Leading Researcher at the Laboratory for Complex Problems of Hygienic Assessment and Examination (e-mail: kurgansk@yandex.ru; tel.: +7 (495) 917-48-31; ORCID: https://orcid.org/0000-0001-7688-586X).

impairment, the functioning of the nervous, cardiovascular systems, etc. [1–13]. The intensity of the educational process is increasing [14]. Electronic learning aids increase visual concentration, significantly prolonging a period during which the eyes have to work. In several scientific publications concerning ergonomics in connection with electronic devices, attention is mainly focused on the hygiene of visual work [15–18]. Previous studies have reported several complaints about health deterioration; general and visual fatigue of schoolchildren when using interactive panels in classes [19]. In addition, literature presents data indicating negative changes in the microclimate parameters in the dynamics of classes with the use of IP [20], unfavorable dynamics of the mental and visual performance of students during classes with unregulated use of IP [21]. With the accumulation of experience in the use of these electronic means in the educational process, research to study their effect on students' body remains relevant.

The issue of optimal IP screen visual characteristics is among the priority areas of research. The screen brightness and the pulsation of the panel screen brightness are significant parameters. The latter has no less negative impact on students' health than the pulsation of general illumination at a workplace because a student is forced to carefully peer at a panel and read information presented on it. The presence of brightness pulsations, as a rule, leads to rapid fatigue of the eyes and parts of the brain responsible for perception and analysis of visual information[1]. You can significantly reduce the panel display pulsation by increasing the brightness of the screen backlight. In its turn, too high brightness also has an adverse effect on a user's body [22].

Therefore, the use of IP in its optimal brightness mode is a significant component in preventing general and visual fatigue and reducing risks of overwork for schoolchildren. An advantage of the interactive panel is the adjustable screen brightness mode depending on lighting conditions. The IP screen brightness can be adjusted independently during classes, for example, by a teacher.

The timeliness of the study is evidenced by the results of scientific works addressing influence of digital means screens, including their brightness, on users' health, vision, etc. However, these data relate mainly to the monitor screen, i.e. working conditions are significantly different from those when using IP. In addition, all studies were conducted with adult subjects [23–28].

Given the above, it is extremely relevant to investigate optimal visual characteristics of the interactive panel screen when used in a class, especially brightness parameters that determine risks of health complaints.

The purpose of the study is to substantiate the optimal range of IP screen brightness when it is used in the classroom to prevent general and visual fatigue of schoolchildren.

To achieve this goal, the following tasks were solved:

• to substantiate the brightness ranges of the IP screen potentially causing various effects on schoolchildren's health;

• to determine the number of health complaints among students when working with IP in reasonable brightness ranges;

• assess risks of schoolchildren's complaints about general and visual fatigue depending on the different IP screen brightness.

Materials and methods. We measured the pulsation coefficient from the screen and the screen brightness of the operating IP.

The measurements were carried out in 30 school classrooms equipped with Irbis IP in accordance with the requirements of GOST 33393-2015, GOST 24940-2016, GOST 26824-2018. To exclude the influence

[1] Pul'satsii osveshchennosti i yarkosti [Pulsations of illumination and brightness]. *NOChU DPO 'EkoSfera'*. Available at: https://ekosf.ru/stati/pulsacii/ (September 12, 2022) (in Russian).

of natural light on the measurement results, they were carried out in the evening and the windows were equipped with thick shading devices (roller blinds). The measurement control points were located evenly on the entire working surface of the IP. The measurements were carried out simultaneously with two devices: the Combined Device, eLight, version 2, 63221-16 and the Combined Device, type TKA-PKM (09). Measuring range of devices was as follows: illumination (10…200 000) lx; pulsation factor (1…100) %; brightness (10…200000) cd/m^2; instrument error 8 %.

Measurement steps:

- sanitary and hygienic assessment of educational premises (in terms of creating an optimal light regime);
- measurement of artificial illumination levels from the general lighting system, measurement of background lighting from natural sources (windows);
- measurement of brightness and pulsation coefficient from the IP screen.

On each IP, measurements were taken at 13 control points at adjusted brightness values from 25 to 155 cd/m^2 with a step of 5 cd/m^2. In total, 10,350 measurements of brightness from the IP screen and 10,350 measurements of the pulsation coefficient from the IP screen were carried out.

We established relationships between values of the pulsation coefficient and the adjusted IP screen brightness. The brightness ranges of the IP screen are substantiated.

We studied children's complaints to assess adverse impacts exerted on their health by the use of IP with different screen brightness. Three hundred and thirty children attending the 4th grade in two secondary schools with standard educational programs underwent medical examination and participated in a social survey. This age group was selected because interactive panels are already actively used in elementary school where students are the most sensitive to the effects of risk factors. The use of interactive panels in the classroom was carried out as much as possible in accordance with the current sanitary legislation. At the end of use, the IP was turned off or put into 'sleep' mode. The survey was conducted in a face-to-face format. The questionnaire included questions related to a) identifying factors caused by the work of IP that negatively affect respondents' health; b) identifying students' complaints about general and visual fatigue. The criteria for including participants in the study were the following: they attended the 4th grade in secondary school; IPs were used in the educational process; availability of written informed consent to research from parents. Exclusion criteria were severe visual impairment; failure to meet the inclusion criteria.

We assessed the number of schoolchildren's complaints about general and visual fatigue that occurred when the IP was operating in different (previously justified) ranges of screen brightness. Nominal data were described in terms of absolute values, percentages, and boundaries of the 95 % confidence interval (CI) calculated by the Wilson method and corrected for continuity.

According to the principles of evidence-based medicine, relative risk values were established, namely likelihood of schoolchildren having complaints related to general and visual fatigue (outcomes) depending on the brightness parameters of the IP screen (risk factors)[2]. Differences between groups of indicators were assessed by calculating the relative risk using four-field contingency tables. After defining the boundaries of the 95 % confidence interval (not including one), the relative risk values were compared with one: values greater than 1 were chosen, if a factor increased frequency of outcomes. We calculated sensitivity and specificity of

[2] Biblioteka postov MEDSTATISTIC ob analize meditsinskikh dannykh. Slovar' statisticheskikh terminov [Library of MEDSTATISTIC posts about medical data analysis. Dictionary of Statistical Terms]. *Meditsinskaya statistika*. Available at: http://medstatistic.ru/theory/relative_risk.html (September 30, 2022) (in Russian).

the method. Significance of a contribution made by a risk factor to the increase in the frequency of the event was determined by calculating the etiologic fraction (EF) expressed as a percentage. To interpret the magnitude of the relative risk and the etiologic fraction, taking into account the continuous and long-term impact of education on the student's body, we used the 'Assessment of the degree of causal relationship of health disorders with work'[3].

Results and discussion. All classrooms equipped with Irbis IP had similar areas (49.0–52.0 m^2). Ceiling LED lamps were used as a source of general artificial lighting. The sanitary and hygienic assessment of the classrooms revealed the absence of burned-out lamps, the uniformity of the lamp arrangement, the absence of pollution on lighting devices, the uniformity of illumination of the IP working surface, the presence of shading devices on the windows.

The background natural lighting was determined to account for less than 10 % of the total artificial lighting. The pulsation coefficient from the general artificial lighting system in the examined premises equipped with LED lamps was 2.1 ± 0.8 %.

Screen brightness parameters were experimentally divided into three ranges.

The first range was obtained using a series of measurements of the pulsation coefficient from the MT depending on the increase in the brightness of the MT screen: a gradual increase in the brightness level of the panel screen was accompanied by an abrupt change in the pulsation coefficient (Figure).

According to the figure, at a brightness level of 115 cd/m^2, the pulsation coefficient reached its minimum values; it approached 20 %, and then, with increasing brightness, its value practically did not change. In addition, according to the reviews of teachers with long work records who have sufficient experience with IP, at an adjusted brightness of less than 115 cd/m^2, a fuzzy, pale image was observed. This made it possible to label a range of less than 115 cd/m^2 as 'potentially sub-optimal'.

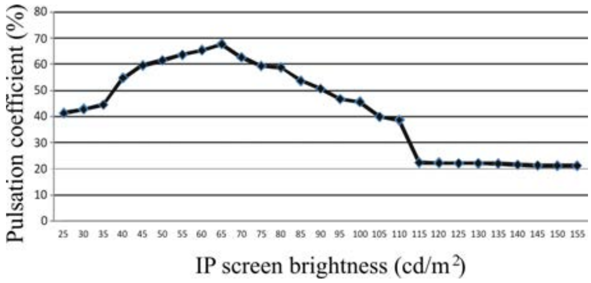

Figure. Change of the pulsation coefficient (%) from the IP screen depending on the adjusted brightness of the IP screen (cd/m^2)

According to the data available in the literature, the range of subjective brightness that the eye can perceive (brightness adaptation – the range of simultaneously distinguishable subjective brightness levels) is about 10–15 cd/m^2 [29, 30], i.e., the range from 115 cd/m^2, extended by the value of brightness adaptation, can hypothetically be considered 'potentially optimal', up to 125–130 cd/m^2. At the same time, according to the reviews of teachers who have significant experience in using IP, the adjusted brightness of its screen over 125 cd/m^2 was already 'uncomfortable' for the eye, so this figure was determined as the upper limit of the optimal brightness level.

Thus, the screen brightness parameters were divided into three ranges: less than 115 cd/m^2; 115–125 cd/m^2 and more than 125 cd/m^2.

To substantiate the optimal level of the IP screen brightness during classes, we studied students' complaints in the process of using it.

The schoolchildren were divided into three groups (90, 100 and 140 schoolchildren); the educational process was carried out for each of them using an IP operating in one (of the above) brightness ranges. The groups were similar in terms of age and sex and learning conditions.

[3] Professional'nyi risk dlya zdorov'ya rabotnikov: rukovodstvo [Occupational health risk for workers: guidelines]. In: N.F. Izmerov, E.I. Denisov eds. Moscow, Trovant Publ., 2003, 448 p.

The plan was to study students' complaints after working with the panel in each brightness mode for one academic week. However, a significant number of complaints was made by children during the operation of the IP in the adjusted brightness modes of less than 115 cd/m^2 and more than 125 cd/m^2 at the very beginning of the study. This led to earlier termination of any operation in these ranges and performing a survey.

Work with IP in the range of 115–125 cd/m^2 continued in the classroom for a school week; after that, students' complaints were also analyzed. The results are presented in Table 1. Analysis of the intensity of schoolchildren's complaints showed that such risk factors as a fuzzy image, in the vast majority of cases, are characteristic of low brightness; bright light from the screen and a rise in air temperature in the class are typical for high brightness. However, these factors, although to a much lesser extent, are also present during the operation of the IP with a screen brightness of 115–125 cd/m^2. This indicates the need to justify the hygienic requirements for the information supplied to the IP screen (contrast, clarity, color, font, etc.); as well as constant monitoring and optimization of the microclimate in classes with IP use taken in dynamics [20].

Complaints about general fatigue and eye fatigue among the respondents using IP in "non-optimal" brightness ranges are distributed approximately equally; headache, pain in the eyes, and lacrimation were significantly more often observed in the children exposed to IP brightness of more than 125 cd/m^2. When the panel was operating in the range of less than 115 cd/m^2, the schoolchildren, respectively, complained significantly more often about the blurring of the image and the feeling of flickering before their eyes.

Table 2 presents the significant relative risks of certain complaints during the operation of the IP in the brightness mode of less than 115 cd/m^2 and more than 125 cd/m^2 compared to the brightness of 115–125 cd/m^2.

Table 1

The number of students' complaints when working with IP in different brightness ranges

Survey Questions	Answer options	IP Screen Brightness Ranges								
		less than 115 cd/m^2			115–125 cd/m^2			over 125 cd/m^2		
		Abs.	%	CI	Abs.	%	CI	Abs.	%	CI
Factors that negatively affect health according to schoolchildren	Bright light from IP	–	–	–	28	28.0	20.14–37.49	138	98.6	93.91–99.69
	Small fuzzy image on the screen	90	100.0	95.1–100.8	17	17.0	10.89–25.55	2	1.4	0.31–6.09
	A temperature rise in the classroom	22	24.4	16.70–34.20	15	15.0	9.31–23.28	80	57.1	47.31–66.36
Students' complaints when working with IP	General fatigue	89	98.9	93.99–99.81	5	5.0	2.15–11.18	134	93.1	86.38–96.63
	Headache	2	2.22	0.60–7.71	2	2.0	0.55–7.0	26	18.6	12.19–27.34
	Pain in the eyes	12	13.3	7.77–21.84	3	3.0	1.03–8.45	71	50.7	41.06–60.29
	Image blur	43	47.8	37.78–58.00	3	3.0	1.03–8.45	2	1.4	0.31–6.09
	Lacrimation	11	12.2	6.95–20.55	3	3.0	1.03–8.45	70	50.0	40.38–59.62
	Feeling of flickering before the eyes	59	65.6	55.33–74.59	4	4.0	1.57–9.84	1	0.7	0.10–4.95
	Eye fatigue	68	75.6	65.80–83.30	5	1.0	0.18–5.45	124	88.6	80.89–93.45
The number of schoolchildren working with IP in each of the ranges, n		90			100			140		

Table 2

Relative risk of schoolchildren's complaints about general and visual fatigue at different screen backlight brightness of the interactive panel

Complaints	IP Screen Brightness	Relative risk	CI*	EF, %	Se	Sp
General fatigue	less than 115 cd/m^2 (compared to brightness 115–125 cd/m^2)	19.8	8.41–46.48	93.9	0.95	0.99
Eye fatigue		15.1	6.38–35.79	70.0	0.93	0.81
Image blur		15.9	5.12–49.56	44.8	0.94	0.67
Feeling of flickering before the eyes		16.4	6.2–43.3	61.6	0.94	0.76
Small fuzzy image		5.8	3.8–9.1	83.0	0.84	0.99
Lacrimation		4.07	1.17–14.14	9.2	0.79	0.55
Pain in the eyes		4.44	1.29–15.95	10.3	0.80	0.55
General fatigue	Over 125 cd/m^2 (compared to brightness 115–125 cd/m^2)	19.1	8.14–45.02	90.7	0.96	0.94
Headache		9.29	2.26–38.23	16.6	0.93	0.46
Eye fatigue		17.7	7.52–41.7	83.6	0.96	0.86
Lacrimation		16.6	5.40–51.43	47.0	0.96	0.58
Pain in the eyes		16.91	5.48–52.14	47.7	0.96	0.58
The bright light from the board interferes		3.52	2.6–4.8	70.6	0.83	0.97
Increasing classroom temperature (getting 'hot')		3.81	2.3–6.2	42.1	0.84	0.59

Note: *CI is a confidence interval ($p < 0.05$); EF, etiological component; Se, sensitivity; Sp, specificity.

The obtained data allow us to substantiate the brightness range of the interactive panel that is optimal for students in the classroom. When the brightness of the IP screen is between 115 and 125 cd/m^2, the risk of complaints about health disorders is detected significantly less frequently.

A decrease in complaints of visual fatigue with adequate adjustment of brightness levels, the use of screens with anti-reflective coating, maintaining an ideal visual distance was also noted in works evaluating the effect of a computer monitor screen in adult users [23–25]. A promising area of research is the assessment of the IP screen brightness at school depending on a viewing distance and field of view, as evidenced by the work carried out on large LED displays [25].

Conclusions. Thus, the use of an interactive panel with optimal visual screen parameters of 115–125 cd/m^2 in classes will reduce the risks of general and visual fatigue and the load on the visual analyzer.

The active development of digital technologies necessitates further research to substantiate the optimal visual characteristics of the screen of new electronic learning tools based on assessing indicators describing the functional state of the child's body.

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Research article

ASSESSMENT OF HEALTH RISKS CAUSED BY OVERWEIGHT IN CHILDREN DEPENDING ON THE FTO GENE RS9939609 POLYMORPHISM

O.G. Bogdanova[1], I.Yu. Tarmaeva[2,3], E.Yu. Sorokina[2], N.V. Efimova[1], I.V. Mylnikova[1]

[1]East-Siberian Institute of Medical and Ecological Research, 3 12a mikroraion, Angarsk, 665826, Russian Federation
[2]Federal Research Centre of Nutrition and Biotechnology, 2/14 Ust'inskii passage, Moscow, 109240, Russian Federation
[3]RUDN University, 9 Miklukho-Maklaya Str., Moscow, 117198, Russian Federation

In this study, we aimed to estimate the association between the rs9939609 FTO (fat mass and obesity associated) polymorphism and a risk of overweight in children living in the Baikal region. We performed a case – control study that included 113 schoolchildren living in industrial centers of the Baikal region (Irkutsk, Angarsk, and Ulan-Ude). Anthropometric parameters were measured and body mass index was calculated with its values being ranked in accordance with the WHO BMI curves depending on a sex and age. Genotyping of the rs9939609 FTO polymorphism was performed by allele-specific amplification with real-time results detection. To assess likelihood of an association between the FTO gene allele and overweight and obesity, relative risk (RR) and 95 % confidence interval (CI) were calculated.

The assessment revealed the A allele of the rs9939609 FTO polymorphism to be by 1.29 times more frequent in the examined children with overweight and obesity (48.44 %) than in the children from the reference group (37.65 %). The FTO rs9939609 polymorphism was authentically associated with likelihood of elevated risks of overweight and obesity in children with the homozygous AA genotype (RR = 2.806, 95 % CI: 1.650–4.772; STD = 0.271). Our study confirms that the rs9939609 polymorphism of the FTO gene is a risk factor of overweight and obesity for children from the Baikal region who have the A allele of the homozygous AA genotype. Prevailing frequency of the TT genotype (29.2 %) as compared with the AA genotype (10.62) is likely due to influence of assimilation processes on urbanized territories in the Baikal region.

***Keywords:** children, FTO gene, rs9939609, polymorphism, risk, overweight, obesity, Baikal region.*

The Roadmap on prevention of non-communicable diseases up to 2025 developed by the WHO includes nine global targets; control of obesity and overweight is one of them [1–3]. Preservation of human potential makes this issue truly vital for healthcare experts, scientists and the society as a whole [4–6]. The WHO Regional Office for Europe reported the results of the studies accomplished within Childhood Obesity Surveillance Initiative (COSI) in 2018–2020. According to it, 29 % of children aged 7–9 years in 33 participating countries had overweight, obesity included [7]. The study that was accomplished in Moscow in 2017–2018 within this Initiative and included 2166 7-year old children established overweight in 27 % of boys and 22 % of girls and obesity in 10 % and 6 % of children accordingly [6].

Genetic background is estimated to account for more than 50 % in common obesity etiology [8]. Child obesity has exogenous rea-

Olga G. Bogdanova – Candidate of Medical Sciences, Senior Researcher at the Laboratory for Environmental and Hygienic Research (e-mail: olga.bogdanova2001@gmail.com; tel.: +7 (3955) 58-69-10; ORCID: https://orcid.org/0000-0002-2358-2280).

Inna Yu. Tarmaeva – Doctor of Medical Sciences, Professor, Academic Secretary; Professor of the Department of Ecology and Food Safety (e-mail: tarmaeva@mail.ru; tel.: +7 (495) 698-53-42; ORCID: https://orcid.org/0000-0001-7791-1222).

Elena Yu. Sorokina – Candidate of Medical Sciences, Leading Researcher at the Laboratory of Demography and Epidemiology of Nutrition (e-mail: sorokina@ion.ru; tel.: +7 (495) 698-53-42; ORCID: https://orcid.org/0000-0002-6530-6233).

Natalia V. Efimova – Doctor of Medical Sciences, Professor, Leading Researcher at the Laboratory for Environmental and Hygienic Research (e-mail: medecolab@inbox.ru; tel.: +7 (3955) 58-69-10; ORCID: https://orcid.org/0000-0001-7218-2147).

Inna V. Myl'nikova – Doctor of Medical Sciences, Associate Professor, Senior Researcher at the Laboratory for Environmental and Hygienic Research (e-mail: inna.mylnikova.phd.ms@gmail.com; tel.: +7 (3955) 58-69-10; ORCID: http://orcid.org/0000-0001-7685-9004).

sons in vast majority of cases and primary factors causing it include absence of physical activity or its low levels, quantities of consumed foods and energy [9–11], and provision of vitamins [12]. A small share of obesity cases can occur due to endogenous reasons where common risk factors include sex, age, ethnic group, and genetic polymorphisms [2, 11]. The fat mass and obesity associated FTO gene encodes an alpha-ketoglutarate-dependent dioxygenase that regulates transcription and translation through the methylation of DNA/RNA [8]. Although the molecular mechanism by which the gene participates in obesity still remains unclear, its polymorphisms are closely connected with a risk of overweight and obesity [8, 13]. The most unambiguous association with a risk of obesity has been identified for rs9939609 polymorphism located in 16q12.2. According to A.K. Baturin with colleagues (2019), the essence and intensity of the association between FTO gene polymorphisms as well as the A allele frequency are largely determined by respondents' race and ethnic group [14]. The aforementioned association between the rs9939609 FTO polymorphism and a risk of obesity has been evidenced by data obtained on various populations including the West [15] and East Asia [11, 13], South America [3, 16], as well as in different regions in Russia [5, 10, 17–19]. Still, S. Doaei with colleagues (2019) [20] combined adjusted odds ratios (*OR*) from 8 eligible case-control studies in their review and established the association between rs9939506 and obesity to be significant in the European subgroup (OR=1.68 [1.2–2.36]), but not in the Asian subgroup (*OR* = 0.94 [0.81–1.10]; *OR* = 0.95 [0.80–1.14]; *OR* = 2.31 [0.96–5.58]).

Since data on genetic factors and their associations with obesity in children and adolescents are still rather controversial [21, 22], it seems interesting to analyze associations between the rs9939506 polymorphism (the FTO gene) in children living in the Baikal region. It is located in two geographical areas, Central Asia and Eastern Siberia and therefore has many ethnical groups such as Slavic, Mongolian, Tungus and Turkic ones with certain assimilation between them.

In this study, our aim was to estimate the association between the rs9939609 FTO polymorphism and a risk of overweight in children living in the Baikal region.

Materials and methods. The study was a case-control one, cross-sectional, observational. Genetic testing was accomplished on 113 schoolchildren aged 7–17 years, 60 boys and 53 girls. They lived in industrial centers in the Baikal region (Angarsk, Irkutsk, and Ulan-Ude). Investigation and sampling of biological material (buccal epithelium) was accomplished only after a parent or a legal representative of each participating child gave their informed voluntary consent to it and after the approval by the Local Committee on Ethics of the East-Siberian Institute of Medical and Ecological Research (the meeting report No. 32 issued on September 10, 2019). The study was accomplished in conformity with the Declaration of Helsinki (1964) Medical Research Involving Human Subjects with all the amendments. We used several criteria for including children in the research: informed consent provided by parents or legal representatives; a child was born after a full-term pregnancy and was breast-fed for longer than 3 months after birth; a child did not have any acute or decompensated chronic diseases at the moment the study was being accomplished[1]; a child attended common physical training classes at school without any limitations and did not do any sports beyond school; an information form was filled in correctly. Anthropometric parameters were estimated considering age and sex; sigma Z-scores of body mass index (BMI) were cal-

[1] Bogdanova O.G., Efimova N.V., Mylnikova I.V. Comparative nutritional characteristics in schoolchildren with different nutritional status. *Gigiena i sanitariya*, 2022, vol. 101, no. 9, pp. 1072–1079. DOI: 10.47470/0016-9900-2022-101-9-1072-1079 (in Russian).

culated according to the respondents' age and then compared with the WHO standards[2]. The aforementioned standards were applied due to the examined respondents belonging to different ethnic groups[1], a situation quite typical for the Baikal region.

Samples of deoxyribonucleic acid (DNA) were obtained from the participants' biological material (buccal epithelium) using conventional procedures. We applied a multicomponent lytic solution able to destroy DNA-protein complexes and then sorbed it on magnetic particles covered with silica gel and washed with ethanol. The final stage involved elution in a buffer solution. DNA was extracted by using a RealBest DNA-extraction3 reagent kit (Vector-Best closed JSC, Russia) and automated pipetting system epMotion 5075 (Eppendorf, Germany). Genotypes were identified by allele-specific PCR and real-time detection of the results. We used TaqMan probes complimentary to DNA polymorphisms and relevant reagents (Syntol, Russia). Amplification was accomplished with a CFX96 Real Time System (Bio-Rad, USA) in real time [5, 10, 14].

To estimate associations between genetic polymorphisms and overweight and obesity (OV and OB), all the examined respondents were divided into Group One ('case', $n = 32$) made of children with OV and OB, and Group Two ('control' or the reference group, $n = 81$) made of children with normal body weight.

The data were tested for normality of distribution by asymmetry and excess and then analyzed with non-parametric statistics. We calculated frequencies of genotypes, alleles, and median percentile BMI trends (*Me* (P25–P75)) by using PASW Statistics 20 [5]. We identified a relative risk (*RR*) and its 95 % confidence interval (CI) and then tested authenticity of the results by Mann – Whitney U-test using DeFinetti software available on the website of Institute of Human Genetics (Munich, Germany)[3] [24]. The critical statistical significance was taken at $p < 0.05$ and $p < 0.01$.

Results and discussion. Overweight (OV) was identified in 23.89 % of the examined respondents and 4.42 % of them had obesity; 25.0 % and 5.0 % of the boys and 22.64 % and 3.77 % of the girls accordingly. Our results are similar to those obtained by the multi-centered study of children aged 5, 10 and 15 years ($n = 5182$) living in Astrakhan, Ekaterinburg, Krasnoyarsk, Saint Petersburg and Samara [25]. Overweight frequency varied between 18.8 and 22.0 % among them; obesity, between 4.7 and 6.7 %.

The results of the genetic tests revealed that the A allele of the rs9939609 FTO polymorphism associated with a risk of obesity was identified in 40.71 % of the examined children. This value is similar to that obtained for European populations where it equals 41.0 % according to the US National Center for Biotechnology Information as of 2022[4]. The A allele of this polymorphism was a bit less frequent among children in Moscow and was identified in 34.4 % of cases [10]. In this study, we were not able to establish any sex-related differences and the share of the A allele was 40.57 % in the examined girls and 40.83 % in the examined boys (Table 1).

Genetic testing in the examined groups detected the A allele of the rs9939609 polymorphism (the FTO gene) in 48.44 % of the children in Group One (OV or OB) and it was 1.29 times higher than the same indicator in the reference group or Group Two (normal

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[2] BMI-for-age (5–19 years). *WHO*. Available at: https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age (February 07, 2023); Tarmaeva I.Yu., Pyrieva E.A., Gmoshinskaya M.V., Bogdanova O.G., Tkachuk E.A., Netunaeva E.A., Safronova A.I., Aleshina I.V. Nutritional features of school children in the Siberian Federal District. *Meditsinskii sovet*, 2021, no. 17, pp. 264–271. DOI: 10.21518/2079-701X-2021-17-264-271 (in Russian).

[3] Case-control studies. *Institute of Human Genetics*. Available at: https://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl (January 12, 2023).

[4] dbSNP. Short Genetic Variations. *U.S. National Library of Medicine*. Available at: https://www.ncbi.nlm.nih.gov/snp/rs9939609#frequency_tab (January 20, 2023).

weight) with its value being equal to 37.65 %. Still, likelihood of obesity and overweight for the examined children was not identified when the alleles were compared (A against T, *RR* = 1.46 [0.81–2.61], 0.297); this is in line with the data of the meta-analysis performed by D. Wang with colleagues (2020) in an Asian population where elevated risks of overweight and obesity were established for adults (*OR* = 1.26, 95 % CI: 1.08–1.47, $p = 0.003$) but not for children or adolescents (*OR* = 1.14, 95 % CI: 0.95–1.36, $p = 0.17$) [26].

The AA genotype of the rs9939609 polymorphism (the FTO gene) was established to be 5.06 times more frequent in the children from Group One than in the reference group (Table 2).

Calculation of relative risk (*RR* = 2.81 [1.65–4.77], STD = 0.271) revealed a probable statistically significant association between the homozygous AA genotype of the rs9939609 FTO polymorphism and overweight and obesity in the examined children. Children with such a genotype had overweight and obesity 2.81 times more frequently than children with TT and AT genotypes.

Table 3 provides the results of comparing median percentile BMI trends (*Me*) in the examined children. Obviously, higher BMI was established only in carriers of the homozygous AA genotype of the rs9939609 FTO gene polymorphism in Group One (20.65 (19.58–22.68) kg/m^2). This was 1.16 times higher than in carriers of the same genotype in the reference group or Group Two (17.77 (17.37–18.03) kg/m^2) at the actual U = 0.5 (critical U = 5 for $p < 0.05$, critical U = 2 for $p < 0.01$). Differences were not statistically significant in all the other cases (actual U > critical U).

The results of our retrospective studies on the examined children established that 4.5 % of them had emaciation at birth; the share was 1.3 % at the moment this study was being accomplished. Emaciation detected at birth persisted only in one child up to the moment of the present study (a girl's age was 8 years). One point nine percent of the examined children had overweight at birth and one was obese with this disorder persisting up to the moment of the study (a girl's age was also 8 years). Table 4 reports the results of the retrospective studies; their analysis revealed that the rs9939609 FTO polymorphism had no influence on children's birth weight or length ($p > 0.05$).

Table 1

Sex-related distribution of genotypes and frequency of the A and T alleles of rs9939609 polymorphism (the FTO gene) among children in the Baikal region

Sex	Genotypes, abs. (%)			Alleles, %	
	TT	AT	AA	T	A
All the respondents	33 (29.20)	68 (51.13)	12 (10.62)	59.29	40.71
Girls	15 (28.30)	33 (62.26)	5 (9.43)	59.43	40.57
Boys	18 (30.00)	35 (58.33)	7 (11.67)	59.17	40.83

Table 2

Distribution of genotypes and frequency of the A allele of the rs9939609 FTO polymorphism among children in the Baikal region depending on body mass index

Genotypes, abs. (%)	Groups as per body mass index		Relative risk between genotypes (*RR* [CI], STD)		
	Group One	Group Two	AA and TT	AT and TT	AA and AT+TT
AT	15 (46.88)	53 (65.43)	2.44 [1.23–4.85]. 0.350	0.81 [0.40–1.65], 0.364	2.81 [1.65–4.77], 0.271*
TT	9 (28.13)	24 (29.63)			
AA	8 (25.0)	4 (4.94)			
Risk A allele, %	48.44	37.65	1.46 [0.81–2.61], 0.297		

Note: * means the significance of this association is $p < 0.05$ since the 95 % CI does not include one.

Table 3

Distribution of median percentile BMI trends (*Me*) depending on genotypes of the rs9939609 FTO polymorphism among children form the Baikal region

Groups	Body mass index (*Me* (P25–P75), kg/m^2)			Mann – Whitney U-test (U_{actual})		
	TT	AT	AA	TT/AT	AA/TT	AA/AT
All the examined children	17.30 (15.60–19.90)	16.95 (15.50–19.10)	19.45 (18.28–21.68)	1071	270.5	555.5
Group One	23.00 (19.60–23.70)	22.00 (18.75–24.25)	20.65 (19.58–22.68)	73	29	56
Group Two	16.10 (14.48–18.28)	16.20 (15.00–17.90)	17.77 (17.37–18.03)	624	66	153
U_{actual} between Group One and Two	196.5	726	0.5*	447	172.5	402.5

Note: * means a statistically significant difference between actual U < critical U for $p < 0.05$ and $p < 0.01$.

Table 4

Anthropometric parameters of newborns depending on the rs9939609 FTO polymorphism (*Me* (P25–P75))

Parameters	Genotypes		
	TT	AT	AA
Length, cm	52.00 (51.00–53.50)	52.00 (50.00–54.00)	52.00 (51.00–53.00)
Weight, kg	3.40 (3.00–3.60)	3.50 (3.10–3.80)	3.70 (3.55–4.00)
BMI, kg/m^2	12.30 (11.65–13.35)	12.70 (12.00–13.60)	13.40 (12.70–14.40)

We did not establish any associations between the allele A heterozygous AT genotype of the rs9939609 FTO polymorphism in the examined children and a risk of overweight or obesity (95 % CI includes 1, Table 2). Prevalence of the homozygous TT genotype (29.20 %), which was more frequent than the AA genotype (10.62 %) in the examined groups, is likely due to influence of ongoing assimilation processes between incoming and indigenous populations on urbanized territories in the Baikal region [27]. These results are consistent with the data reported by E.A. Bondareva with colleagues (2018) [28]. In their study, detected frequency of the TT genotype varied depending on ethnic peculiarities: it equaled 3.7 % in Altaians; 16.4 % in Russians from Moscow, Arkhangelsk, and Saransk; 49.7 % in Mongolians; and 50.6 % in Kalmyks. The AA genotype, though, was detected in 36.5 % Altaians, 33.2 % Russians, 10.5 % Mongolians, and 24.1 % Kalmyks.

Conclusions. Our study results established emaciation at birth in 4.5 % of the examined children from the Baikal region and it persisted only in one child (8 years old) at the moment the study was being accomplished.

Overweight at birth was identified in 1.9 % of the examined children whereas 23.89 % of them had it at the moment the study was being accomplished and 4.42 % were obese.

We did not establish a statistically authentic association between the rs9939609 (FTO gene) polymorphism and children's anthropometric parameters at birth (birth weight and length and BMI).

Our analysis of the results obtained by genetic tests of children from the Baikal region established a statistically significant association between the A allele AA genotype of the rs9939609 polymorphism (FTO gene) and a risk of overweight and obesity (RR = 2.806, 95 % CI: 1.650–4.772; STD = 0.271). The frequency of the A allele of the rs9939609 FTO polymorphism that was associated with obesity equaled 40.71 % and this value is not higher than that identified for European populations (41.0 %). Prevailing frequency of the homozygous TT genotype (29.2 %) as compared with the AA genotype (10.62) is likely due to influence of assimilation processes on urbanized territories in the Baikal region.

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Research article

JOB DESIGN FOR CRANE OPERATORS BASED ON FATIGUE ASPECTS AND MENTAL WORKLOAD IN INDONESIA

I. Pratiwi, S. Oktaviara

Universitas Muhammadiyah Surakarta, Jl. Jend. A Yani Tromol Pos 1 Pabelan, Surakarta, 57102, Indonesia

Terminal Teluk Lamong (TTL) in Indonesia is a company that operates in service sector managing a multipurpose terminal. It provides various services such as loading and unloading containers and dry bulk using integrated crane tools that employ the first semi-automatic facilities and infrastructure in Indonesia. Crane operators' work involves risks of work accidents because they operate at heights and their job tasks require high concentration.

This study aimed to find out fatigue levels and mental workloads typical for workplaces of crane operators and to analyze and assess working conditions. The study results gave grounds for developing recommendations on how to improve workplaces of STS and GSU crane operators who deal with loading and unloading containers and dry bulk cargoes at a seaport.

The relevant data were obtained by questioning 56 STS and GSU crane operators working in four shifts, 6 hours each. We used an employee identity questionnaire as well as SOFI and NASA TLX questionnaires. The results were analyzed to obtain scores for estimating fatigue levels and mental workloads. Statistical analysis involved correlation and regression tests on two variables on STS and GSU crane operators. Upon completion, some recommendations were suggested as regards necessary changes into work process and longer rest in order to reduce fatigue and mental workloads for operators.

The SOFI questionnaire established medium fatigue levels of STS and GSU operators but mental workloads turned out to be high. The correlation test did not reveal any correlation between fatigue and mental workloads for STS crane operators.

It was shown that fatigue could be overcome by adequate rest, well-balanced diet rich with nutrients, and relevant exercise. At the same time, arranging work shifts more rationally, socializing, and training on the importance of fatigue awareness can reduce high mental workloads. The study results can help prevent or reduce increased fatigue and mental workloads that can lead to work accidents.

Keywords: *SOFI method, NASA-TLX method, crane operator, Terminal Teluk Lamong, risks of work accidents, fatigue, mental workloads, statistical analysis, Indonesia.*

Based on the Social Security Administering Body (BPJS) for Employment data, work accident cases in Indonesia have increased from 114,000 cases in 2019 to 177,000 cases in 2020 [1]. More than 65 % of workers in Indonesia come to company polyclinics with complaints of work fatigue. This complex phenomenon occurs due to wake time, long working hours, extreme workloads, health, high responsibility at a workplace, and lifestyle both on and off work duty [2].

Tedious or repeating tasks may increase work fatigue. Fatigue can be described as critical or chronic. Fatigue can be defined as feeling very tired or sleepy due to lack of sleep, prolonged mental or physical work, and prolonged stress or anxiety [2, 3]. In general, fatigue manifests as tiredness, dysautonomia, and decreased work efficiency. This may result in some diseases such as chronic fatigue syndrome, psychosis, depression, stress-related disorders, autoimmune diseases, etc. [4].

Indah Pratiwi – Doctor of Industrial Engineering, Associate Professor at Faculty of Engineering (e-mail: indah.pratiwi@ums.ac.id; tel.: +62 813 9349-8843; ORCID: https://orcid.org/0000-0002-6412-3304).

Senja Oktaviara – Researcher at the Researches Centre for Logistics and Industrial Optimization Studies (e-mail: senjaoktaviara15@gmail.com; tel.: +62 896 9011-2775).

Work-related fatigue is highly relevant not only for workers' health assessment but also for safety issues related to preventable deaths and injuries [5]. Apart from that, fatigue consists of acute and chronic symptoms with subjective and objective features that correspond to situational and individual characteristics. Therefore, it is hard to evaluate fatigue subcomponents comprehensively [6].

Fatigue is characterized by multi-dimensional aspects of physical, mental, and functional health, all of which interact [6]. Fatigue leads to changes in a strategy for use of resources in some processes such as the initial level of mental processing or physical activity that is either maintained properly or reduced [3].

The multi-dimensional concept of mental workload (MWL) and subjective perception are the fundamentals that differ between task and operator characteristics at a crane operator's workplace. Environmental context, time pressure, and other subjective aspects influence stress perceptions or fatigue[1].

To unify these dimensions and better understand them, MWL must meet both objective and subjective criteria, mediated by task demands, external support, and experience [7]. There are three aspects of fatigue, namely: physiological fatigue (reduction in physical capacity), objective fatigue (reduction in work), and subjective fatigue (feeling tired) [8]. The underlying structure of the instrument corresponds to a new qualitative and quantitative description of the perceived physical (exertion and discomfort) and mental (lack of motivation and sleepiness) dimensions of the instrument. Within this concept, lack of energy factors relates to fatigue dimension with physical and mental characteristics [9]. Any workload must be adapted to a worker's health because it can affect a company positively or negatively [10]. E. Ahsberg developed the Swedish Fatigue Inventory (SOFI) method to evaluate fatigue levels in a practical, fast and straightforward way [9].

Intuitively, mental workload is the amount of mental work required by a person to complete a task over a certain period of time [11]. To be more precise, the construct arises from the interaction between the requirements of a given task, the circumstances in which it is performed, the context and skills, behavior, emotional state, and perception of a crane operator [12]. Mental workload is assessed by different techniques, including physiological, performance-based, and subjective measurements [13]. Mental workload is formed mentally and can be seen from the work activities performed. The most widely used mental workload measurement questionnaires are the Subjective Workload Assessment Technique (SWAT) and the National Aeronautics and Space Administration-Task Load Index (NASA-TLX) [13]. Besides that, the research [14] discusses how the work sampling method was used to determine physical workload and the NASA-TLX was utilized to analyze mental workload.

The study [15] has been carried out to analyze the performance of container cranes and supply chain efficiency modeling at the port. It was applied to four Rubber Tired Gantry (RTG) cranes from three different ports. The study results indicate that the RTG model obtains decent results with the same accuracy as the STS model. Previous research on NASA-TLX was conducted by [16] to analyze the workload level of Automated Stacking Crane (ASC) operators in TTL Indonesia. The results of this study indicate that the ASC operator's workload level is high with influencing indicators, namely performance (P) and mental demand (MD) [16]. The NASA-TLX is an excellent multi-dimensional scale for measuring mental workload. The guide by V.J. Gawron notes found that TLX is highly favorable, sensitive to changes in workload, and has high diagnostic value[2].

[1] ISO 10075-1:2017. Ergonomic principles related to mental workload – Part 1: General issues and concepts, terms and definitions, 2017, 9 p.

[2] Gawron V.J. Workload Measures, 3rd ed. Boca Raton, CRC Press, 2019, pp. 1–65. DOI: 10.1201/9780429019579

a

b

Figure 1. (*a*) Ship to Shore (STS) Crane, (*b*) Grab Ship Unloader (GSU) Crane

Terminal Teluk Lamong (TTL) in Indonesia is a company that operates a multipurpose terminal rendering various services such as loading and unloading containers and dry bulk. The terminal uses integrated crane tools with the first semi-automatic facilities and infrastructure in Indonesia [16]. Stevedoring or loading and unloading of containers and dry bulk is an activity to unload or remove containers from the ship and load or load them into the ship. This activity uses a crane consisting of a Grab Ship Unloader (GSU) and a Ship To Shore (STS) Crane. GSU is a crane used for dry bulk unloading activities at the dock. STS is a tool used to lift containers in loading and unloading activities at the dock (Figure 1). This tool is handled by an operator from the top of a crane. Several work processes occur in the loading and unloading process using cranes, namely hoist, trolley, gantry, and boom [17].

Operators work in a shift system divided into five groups. This job is an activity with a high risk because it is performed at a height and requires high concentration. Based on the company's accident data in 2020, 39 accidents occurred during stevedoring activities at TTL Indonesia.

This study aimed to to determine the level of fatigue and mental workload experienced by crane operators. Is this partly a cause of accidents in stevedoring activities at TTL Indonesia? To achieve this, it was necessary to analyze and evaluate workloads using the SOFI method to determine the level of operator fatigue and the NASA-TLX method to determine the level of mental load experienced by the operator. The implication of the research would be to provide recommnedations on a more appropriate job design to reduce problems that occur in the stevedoring process for crane operators.

Materials and methods. This research was conducted on crane operators in TTL Indonesia, where they handled two types of cranes, namely GSU and STS. Fifty-six operators were divided into four work shifts, 6 hours each. The first shift started at midnight and finished at 6 am; the second one, 6 am – noon; the third one, noon – 6 pm; and the last one, 6 pm – midnight. The workers were divided into five groups: E, F, G, H, and I. The five groups included 46 STS crane operators and 10 GSU crane operators. The tools handled by operators were cranes for dry bulk unloading activities, lifting containers for loading and unloading of containers at the dock.

This study type is qualitative research that emphasizes objective phenomena and their quantitative estimation. The objectivity of this research design is maximized by using statistical processing numbers, structures, and controlled experiments. Relevant data were collected by questioning performed among crane operators at TTL Indonesia between January and March 2021.

The research procedure involved several stages. The first stage was a literature study on theories related to fatigue, mental workload, SOFI method, and NASA-TLX method. Then we conducted a field study to determine the location of the research object. The next step was to identify the problems that occur at crane operators' workplaces. Then we collected relevant data using SOFI and NASA TLX questionnaires. Fifty-six crane operators were asked to fill out the questionnaire honestly, especially regarding the conditions they felt when working. The data on fatigue levels were estimated as per the SOFI scales; mental workloads, as per the NASA TLX scales. The hypothesis was that there was a relationship between the level of fatigue and mental workload using statistical tests.

Finally, analysis was carried out and improvements were suggested to overcome fatigue and mental workload experienced by crane operators.

Research methods. The SOFI method is a tool used to identify factors that cause fatigue during work activities subjectively. The SOFI method was developed by E. Ahsberg in 1999; it involves observing several indicators, each of them having five multi-dimensional questions [9]. SOFI's five dimensions are sleepiness, physical discomfort, lack of motivation, lack of energy, and physical exertion. Each dimension is described in 25 questions. Each participant was asked to provide a subjective self-assessment on a scale from 0 to 6. A scale of 0 means it is not felt, while a scale of 6 means it is very felt [18]. To find out which statement has the highest level, a rating with a sub-maximum level is made [19].

Thus, the first dimension describes lack of energy with such words as 'overworked', 'worn out', 'exhausted', 'spent', 'drained'. The second dimension is physical exertion described with 'sweaty', 'breathing heavily', 'palpitations', 'hot', 'out of breath'. The third dimension is physical discomfort, consisting of 'tense muscles', 'stiff joints', 'numbness', 'hurting', 'aching'. The fourth dimension is the lack of motivation, which consists of 'uninterested', 'passive', 'listless', 'indifferent', 'lack of concern'. The last fifth dimension is sleepiness described with 'sleepy', 'falling asleep', 'drowsy', 'yawning', 'lazy' [18].

The steps used to apply the SOFI method are: (1) calculating the average of each dimension, (2) calculating the total average, (3) interpreting the score. After calculating using the SOFI method, the level of fatigue experienced by a crane operator can be analyzed. This method makes it easy to classify the types of fatigue that can be seen based on the fatigue rating classification. The rating categories are < 1.13 for the low fatigue category, a rating value of 1.13–4.87 for the moderate fatigue category, and a rating value of > 4.87 for the high fatigue category [21].

The NASA-TLX method developed by Hart and Staveland is a tool to analyze the characteristics of workloads precieved by a crane operator [12]. The method was used to subjectively collect workload scores based on the average of the six-factor ranking considerations [22]. Workers were asked to assess (between 0–100) six factors of the job [15]. These factors include mental demand (MD), physical demand (PD), temporal demand (TD), performance (P), effort (E), and frustration level (FL). MD component is measured with a low-high scale and means the mental activity and perception needed to do a task. PD component also measured with a low-high scale is physical activity needed to do a task. TD component is the time needed to do a task. These three components of the workload are related to specific demands a worker has to meet to perform the relevant job tasks [22]. The FL dimension measured with a low-high scale is the mental and physical activity needed to do a task at a certain level. The P dimension is measured with a good-poor scale and determines overall stress and / or satisfaction related to the complexity of the task. The E dimension measured with a low-high scale is the level of success or satisfaction and the level of completion of the given assignment. These three dimensions are associated with interactions between a worker and a job task [22]. When respondents fill in a questionnaire, they have to give their scores at five high – low scale and pone good – poor scale. NASA-TLX consists of two phases in its application, namely the weighting phase and the assessment phase. The weighting phase aims to determine the source of the load, while the assessment phase aims to provide an assessment of the six dimensions [23]. The data collected in the NASA-TLX questionnaire are average weighting data on mental workload and mental workload assessment. The mental workload weighting data select the most dominant mental workload dimension felt by respondents while the assessment data give a rating on questions related to the mental workload dimension.

NASA-TLX data processing included several steps: (1) weighting, (2) rating, (3) calculating the value of the moment product (see eq. 1), (4) calculating the weighted workload (WWL), (5) calculating the mean WWL (see eq. 2), (6) interpretation of the score [24]:

$$\text{Product} = \text{raiting} \cdot \text{weight} \quad (1)$$

$$\text{WWL} = \sum \text{Product} \quad (2)$$

The work is categorized as heavy if the score is 80; moderate if the score is 50–80; and relatively easy if the score is < 50 [14]. The categories of high and low mental workload experienced by crane operators are given to facilitate the type grouping, namely: low, medium, relatively high, high, and very high. The assessment categories for the NASA-TLX method are rating value 0–9 for very low workload category, rating value for 10–29 for low workload category, rating value for 30–49 for medium workload category, rating value for 50–79 for high workload category, and a rating of 80–100 in the very high workload category [23, 25].

Data analysis. The calculation results in SOFI and NASA TLX scores were then analyzed using the Statistical Package for the Social Sciences Version 21.0 (SPSS Version 21.0) software. We perfromed correlation and regression tests on two variables to test a hypothesis about a possible relationship between the level of fatigue and the level of workload of the STS and GSU crane operators.

H0: there is no relationship between the level of fatigue and the level of mental workload of a crane operator;

H1: there is a relationship between the level of fatigue and the level of mental workload of a crane operator.

Regression analysis was aimed at determining whether there is a positive effect between two variables, mental workload and the fatigue level for STS and GSU crane operators.

H0: There is no positive effect between two variables;

H1: There is a positive influence between two variables.

The results of the correlation test with SPSS Version 21.0 were put into the Pearson Correlation tavle. The regression test produced an ANOVA table to determine the relationship between the dependent and independent variables.

The analysis was continued by providing alternative solutions in the form of proposed improvements, namely the right job design to reduce fatigue levels and workloads that are too high.

Results and discussion. General data on respondents included the following: age (years), body mass index obtained from weight (kg) and height (cm), history of illness, work records (years), sleep duration (hours per day), and commuting time (minutes). The questionnaire was filled out by 56 respondents, consisting of 46 STS crane operators and 10 GSU crane operators, as shown in Table 1.

Table 1

General profiles of STS and GSU crane operators

Characteristics		STS crane		GSU crane	
		Abs.	(%)	Abs.	(%)
Age (years)	17–25	1	2.17	0	0.00
	26–35	28	60.87	7	70.00
	36–45	17	36.96	3	30.00
	46–55	0	0.00	0	0.00
Body mass index	Skinny	1	2.17	0	0.00
	Normal	21	45.65	5	50.00
	Overweight	9	19.57	1	10.00
	Obese	15	32.61	4	40.00
Work records (years)	< 5	32	69.57	3	30.00
	≥ 5	14	30.43	7	70.00
Diseases in a case history	Yes	5	10.87	1	10.00
	No	41	89.13	9	90.00
Sleep duration (hours/day)	< 7	25	54.35	6	60.00
	≥ 7	21	45.65	4	40.00
Commuting time (minutes)	< 43	21	45.65	3	30.00
	≥ 43	25	54.35	7	70.00

Table 1 shows that work records affect work performance. The longer a person's work records, the better he can understand his body condition so that it can prevent fatigue symptoms that arise [26]. Respondents of STS cranes with a history of disease were 10.87 %, namely 5 people, and respondents without a history of disease were 89.13 %, namely 41 people. Respondents of GSU cranes with a history of disease accounted for 10 %, namely 1 person, and respondents without a history of disease, 90 %, namely 9 people. If a person has a history of certain diseases such as flu, glandular fever, anemia, sleep disorders, Chronic Fatigue Syndrome or Myalgic Encephalopathy (CFS/ME), hypothyroidism, hepatitis, tuberculosis or chronic pain, celiac disease, Addison's disease, Parkinson's disease and heart disease, HIV/AIDS, or cancer, they can be a cause of fatigue. Taking certain medicaiton cal also lead to it [27].

STS crane respondents with sleep duration below the normal limit of < 7 hours accounted for 54.35 %, and respondents with a normal sleep duration of 7 hours accounted for 45.65 %. Sixty percent of GSU crane respondents slept for less than 7 hours, and 40 % of respondents had normal sleep duration of 7 hours and longer. Normal sleep time for adults, according to the National Sleep Foundation, is 7–9 hours per day. Reduction of sleep time by about 2–3 hours from the normal limit can cause a lack of sleep. A lack of sleep that occurs continuously for 5–10 days can reduce a person's awareness, worsen cognitive performance, slow response time, reduce mood, motivation, morale, and initiative [28].

Respondents with STS cranes with commuting time < 43 minutes accounted for 45.65 %, and respondents with commuting time ≥ 43 minutes accounted for 54.35 %. Meanwhile, 30 % of GSU respondents reported commuting time < 43 minutes and 70 % of respondents reported commuting time ≥ 43 minutes. Workers who spend 43–90 minutes on a single walk can waste 14 minutes of sleep each night and report experiencing mental fatigue on weekdays from arriving late [29]. Time spent by workers on commuting to and from work coupled with overtime work can reduce sleep or rest time as well as time for family [30].

Data analysis. Data were analyzed after collecting those related to the level of fatigue and mental load of workers. Data analysis was perfomed first using SOFI and NASA-TLX methods and then by statisitcal methods.

1) Calculation results using the SOFI method

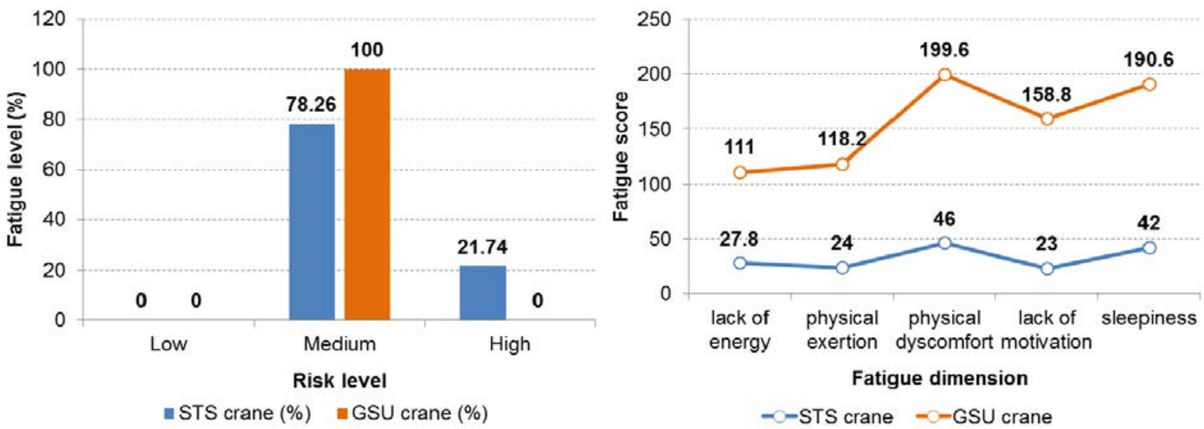
Based on the SOFI method questionnaire filled by 46 STS crane operators, the level of fatigue in the low category was 0 %, the medium category was 78.26 % with a total of 36 operators, and the high category was 21.74 % with a total of 10 operators (see Figure 2(*a*)). Based on Figure 2, it can also be seen that the fatigue level of the GSU crane operator is in the medium category at 100 %, with a total of 10 operators. So it can be concluded that based on the SOFI questionnaire, the fatigue level of the STS and GSU crane operators is in the medium category.

The results obtained for the dimensions of the level of fatigue using the SOFI method indicate that the highest total value for GSU crane operators was identified for the 'physical discomfort'scale equaling 199.6; the next was sleepiness with a total value of 190.6, and lack of motivation with a total value of 158.8 (Figure 2(*b*)). Figure 2 also shows the results of the fatigue level dimension for STS crane operators using the SOFI method; they given with the blue curve. The highest total value was identified for physical discomfort equaling 46, then sleepiness folowed with a total value of 42, and lack of energy with a total value of 27.8. So it can be concluded that physical discomfort and lack of motivation are two dimensions that greatly affect the fatigue level of STS and GSU crane operators. Other studies describe related but a bit difefrent states, such as physical exhaustion, lack of strength, and lethargy that shift workers may be quite sensitive to. Thus, long shifts or a series of long working days can have a greater effect on physical fatigue.

The state of high sleepiness occurs in night shift workers [19]. In addition, shift cycles with short rest periods between shifts can lead to sleep deprivation, resulting in drowsiness during a work day [9].

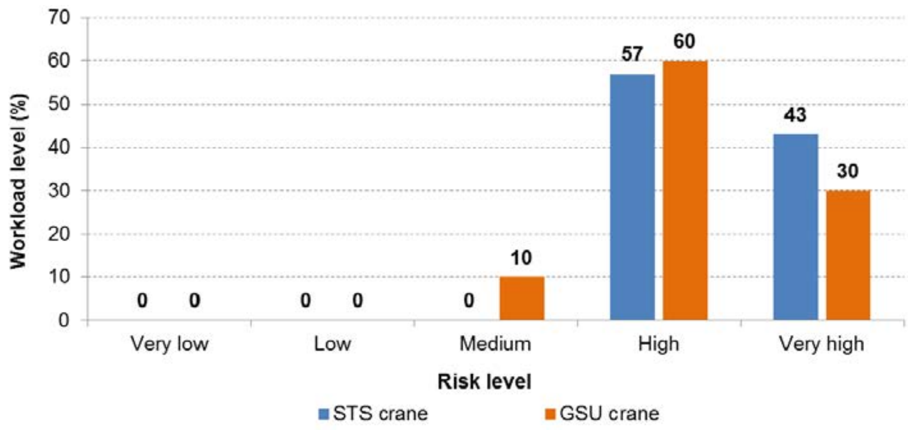
2) Calculation results obtained by using the NASA-TLX method

Based on the NASA TLX questionnaire filed by 46 STS crane operators, it was established that the mental workload level in the very low category was 0 %, the low category was 0 %, the medium category was 0 %, the high category was 57 % identified for 26 operators, and the very high category was 43 % identified for 20 operators. For GSU crane operators, the medium category was identified for 10 % (1 operator); the high category, 60 % (6 operators); and the very high category was identified for 30 % (3 operators). So it can be concluded that based on the NASA-TLX questionnaire, the mental workload level is in the high category for the STS and GSU crane operators (see Figure 3(*a*)).

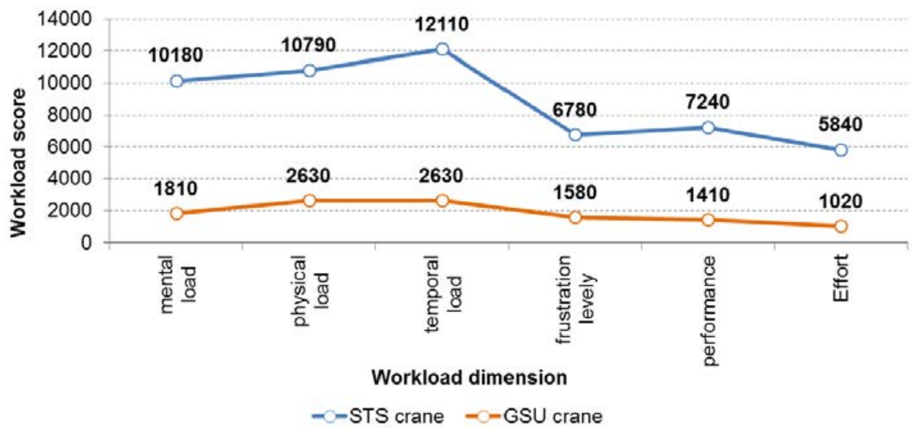


a *b*

Figure 2. SOFI method to measure (*a*) fatigue level, (*b*) fatigue dimension in STS and GSU crane operators



a



b

Figure 3. NASA-TLX method to measure (*a*) risk level, (*b*) workload level for STS and GSU crane operators

Figure 3(*b*) shows mental workload scores using the NASA-TLX method. The dimensions of workload with the three highest scores are temporal demand, physical demand, and mental demand and they are are experienced by both STS and GSU crane operators. Each has a score: temporal demand is 12,110 for STS crane operators and 2630 for GSU crane operators, physical demand is 10,790 for STS crane operators and 2630 for GSU crane operators, and mental demand has a score of 10,180 for STS crane operators and 1810 for GSU crane operators.

Table 2 shows the results of the Kolmogorov – Smirnov normality test using the SOFI method and the NASA-TLX for STS crane operators, and the P-value is 0.2. The data is said to be normally distributed if the P-value > α (α = 0.05). From the resulting normality test, the data is normally distributed, or the data is not biased because the P-value > α. The correlation test was aimed to identify a possible relation between the level of fatigue and mental workload on STS crane operators; as a result, P-value of 0.761 > 0.05 was obtained so that H0 is accepted. There is no relationship between fatigue and mental workload for STS crane operators.

The correlation analysis stabliched the value of the correlaiton coefficient equal to 0.046 indicating that the relationship between the two variables is insignificant. Based on the results of the calculations in the ANOVA table for STS crane operators, it is known that the F-Count value is 0.094 < F-Table value which is 4.06. So, H0 is accepted meaning there is no positive effect between the independent variable and the dependent one. The obtained regression model is $Y' = 3.154 + (0.006)X$.

Table 2

Normality test results

Results		STS crane		GSU crane	
		SOFI	NASA-TLX	SOFI	NASA-TLX
Normality test	N	46	46	10	10
	significance	0.200	0.200	0,200	0,200
Correlation analysis	P	0.761		0.751	
	Correlation coefficient	0.046		-0.115	
Variation analysis	F-count	0.094		0.108	
	F-table	4.06		5.32	

Based on the results of the Kolmogorov-Smirnov normality test on the GSU crane operator, a P-value of 0.2 was obtained. The data are considered to be normally distributed if the P-value > α (α = 0.05). From the normality test, the data are normally distributed and not biased because P-value > α. The correlation test between the level of fatigue and mental workload for GSU crane operators establsihed a P-value of 0.751 > 0.05 so that H0 is accepted indicating there is no relationship between the level of fatigue and mental workload for GSU crane operators. The correlation coefficient was established to be equal to -0.115 indicating there is no relationship between the two variables. Based on the results of the calculations in the ANOVA table, it was established that the H-Count value is 0.108 < F-Table of 5.32. So, H0 is accepted meaning there is no positive effect between the independent variable and the dependent one. The obtained regression model is $Y' = 3.791 + (-0.007)X$.

Suggestions on improvement. The results of the study using the SOFI method showed that the largest percentage of moderate fatigue levels was 78 % for STS crane operators and 100 % for GSU crane operators. Meanwhile, using the NASA TLX method, the largest percentage was established for the high mental load level, namely 57 % for STS crane operators and 100 % for GSU crane operators. Measurement of fatigue levels and workloads is subjectively considered a flexible and comfortable method to estimate a workplace; it is not time-consuming and the cheapest to evaluate workload[3]. High levels of fatigue can contribute to work errors and accidents, especially if jobs require high level of alertness [31].

The improvements that can be made to reduce high fatigue on crane operators based on the highest indicators experienced are provided in Table 3.

Based on the results of mental workload research using the NASA-TLX method, it can be seen that crane operators have a fairly high level of mental workload. The improvements that can be made to overcome the high mental workload at crane operators' wokrplaces considering the highest indicators experienced are provided in Table 4.

The high mental load can also lead to chronic fatigue, demotivation, poor health, and absenteeism [31]. Ergonomic workplace design can reduce the impact of fatigue and can help reduce mental workload and avoid work accidents [31]. In addition to the job demands, operator performance is related to fatigue, alertness, and duration of work[4]. It is necessary to search for balance between workers' fatigue, stress, and independent control [35].

Work fatigue can be overcome with adequate rest, eating nutritious food, exercising, ergonomic exercises, and adequate sleep duration. High mental workloads can be overcome by adjusting them with operators' own ability, providing rewards and employee motivation. The company can conduct socialization or training about the importance of mental workload and fatigue awarness, maximaze safety talk, and roster shift adjustment.

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[4] Handbook of Cognitive Task Design. In: E. Hollnagel ed. Boca Raton, CRC Press Publ., 2003, 840 p.

Table 3

Suggested measures to reduce fatigue levels

Indicator	Suggested improvements
Physical discomfort	Operators experience physical discomfort, namely: pain, cramps, joint stiffness, and muscle tension due to a bent working posture. In addition, operators also experience complaints and pain in the neck and back of the body, leading to musculoskeletal disorders (MSDs). MSDs occur in nine body parts, namely: neck, shoulders, forearms, elbows, lower back, waist, wrists, thighs, and knees [22]. Work methods should be revised to reduce muscle fatigue caused by repetitive motion [32]. The solution is for operators to exercise regularly, participate in fitness challenge programs, perform ergonomic exercises and relaxation/neck stretching movements such as head drops, shoulder blade squeeze, prone extension in between of work activities. Operators can carry out regular medical check-ups at the company's clinic and participate in a fit to work program [30].
Lack of energy	There is an increase in the amount of energy released during the night shift and this is correlated with the dimension of sleepiness [19] due to larger workload on the body during a night shift. Operators are overworked, feeling tired, and drained of energy. The improvement is that an operator gets enough rest time which he uses efefctively. They should consume nutritious food, avoid spicy and high-fat foods because they can interfere with digestion and sleep patterns.
Sleepiness	Operators can do a power nap, which is a short nap for 30–45 minutes before work. This is considered to increase a person's awareness. Maintain a normal sleep pattern of 7–9 hours per day. The duration of the break between working hours needs to be controlled, the average rest time for 8 hours of work is 30 minutes to 2 hours [22] which is divided into coffee breaks, toilet breaks, and food breaks[5]
Lack of motivation	The company can appreciate the operator's achievements/performance by providing bonuses and allowances, evaluate operators' work, maximize safety talk activities, and provide punishment if an operator does not work according to procedures. It is also necessary to conduct training on ergonomic principles [33].

Table 4

Suggestions for reducing mental workload for crane operators

Indicator	Suggested improvements
Temporal demand	Operators have high time requirements, and this is related to repetitive work. Operators can take advantage of waiting time and prayer breaks as a way to reduce this load.
Physical demand	Crane operators have high physical needs because they work with continuous physical activity. An operator who works at height requires a healthy physique to be able to focus on work. Awkward posture, lifting heavy weights, and long shifts cause pain in body parts [34]. The suggestion is to adjust the roster shift schedule so that the operator's circadian rhythm pattern is not disturbed.
Mental demand	Crane operators require high concentration, focus, and vision in their work. Single workers have 3.43 times more distractions than married ones. It is necessary to understand that emotional support from a family can be considered a factor able to adjust workload and behave more safely [34]. In addition, operators should be able to control boredom while working. Companies can provide rewards for operator performance to increase work motivation.

Conclusion. Measurements of fatigue levels and mental load were carried out at workplaces of 46 STS crane operators and 10 GSU crane operators in TTL Indonesia. The results showed that the crane operator's fatigue level was in the medium category, while the crane operator's mental workload was in the high category. The dimensions of fatigue level for STS crane operators in a descending order are physical discomfort, sleepy, lack of motivation, physical exertion, and lack of energy; for GSU crane operators, physical discomfort, sleepy, lack of energy, physical exertion, and lack of motivation. STS and GSU crane operators have the

[5] Haworth N., Hughes S. The International Labour Organization. London, Routledge, 2012.

same descendimg order for the mental workload dimension, namely: temporal demand, physical demand, mental demand, performance, effort, and frustration level. Work fatigue can be overcome with adequate rest, nutritious food, and exercise. At the same time, high mental workload can be reduced by arranging work shifts, socializing, and training on the importance of fatigue awareness. All this aims to reduce high fatigue levels and mental workload, which can lead to industrial accidents.

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Research article

USE OF AQUEOUS COMPOSITIONS OF POLYACRYLAMIDE WITH ZINC AND COPPER CATIONS AS A POSSIBLE WAY TO REDUCE THE RISKS OF MICROBIAL CONTAMINATION IN OBJECTS IN THE HOSPITAL ENVIRONMENT

M.V. Kuznetsova[1,2], E.V. Afanasievskaya[2], N.V. Nikolaeva[2], E.S. Gorovitz[2], A.S. Averkina[3], I.N. Feklistova[4], V.A. Valtsifer[3]

[1]Institute of Ecology and Genetics of Microorganisms of Ural Branch of Russian Academy of Sciences – affiliation of Perm Federal Research Centre of Ural Branch of RAS, 13 Goleva Str., Perm, 614081, Russian Federation
[2]Perm State Medical University named after Academician E.A. Wagner, 26 Petropavlovskaya Str., Perm, 614990, Russian Federation
[3]Institute of Technical Chemistry of Ural Branch of Russian Academy of Sciences – affiliation of Perm Federal Research Centre of Ural Branch of RAS, 3 Akademika Koroleva Str., Perm, 613013, Russian Federation
[4]Belarusian State University, 4 Nezavisimosti Ave., Minsk, 220030, Belarus

Microbial contamination means that infectious agents are identified on objects in the hospital environment. This serious issue is the most significant for healthcare organizations. Covering abiotic surfaces with a thin polymer film can be a promising way to fight against microbial adhesion and colonization. This film acts as a depot of an antibacterial substance.

In this study, our aim was to investigate antimicrobial effects of new water compositions of polyacrylamides (PAM) with $CuSO_4$ and $ZnSO_4$.

We examined antibacterial activity of 5%-solutions of $CuSO_4$ and $ZnSO_4$ and their compositions with various PAM types in a concentration equal to 0.075 % against such reference cultures as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. We estimated use of PAM as a growth substrate as well as antimicrobial activity of the analyzed solutions and compositions in agar and liquid nutrient media.

As a result, we established that bacterial cultures did not use PAM as sole nutrition source when growing in a liquid mineral medium and on PAM-films covering glass and plastic surfaces. More apparent inhibitory effects were produced on microorganisms cultivated on solid and liquid nutrient media by 5%-solution of $ZnSO_4$. When PAM Praestol 857 and PAM Praestol were added to solutions of Cu^{2+} and Zn^{2+} cations, it resulted in an authentic increase in a diameter of a zone with inhibited bacterial growth in the agar medium. In the liquid medium, salts of both metals inhibited the growth and viability of all the analyzed microorganisms already in a concentration equal to 0.16 % or lower. Adding PAM Praestol 2530 led to a slight decrease in antibacterial efficiency of the examined metal salts whereas PAM Praestol 857 had practically no influence on bacteriostatic and bactericidal effects produced by them.

Therefore, use of the obtained composite solutions where $CuSO_4$ or $ZnSO_4$, immobilized on a PAM matrix act as an antibacterial component seems a promising way to disinfect objects in the hospital environment. This can significantly reduce risks of hospital-acquired infections.

Keywords: *risks of microbial contamination, $CuSO_4$, $ZnSO_4$, polyacrylamides (PAM), antimicrobial solutions, antimicrobial activity, hospital environment.*

Marina V. Kuznetsova – Doctor of Medical Sciences, Associate Professor, Leading Researcher of the Laboratory for Molecular Biotechnology; Professor of Microbiology and Virology Department (e-mail: mar@iegm.ru; tel.: +7 (342) 212-44-76; ORCID: https://orcid.org/0000-0003-2448-4823).

Elizaveta V. Afanasievskaya – Candidate of Medical Sciences, Associate Professor of Microbiology and Virology Department (e-mail: lizavika@mail.ru; tel.: +7 (342) 36-44-85; ORCID: https://orcid.org/0000-0002-3498-6459).

Nina V. Nikolaeva – Candidate of Biological Sciences, Associate Professor of Microbiology and Virology Department (e-mail: solomonia.ya@yandex.ru; tel.: +7 (342) 36-44-85; ORCID: https://orcid.org/0000-0002-6799-5503).

Eduard S. Gorovitz – Doctor of Medical Sciences, Professor, Head of the Microbiology and Virology Department (e-mail: eduard.gorovitz@mail.ru; tel.: +7 (342) 36-44-85; ORCID: http://orcid.org/0000-0003-4320-8672).

Anastasiia S. Averkina – Candidate of Technical Sciences, researcher (e-mail: Averkina_A_S_ITChUBRAS59@mail.ru; tel.: +7 (342) 237-82-81; ORCID: https://orcid.org/0000-0002-6833-2867).

Iryna N. Feklistova – Candidate of Biological Sciences, Head of the Research Laboratory for Molecular Genetics and Biotechnology (e-mail: feklistiva@bsu.by; tel.: +375 (17) 209-58-86; ORCID: https://orcid.org/0000-0002-8669-3666).

Viktor A. Valtsifer – Doctor of Technical Sciences, Professor, Deputy Director responsible for research work (e-mail: valtsifer.v@itcras.ru; tel.: +7 (342) 237-82-50; ORCID: https://orcid.org/0000-0002-8671-739X).

Contamination of abiotic surfaces with pathogens and opportunistic pathogens is a substantial threat for human health and health of farm animals [1]. Microbial contamination or, in other words, infectious agents occurring on various environmental objects has specific significance when it comes to healthcare organizations, first of all, intensive care units, emergency surgery and burn units [2, 3]. It creates high risks of healthcare-associated (nosocomial) infections. The official multi-centered study on prevalence of nosocomial infections was accomplished by the World Health Organization experts in 50 clinics located in 14 different countries. It established that 8.7 % of all hospitalized patients or more than 1.4 million people worldwide had infectious complications [4]. Their development requires additional diagnostic and treatment procedures thereby extending a period a person has to spend in hospital and resulting in substantial expenses. In addition, these developing complications deteriorate a patient's quality of life considerably and create elevated risks that a primary disease would have an adverse outcome [2].

M. Robakowska with colleagues (2017) detected microbial contamination in 20 % of the swabs taken off various objects in the hospital environment when performing bacteriological control in a multi-field in-patient hospital [5]. Healthcare organizations use varied disinfectants to prevent infections caused by microbe circulation and persistence on objects and equipment [3, 5]. Most disinfectants produce their effects directly at the moment when a surface is being treated with them or during a very short time. Moreover, at present most clinically significant bacteria are developing greater resistance to disinfectants that are commonly used in hospitals [6]. Covering abiotic surfaces with a thin polymer film containing antibacterial substances can be a promising way to fight against microbial adhesion and colonization. Such disinfectants are more effective since their effects last longer due to a polymer basis provided for a material that acts as a depot of a biocide.

Inorganic salts, salts of Cu and Zn in particular, are known to produce a wide range of antibacterial effects [7–10]; polyacrylamides (PAM) play a significant role in compositions of water soluble polymer with metallic cations acting as reducers and / or being a matrix for aggregation of metal ions or nanoparticles [11]. Properties of these compositions and their application as antibacterial materials are being studied actively now [12]. Nevertheless, as far as we know, experts have not yet made an attempt to compare antimicrobial effects produced on certain pathogens and opportunistic pathogens by water solutions of PAMs with Zn^{2+} and Cu^{2+} cations with different physical and chemical properties.

In this study, our aim was to investigate antimicrobial effects of new compositions of water soluble polyacrylamides (PAM) with $CuSO_4$ and $ZnSO_4$.

Methods and materials. ***Analyzed solutions and compositions***. In this study, we analyzed a 5%-solution of $CuSO_4$, 5%-solution of $ZnSO_4$, various types of polyacrylamides (PAM) in a concentration equal to 0.075 %: PAM Praestol 806, PAM Praestol 857, PAM Praestol 2510, PAM Praestol 2530, as well as a 5%-solution of $CuSO_4$ in PAM Praestol 857, 5%-solution of $ZnSO_4$ in PAM Praestol 857, 5%-solution of $ZnSO_4$ in PAM Praestol 2530 (provided by the Institute of Technical Chemistry of Ural Branch of RAS, Perm). PAM are acrylamide polymers, which, when being solved in water, are used for gelation of liquids and creation of film coatings. Their approximate molecular mass varies between 8 and 14 million. PAM Praestol 806 and PAM Praestol 857 become positively charged when dissolved in water; PAM Praestol 2510 and PAM Praestol 2530 become negatively charged.

Bacterial strains. We selected several strain cultures as our test objects including *Escherichia coli* ATCC®25922, *Klebsiella pneumoniae* ATCC®700603, *Pseudomonas aeruginosa* ATCC®27853, *Staphylococcus aureus* ATCC®25923 (provided by the Scientific Centre for Expert Evaluation of Medicinal Products of the Ministry of Health of the Russian Federation, Moscow).

Using PAM as a growth substrate. Bacteria's capability to use PAM as a sole growth substrate was studied both in a liquid and agar medium as well as on abiotic surfaces. In the first test, 100 µl of a suspension (10^6 cells/ml) of each culture and PAM in a concentration up to 0.075 % were added to penicillin vials containing 2 ml of a Nitrogen-free mineral salts medium (N) with the following composition (g/l): KH_2PO_4 – 1.0; $K_2HPO_4 \times 3H_2O$ – 1.6; NaCl – 0.5; $MgSO_4 \times 7H_2O$ – 0.5, $CaCl_2$ – 0.005; $FeSO_4 \times 7H_2O$ – 0.01; $CoCl_2 \times 6H_2O$ – 0.01 (pH 7.2 ± 0.2). A variant with the N medium without PAM was used as a negative control; a variant with Lysogeny broth (LB) was used as a positive control. The cultures were then incubated under 37 ºC without mixing and in a shaker at a mixing speed equal to 120 turns per minute for 7 days. A film of each PAM type was created out of 1 ml on an agar medium N (the 2nd variant); it was then dried and after that a bacterial suspension (100 µl, 10^6 cells/ml) was deposited on its surface with a spatula. N-agar without PAM was used as a negative control and LB-agar was used as a positive control. Glass and plastic Petri dishes were used as abiotic surfaces; a PAM-film of each type was created on their bottoms out of 1 ml of a solution and a bacterial suspension was deposited as described above. A surface without preliminary treatment with PAM was used a control.

A capability to use PAM as a source of carbon or nitrogen was investigated in immunological flat bottom plates. To do that, we added ammonia chloride to wells with 200 µl of a nitrogen-free mineral medium N until its ultimate concentration reached 5 mM, or glucose up to the ultimate concentration 0.1 % as well as various PAM up to a concentration equal to 0.075 % as a source of carbon or nitrogen accordingly. Then, 10 µl of cell suspensions (10^6 cells/ml) of each bacterial culture were inoculated in the wells. The wells that were added only with PAM were used as control (PAM as a sole nutrition source, similar to the first experiment); the wells without PAM were used as a negative control and the wells with LB-medium were used as a positive control. The plates were incubated under 37 ºC without mixing and in a shaker at a mixing speed equal to 120 turns per minute for 7 days. Bacterial growth was estimated as per optical density (OD) of a cell suspension measured with automated microplate PowerWave X spectrophotometer (Biotech, USA) at λ = 600 nm.

We assessed effects produced by inorganic salts and compositions on bacteria by using agar diffusion tests and twofold serial cultivations in the microplates [13, 14].

Assessment of antimicrobial effects produced by solutions and compositions on a solid medium (disk diffusion test and gel diffusion test). Bacterial cultures were grown in a liquid nutrient LB-medium for 18–24 hours and then standardized to 10^6 cells/ml. The prepared bacterial suspension was inoculated as an 'unbroken' lawn on LB-agar in Petri dishes. Sterile paper disks (d = 6 mm) were placed on the agar surface and saturated with the above listed solutions and compositions (10 µl); also, droplets with the same volume were deposited without a disk. Next, the inoculations were cultivated under 37 °C for 24 hours and antimicrobial effects were then estimated as per a diameter of a zone where the bacterial growth was inhibited (taken in mm).

Assessment of antimicrobial effects produced by solutions and compositions in a liquid medium. Bacterial cultures were grown in the same way as in the previously described test. Bacteriostatic (MIC or minimum inhibitory concentration) and bactericidal (MBC or minimum bactericidal concentration) effects produced by inorganic salts with added PAM and without them were investigated in wells of a polystyrene immunological plate as per the conventional procedure. It involved estimating OD_{600} of the cultures and their growth after inoculation on LB agar from wells without any apparent bacterial growth. Concentrations of inorganic salt solutions in a monovariant and in compositions varied in a range between 0.01 and 5 %.

Statistics. All the experiments were performed not less than threefold, simple means and standard deviations were calculated. Validity of differences between the samples was estimated as per *t*-test (differences were considered statistically authentic at $p \leq 0.05$). The data were analyzed with standard software packages, Microsoft Office XP Excel and STATISTICA 10.0.

Results. ***Bacteria's capability to use PAM as a growth substrate.*** All the analyzed reference bacterial cultures did not grow in either test variant (absence of any visible growth) during 7-day incubation in a liquid medium N with added PAM (Table 1). This indicates that the analyzed microorganisms did not use PAM as a sole nutrition source. Nevertheless, bacterial cells preserved their viability in PAM under the given conditions up to the 7th day in the experiment. Consequently, colony formation occurred in inoculation on LB (the data are not presented here). Cultures were established to grow on PAM-films created on the agar medium N but only on the 7th day of exposure. We did not detect any visible growth on PAM-films created on glass or plastic. Moreover, gram-negative bacteria cells did not preserve their viability under the given conditions, as opposed to *S. aureus* (excluding PAM Praestol 2530).

Table 1

Growth and viability* of bacteria on PAM as a sole nutrition source in different model systems

A test variant	Strain			
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
PAM Praestol 806				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass*	-	-	-	33 CFU/dish
plastic*	-	-	-	**7** CFU/dish
PAM Praestol 857				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass	-	-	-	12 CFU/dish
plastic	-	-	-	No calculation
PAM Praestol 2510				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass	-	-	-	3 CFU/dish
plastic	-	-	-	54 CFU/dish
PAM Praestol 2530				
liquid medium N with aeration	-/-	-/-	-/-	-/-
liquid medium N without aeration	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass	-	-	-	-
plastic	-	-	-	-
Controls (no PAM added)				
liquid medium N	-/-	-/-	-/-	-/-
agar N	-/+	-/+	-/+	-/+
glass **	+/-	+/-	+/-	+/+
plastic **	-/-	-/-	+/-	+/-

Note: The Table provides the results of the 3rd/7th day in the experiment: "-" means absence of visible growth, "+" is visible growth; * is one-day exposure, viability on LBA identified after a swab off a surface; ** is 1/7 day exposure, viability on LBA identified after a swab off a surface.

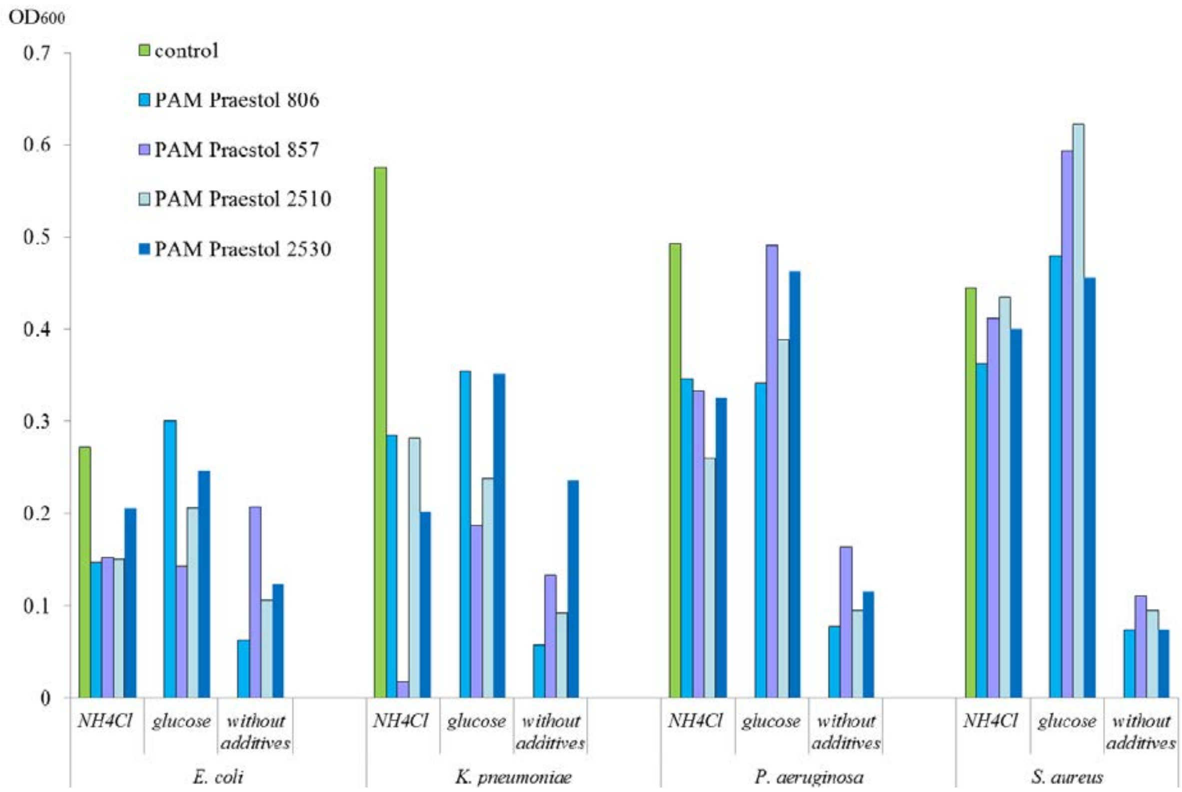

Figure 1. Bacteria growth on PAM as a sole substrate of nutrition, as a sole carbon or sole nitrogen source (without aeration)

Bacteria's capability to use various PAM as a source of carbon or nitrogen nutrition had to be investigated profoundly. To do that, we cultivated the analyzed test-cultures in wells of a polystyrene plate in a liquid medium N with PAM and 0.1-% glucose as a source of carbon or with 5 mM ammonia chloride as a source of nitrogen. A control test variant contained glucose or ammonia without any PAM added. Similar to the previous tests, bacteria growth was not detected in any test variant in a liquid mineral medium with PAM without any additional nutrition sources (Figure 1). Bacteria grew on a medium with PAM and glucose more often; in a smaller number of cases, growth was also detected on a medium with added ammonia chloride. This indicates that the analyzed acryl polymers can be used either solely as sources of nitrogen or sources of carbon. Table 2 provides the results obtained by statistical analysis of the test data.

Regardless of where PAM are used, either in healthcare or agriculture, these polymers can come in contact with various microorganisms and be exposed to biodegradation. Most studies on PAM degradation providing evidence that this polymer could be used by bacteria have been accomplished either in soil or with soil bacterial strains. It has been established that bacteria can use polyacrylamide as a source of energy or nitrogen nutrition due to their amidase activity despite its resistance to microbial degradation [15–19]. Very few studies mention possible microbial PAM degradation without any additional sources of nutrition / energy [20, 21]. Shanker with colleagues (1990) reported that when ammonia sulfate was added as an additional source of nutrition, this enhanced bacteria capabilities to degrade acrylamide [22]. The process was established to develop even more actively in case glucose was added. PAM-degrading bacteria are assumed to largely hydrolyze amide groups in the side chain of the polymer and to a lesser extent affect the main carbon chain [11]. It is noteworthy that microbial amidases (for example, N-acyl-L-amino-acid amidohydrolase [EC 3.5.1.4]) deaminate aliphatic amides down to their carbon acids and ammonia and this reaction is substrate-specific. Amidase production is considered a species

Table 2

Bacteria growth on PAM as a sole substrate or a source of carbon / nitrogen nutrition

A test variant		Absorbance, units (λ = 600)											
		A	*E. coli*		**B**	*K. pneumoniae*		**C**	*P. aeruginosa*		**D**	*S. aureus*	
PAM Praestol 806													
N with aeration	NH_4Cl	1	0.201 ± 0.011	$p^{3,19,25,C,D}$	1	0.248 ± 0.116	$p^{25,D}$	1	0.364 ± 0.026	$p^{3,7,25,A}$	1	0.439 ± 0.045	$p^{3,A,B}$
	glucose	2	0.429 ± 0.201	p^{3}	2	0.439 ± 0.286		2	0.262 ± 0.130	p^{25}	2	0.375 ± 0.065	p^{3}
	without additives	3	0.054 ± 0.017	$p^{1,2,15,25,C}$	3	0.052 ± 0.026	p^{21}	3	0.091 ± 0.002	$p^{1,15,25,A}$	3	0.088 ± 0.005	p–1,2
N without aeration	NH_4Cl	4	0.147 ± 0.056	$p^{5,6,26,D}$	4	0.285 ± 0.236	p^{26}	4	0.346 ± 0.213		4	0.362 ± 0.016	$p^{5,6,16,22,A}$
	glucose	5	0.300 ± 0.096	$p^{4,6,11,26,D}$	5	0.355 ± 0.266		5	0.341 ± 0.247		5	0.480 ± 0.017	$p^{4,6,11,17,26,A}$
	without additives	6	0.063 ± 0.025	$p^{4,5,12,24,26}$	6	0.058 ± 0.029	$p^{18,24,26}$	6	0.078 ± 0.001	$p^{12,26,D}$	6	0.074 ± 0.001	$p^{4,5,18,26,C}$
PAM Praestol 857													
N with aeration	NH_4Cl	7	0.173 ± 0.042	$p^{9,25,D}$	7	0.109 ± .062	$p^{25,C,D}$	7	0.227 ± 0.049	$p^{1,9,25,B,D}$	7	0.379 ± 0.059	$p^{9,A,B,C}$
	glucose	8	0.296 ± 0.226	p^{25}	8	0.246 ± 0.131	p^{25}	8	0.261 ± 0.167		8	0.394 ± 0.027	p^{9}
	without additives	9	0.082 ± 0.025	$p^{7,12,25}$	9	0.063 ± 0.002	$p^{12,25,C,D}$	9	0.093 ± 0.005	$p^{7,12,25,B}$	9	0.092 ± 0.016	$p^{7,8,12,25,B}$
N without aeration	NH_4Cl	10	0.152 ± 0.032	$p^{26,D}$	10	0.0178 ± 0.072	$p^{26,D}$	10	0.333 ± 0.180		10	0.412 ± 0.036	$p^{11,12,A,B}$
	glucose	11	0.143 ± 0.016	$p^{5,26,D}$	11	0.187 ± 0.060	$p^{26,D}$	11	0.491 ± 0.211		11	0.593 ± 0.020	$p^{5,10,26,A,B}$
	without additives	12	0.207 ± 0.055	$p^{6,9,26,D}$	12	0.133 ± 0.063	$p^{9,26}$	12	0.164 ± 0.034	$p^{6,9,26,D}$	12	0.110 ± 0.026	$p^{9,10,11,26,A,C}$
PAM Praestol 2510													
N with aeration	NH_4Cl	13	0.166 ± 0.064	$p^{25,D}$	13	0.144 ± 0.082	$p^{25,C,D}$	13	0.251 ± 0.091	$p^{25,B}$	13	0.381 ± 0.076	$p^{15,A,B}$
	glucose	14	0.453 ± 0.203	$p^{15,17}$	14	0.578 ± 0.213	$p^{15,17,25}$	14	0.275 ± 0.109	$p^{17,25,D}$	14	0.486 ± 0.037	$p^{15,17,25,C}$
	without additives	15	0.099 ± 0.006	$p^{3,14,25}$	15	0.093 ± 0.007	$p^{14,25,C}$	15	0.103 ± 0.006	$p^{3,25,B,D}$	15	0.097 ± 0.007	$p^{13,14,25,C}$
N without aeration	NH_4Cl	16	0.150 ± 0.029	$p^{26,B,D}$	16	0.282 ± 0.025	$p^{18,25,A,D}$	16	0.260 ± 0.203	p^{26}	16	0.435 ± 0.017	$p^{4,26,A,B}$
	glucose	17	0.206 ± 0.077	$p^{14,26,D}$	17	0.238 ± 0.071	$p^{14,18,26,D}$	17	0.389 ± 0.255	p^{14}	17	0.622 ± 0.078	$p^{5,14,16,18,26,A,B}$
	without additives	18	0.106 ± 0.037	p^{26}	18	0.093 ± 0.037	$p^{6,16,17,26}$	18	0.095 ± 0.014	p^{26}	18	0.095 ± 0.006	$p^{6,16,17,26}$
PAM Praestol 2530													
N with aeration	NH_4Cl	19	0.124 ± 0.034	$p^{1,25,D}$	19	0.211 ± 0.094	$p^{25,D}$	19	0.228 ± 0.114	p^{25}	19	0.380 ± 0.028	$p^{21,A,B}$
	glucose	20	0.310 ± 0.265		20	0.374 ± 0.225		20	0.340 ± 0.135		20	0.392 ± 0.023	p^{21}
	without additives	21	0.100 ± 0.041	p^{25}	21	0.104 ± 0.003	$p^{3,25}$	21	0.172 ± 0.061	p^{25}	21	0.108 ± 0.007	$p^{19,20,25}$
N without aeration	NH_4Cl	22	0.206 ± 0.135	p^{26}	22	0.202 ± 0.016	$p^{24,26,C,D}$	22	0.326 ± 0.023	$p^{24,26,B,D}$	22	0.400 ± 0.008	$p^{4,23,24,B,C}$
	glucose	23	0.247 ± 0.166	p^{26}	23	0.352 ± 0.097		23	0.463 ± 0.138	p^{24}	23	0.456 ± 0.016	$p^{22,24}$
	without additives	24	0.123 ± 0.011	$p^{6,26,B,D}$	24	0.236 ± 0.018	$p^{6,22,26,A,C,D}$	24	0.116 ± 0.021	$p^{6,22,23,26,B,D}$	24	0.075 ± 0.001	$p^{22,23,26,A,B,C}$
Controls													
N with aeration	glucose, NH_4Cl	25	0.391 ± 0.008	$p^{1,3,7,8,9,13,15,19,21}$	25	0.462 ± 0.012	$p^{1,3,7,8,9,13,15,19,21}$	25	0.452 ± 0.015	$p^{1,3,7,9,13,14,15,19,21,D}$	25	0.411 ± 0.051	$p^{9,14,15,21,D}$
N without aeration	glucose, NH_4Cl	26	0.272 ± 0.027	$p^{4,5,6,10,11,12,16,17,18,22,23,24,B,C,D}$	26	0.576 ± 0.032	$p^{4,6,10,11,12,16,17,18,22,24,A,C,D}$	26	0.493 ± 0.087	$p^{6,12,18,22,24,A,B,D}$	26	0.445 ± 0.034	$p^{5,6,11,12,16,17,18,24,A,B,C}$

Note: p^{n} means the indicator is authentically different from the variant n (*t*-test).

character of pseudomonades and acetamide is included into selective media for identifying and controlling purity of *P. aeruginosa* culture in clinical practice as well as in environmental studies in the USA (APHA (1995) Standard Methods for the Examination of Water and Wastewater, Washington, DC). There are other bacteria types among amidase producers including those colonizing the human body such as *E. coli*, *K. pneumoniae*, *S. aureus*, *Enterococcus faecalis* and *Helicobacter pylori* [23–25]. However, substrate specificity of their amidases and a possibility to enter a reaction with acrylamide and PAM has not been confirmed since in most cases these bacteria produce only enzymes able to hydrolyze the amide bond between the residue of N-Acetylmuramic acid in the glycan chain and L-alanine in the peptide segment of peptidoglycan of the cell wall, for example, N-acetylmuramoyl-L-alanine amidase [24]. Though some of them are constitutive, synthesis of *P. aeruginosa* amidase is induced by amides through a regulatory protein coded by *amiR* whereas *amiC* negatively regulates expression of this enzyme [26]. The cellular mechanism of amidase induction by polyacrylamides is not clear since the polymer is too large to be able to penetrate bacterial cells and act as a direct inducer. We can assume that the more intensive growth in test variants with PAM and additional sources of carbon / nitrogen are associated with carbon / nitrogen being released from the side-chain amide (cation PAM) and carboxyl groups (anion PAM). It is interesting that PAM were offered by H.H. Tuson with colleagues (2012) as an alternative to agar-agar in studies that address bacterial growth on solid nutrient media. Thus, most gram-negative (*E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella enterica* serovar Typhimurium and *Serratia marcescens* ATCC) and gram-positive (*B. subtilis*, *S. epidermidis*) bacteria strains grew on PAM-gels so-polymerized with acrylic acid but absorbance of the cultures (OD_{595}) did not exceed 0.3 a.u. [27]. So, the experiment data indicated that clinically significant bacteria did not use PAM as a sole nutrition source and their growth was also rather slow in a medium with other substrates added to it. All this made it possible to move on to the next stage in our research, namely, to assessing antimicrobial effects produced by water solutions of PAMs with Zn^{2+} and Cu^{2+} cations.

Assessment of antimicrobial effects produced by investigeted composition by disk diffusion test and gel diffusion test. We established antibacterial effects produced on the analyzed test-cultures by 5%-solutions of Cu and Zn sulfates combined with water solutions of PAM Praestol 857 and PAM Praestol 2530. As expected, polyacrylamide solutions did not have any influence on bacteria whereas solutions of Cu and Zn sulfate inhibited growth of all the analyzed strains. More apparent effects were identified for 5%-solution of $ZnSO_4$ (*t*-test; $p = 0.019$) (Figures 2 and 3).

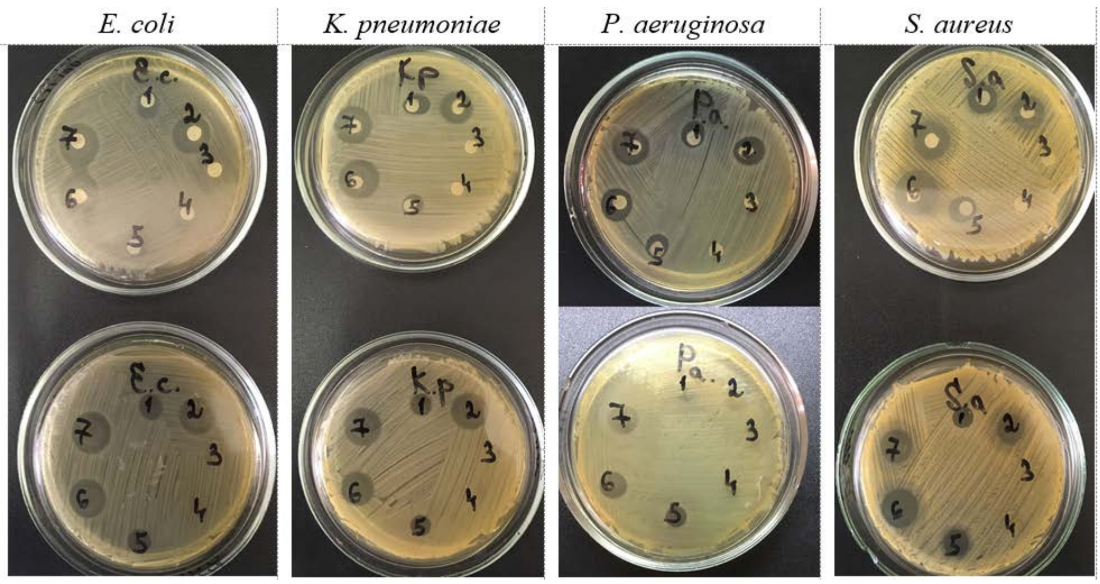

Figure 2. Dishes with the results obtained by a test aimed at identifying antibacterial effects produced by the analyzed solution with disk diffusion test (top) and with droplet deposition (bottom): 1 is 5%-solution of $CuSO_4$; 2 is 5%-solution of $ZnSO_4$; 3 is 0.075%-PAM Praestol 857; 4 is 0.075%-PAM Praestol 2530; 5 is $CuSO_4$ + PAM Praestol 857; 6 is $ZnSO_4$ + PAM Praestol 857; 7 is $ZnSO_4$ + PAM Praestol 2530

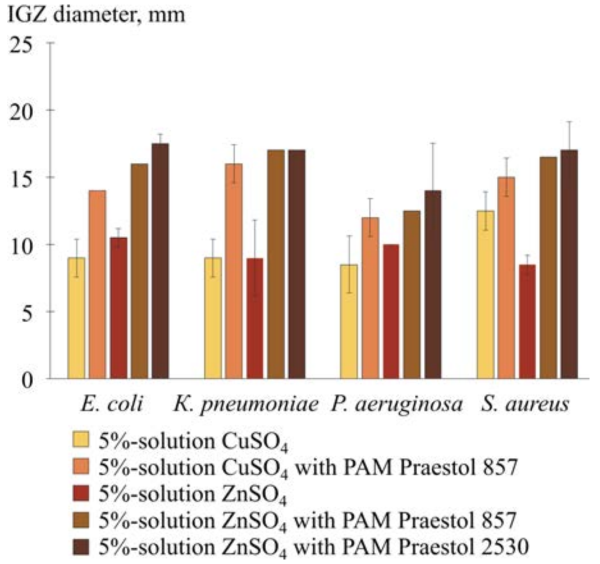

Figure 3. Antimicrobial effects produced by solutions of Cu and Zn salts combined with PAM and without them (IGZ is a diameter of zones with inhibited growth, droplet test)

It is noteworthy that adding polymers to $ZnSO_4$ solution led to an authentic increase in a zone with inhibited bacterial growth (*t*-test; p = 0.030 and p = 0.025 for PAM Praestol 857 and PAM Praestol 2530 accordingly). Obviously, an increase in a zone with inhibited bacterial growth in the test variants 'inorganic salt + PAM' is associated with a wider area on agar surface being covered with a water composition PAM and cations for both types of deposition. We should note that an antibacterial effect produced by $ZnSO_4$ combined with PAM Praestol 2530 was a bit more apparent than for a combination with PAM Praestol 857 although the difference was not statistically significant.

Assessment of antimicrobial effects in a liquid medium. The next task was to estimate influence exerted by polymers on antibacterial effects of salts that were established to become more intensive in an agar medium. To do that, we identified MICs and MBCs of inorganic salt solutions and four our test-compositions in a liquid nutrient medium (Table 3). As expected, salts of Zn and Cu inhibited growth and viability of all the analyzed microorganisms; in most cases, both MIC and MBC of zinc sulfate were by 1–2 dilutions lower than those of copper sulfate. MICs of metal salts did not exceed 0.16 % in all the test variants. Bacteria viability was mostly inhibited under a concentration of salts being per 1 or 2 dilutions (for *P. aeruginosa*) higher than MIC. We should also highlight apparent bactericidal effects (MBC/MIC = 1) of copper sulfate with respect to *E. coli* and *K. pneumoniae.* PAM addition to the analyzed medium mostly did not have any influence on antibacterial effects produced by salts. We detected a growth in MIC and MBC for some cultures but only in the test variant with added $ZnSO_4$. As for a relationship between antibacterial effects and a type of a polymer under these conditions, MIC and MBC were higher when PAM Praestol 2530 was added to the analyzed medium. In some cases, induction of antibacterial effects was identified for copper salts.

Table 3

MICs and MBCs of $CuSO_4$ and $ZnSO_4$ solutions and their compositions with PAM as regards the analyzed strains

A test variant	Strain							
	E. coli		*K. pneumoniae*		*P. aeruginosa*		*S. aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
$CuSO_4$	0.08	0.16	0.16	0.16	0.16	0.31	0.08	0.16
$ZnSO_4$	0.02	0.04	0.04	0.08	0.08	0.31	0.02	0.08
$CuSO_4$, PAM* Praestol 857	0.08	0.16	0.08	0.16	0.16	0.63	0.08	0.16
$CuSO_4$, PAM Praestol 2530	0.08	0.16	0.08	0.16	0.16	0.63	0.08	0.16
$ZnSO_4$, PAM Praestol 857	0.02	0.08	0.08	0.16	0.08	0.31	0.02	0.04
$ZnSO_4$, PAM Praestol 2530	0.04	0.16	0.04	0.16	0.08	0.31	0.04	0.31

Note: PAM were added in all test variants in a concentration equal to 0.075 %. The Table provides the results of four experiments with similar indicators out of total six.

Microorganisms are known to be sensitive to effects produced by salts of heavy metals. Therefore, the latter are commonly used to treat some communicable human and animal diseases. Inhibition of bacterial growth with metal ions is associated with various metabolic processes in prokaryotic cells including impaired protein functions, production of reactive oxygen species and antioxidant depletion as well as damage to membranes and genotoxicity [28]. Toxicity of different inorganic salts has different mechanisms and hazard levels for representatives of different taxons. Thus, A. Singh with colleagues (2015) showed *E. coli*, *S. aureus* and *K. pneumoniae* to be highly sensitive to zinc salts; the first two cultures were more sensitive than *K. pneumoniae*, and in addition Zn sulfate in a concentration equal to 10 mM completely inhibited the growth of all three strains [13]. These data are in line with observations by D. Chudobova and others (2015) [29] and our research as well: $ZnSO_4$ MICs did not exceed 0.08 % for all the analyzed strains (the figure corresponds to 5 mM). Most studies in the area show that Cu salts are less deleterious for bacteria than Zn salts even when similarly tolerant strains are described in them. Thus, S.B. Cheknev with colleagues (2015) established that inhibiting effects produced by zinc sulfate on S. *aureus* was by 1.3–1.6 times stronger than effects of copper sulfate whereas these antibacterial effects produced by both sulfates were quite similar as regards *P. aeruginosa* culture [7]. Also, similar results were described for staphylococci in the studies by H. Xue with colleagues (2015) where it was reported that MICs of Zn and Cu salts differed by two times for strains isolated from animals and equaled 2 and 4 mM accordingly [30].

Many researchers share the opinion that thorough cleaning and disinfection of surfaces in the hospital environment allow considerable reduction in risks of infection; they are significant elements in effective prevention. However, traditional disinfection in in-patient hospitals is not always optimal and requires improvement including new treatment procedures that make it possible to achieve longer biocide effects [31]. A promising approach is use of film disinfectants, which are compositions of traditional antibacterial substances and a polymer providing longer persistence of a biocide on a surface. In our tests, PAM addition to a medium did not have any substantial influence on bacteriostatic or bactericidal effects produced by metal salts (some decline in them fixed in isolated cases can be due to inaccuracies when dilutions were being prepared in a dense PAM medium) and when PAM were deposited on an agar surface, it led to an authentic increase in a zone with inhibited bacterial growth. Bearing these two findings in mind, we can conclude that the outlined strategy seems promising.

Conclusion. Providing up-to-date and effective sanitary and anti-epidemiological measures in healthcare organizations is an extremely vital task in health protection. A true challenge in the sphere is to develop and test new disinfectants with prolonged effects produced on infectious agents. No wonder that this research trend, namely Medicine and Technologies of Live Systems, Creation of New Drugs, Biomedical Technologies for Life Support and Human Health Protection, is mentioned in the Order by the Head of the Perm Regional Administration issued on November 01, 2010 No. 83 ‘On major trends in the research and technical policy in the Perm region’.

We detected antibacterial effects produced by water solutions of PAMs combined with Cu and Zn sulfates as regards reference strains of the most common infectious agents causing healthcare-associated infections. All the bacterial cultures did not use PAM as a sole nutrition source when growing in a liquid mineral medium and on PAM-films created on glass and plastic. PAM and products of their biotransformation are highly toxic and this might be a reason for the situation outlined above; also, another reason might be that the analyzed clinically significant bacterial strains do not have any mechanism for utilization of high-molecular substances. In most cases, the analyzed cultures could grow on these substrates only if glucose was added to them. We established that 5%-solution of $ZnSO_4$ had more apparent inhibitory effects on microorganisms cultivated on solid and liquid nutrient media. Adding PAM Praestol 857 and PAM Praestol 2530 to solutions of investigated inorganic salts led to an authentic increase in a diameter of a zone with inhibited bacterial growth on an agar medium. This effect might be due to a greater area of a surface being covered with water solutions of PAMs combined with Cu and Zn sulfates; therefore, the analyzed compositions seem quite promising as regards their use for disinfection. In a liquid medium, salts of both metals inhibited growth and viability of all the analyzed microorganisms already in a concentration equal to 0.16 % or lower. Adding PAM Praestol 2530 to a medium to a certain extent weakened antibacterial effects produced by inorganic salts whereas PAM Praestol 857 had practically no influence on their bacteriostatic or bactericidal effects.

Therefore, use of the obtained composite solutions where $CuSO_4$ or $ZnSO_4$, immobilized on a PAM matrix act as an antibacterial component seems a promising way to disinfect objects in the hospital environment. This can significantly reduce risks of hospital-acquired infections. Further research will focus on investigating effectiveness of these created disinfecting compositions as regards clinically significant bacteria types. Various abiotic surfaces will be used in future studies to imitate similar surfaces of medical equipment, furniture etc. in order to identify whether these disinfecting compositions can reduce risks of healthcare-associated infections.

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Competing interests. The authors declare no competing interests.

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Research article

THE RISK OF DEVELOPING SEVERE CLINICAL FORMS OF COVID-19 IN HEALTHCARE WORKERS IN THE INITIAL PERIOD OF THE PANDEMIC: NON-OCCUPATIONAL FACTORS AND LABORATORY PROGNOSTIC INDICATORS

T.A. Platonova[1,2], A.A. Golubkova[3,4], M.S. Sklyar[1,5], E.A. Karbovnichaya[1], S.S. Smirnova[2,6], K.V. Varchenko[7], A.A. Ivanova[7], A.B. Komissarov[7], D.A. Lioznov[7,8]

[1]European Medical Center 'UMMC-Health', 113 Sheinkmana Str., Ekaterinburg, 620144, Russian Federation
[2]Ural State Medical University, 3 Repina Str., Ekaterinburg, 620028, Russian Federation
[3]Central Research Institute of Epidemiology, 3A Novogireevskaya Str., Moscow, 111123, Russian Federation
[4]Russian Medical Academy for Continuous Professional Education, 2/1 Barrikadnaya Str., bldg 1, Moscow, 125993, Russian Federation
[5]Ural Mining and Metallurgical Company, 1 Uspenskii Ave., Verkhnyaya Pyshma, 624091, Russian Federation
[6]Federal Scientific Research Institute of Viral Infections "Virome", 23 Letnyaya Str., Ekaterinburg, 620030, Russian Federation
[7]Smorodintsev Research Institute of Influenza, 15/17 Prof. Popova Str., Saint Petersburg, 197376, Russian Federation
[8]Pavlov First Saint Petersburg State Medical University, 6-8 L'va Tolstogo Str., Saint Petersburg, 197022, Russian Federation

Under the COVID-19 pandemic, healthcare workers were at the highest risk of getting infected with the disease; this necessitates specialized studies in this occupational group.

The aim of the study was to identify non-occupational risk factors and laboratory markers indicating that severe clinical forms of new coronavirus infection would probably develop in healthcare workers in the initial period of the pandemic.

The study included 366 workers who suffered COVID-19 in 2020–2021. The disease was confirmed by examining smears from the pharynx and nose with PCR. Some of the samples were examined using the SARS-CoV-2 whole genome sequencing technology. To determine laboratory prognostic indicators evidencing the development of more severe forms of the disease (pneumonia), a number of healthcare workers underwent laboratory examination during the acute period of the disease, namely: general clinical and biochemical blood tests, immunophenotyping of lymphocytes, analysis of the hemostasis system and cytokine levels. To study non-occupational risk factors of pneumonia, all healthcare workers after recovery were asked to fill in a Google form developed by the authors.

Tatyana A. Platonova – Candidate of Medical Sciences, Head of the Epidemiological Department-epidemiologist; Assistant at the Department of Epidemiology, Social Hygiene and Organization of Sanitary-Epidemiological Service (e-mail: fill.1990@inbox.ru; tel.: +7 (343) 344-27-67 (ext. 1894); ORCID: https://orcid.org/0000-0001-5441-854X).

Alla A. Golubkova – Doctor of Medical Sciences, Professor, Leading Researcher at the Laboratory for Healthcare-Associated Infections; Professor of the Department of Epidemiology (e-mail: allagolubkova@yandex.ru; tel.: +7 (912) 617-39-85; ORCID: https://orcid.org/0000-0003-4812-2165).

Mikhail S. Sklyar – Doctor of Medical Sciences, general director; advisor to the general director (e-mail: info@ugmk-clinic.ru; tel.: +7 (343) 344-27-67 (ext. 1000); ORCID: https://orcid.org/0000-0003-1692-522X).

Elena A. Karbovnichaya – Head of the Clinical and Diagnostic Laboratory (e-mail: KarbovnichaiaEA@ugmk-clinic.ru; tel.: +7 (343) 344-27-67 (ext. 1940); ORCID: https://orcid.org/0000-0001-6236-4916).

Svetlana S. Smirnova – Candidate of Medical Sciences, Associate Professor of the Department of Epidemiology, Social Hygiene and Organization of Sanitary-Epidemiological Service; Leading Researcher, Head of Ural-Siberian scientific-methodological center for the prevention of healthcare-associated infections (e-mail: smirnova_ss69@mail.ru; tel.: +7 (343) 261-99-47 (ext. 106); ORCID: https://orcid.org/0000-0002-9749-4611).

Kirill V. Varchenko – Leading Programmer at the Department of Etiology and Epidemiology (e-mail: kirill.varchenko@gmail.com; tel.: +7 (911) 176-25-75; ORCID: https://orcid.org/0000-0002-3273-0660).

Anna A. Ivanova – Junior Researcher at the Laboratory of Molecular Virology (e-mail: anna.e.svobodniy@gmail.com; tel.: +7 (911) 758-44-84; ORCID: https://orcid.org/0000-0002-3495-4393).

Andrey B. Komissarov – Head of the Laboratory of Molecular Virology (e-mail: a.b.komissarov@gmail.com; tel.: +7 (921) 927-37-29; ORCID: https://orcid.org/0000-0003-1733-1255).

Dmitry A. Lioznov – Doctor of Medical Sciences, director; Head of the Department for Infectious Diseases and Epidemiology (e-mail: office@influenza.spb.ru; tel.: +7 (812) 499-15-00; ORCID: https://orcid.org/0000-0003-3643-7354).

The most severe clinical forms of COVID-19 were registered in healthcare workers who were older than 40 years, with low physical activity and a body mass index higher than 25.0, had diabetes mellitus and chronic diseases of the genitourinary system.

When analyzing the results of laboratory tests, markers indicating development of pneumonia were identified and their critical values (cut-off points) were determined: the level of lymphocytes (below 1.955·109/l), T-cytotoxic lymphocytes (below 0.455·109/l), T-helpers (below 0.855·109/L), natural killers (below 0.205·109/l), platelets (below 239·109/L), erythrocyte sedimentation rate (above 11.5 mm/h), D-dimer (above 0.325 mcg/ml), total protein (below 71.55 g/L), lactate dehydrogenase (above 196 U/L), C-reactive protein (above 4.17 mg/l), and interleukin-6 (above 3.63 pg/l).

The study identified non-occupational risk factors causing development of severe COVID-19 and established laboratory prognostic indicators.

Keywords: *coronavirus infection, COVID-19, healthcare workers, clinical manifestations, non-occupational risk factors, laboratory markers, prognostic indicators of severe clinical forms.*

The new coronavirus infection (COVID-19) was first detected at the end of 2019 in Wuhan, the People's Republic of China. Over the next few months, it spread rapidly all over the world bringing about significant social and economic losses everywhere it occurred. According to the official statistical data, on January 01, 2023 there were more than 650 million COVID-19 cases registered all over the world and more than 6.5 million deaths caused by the disease[1] [1, 2].

Since the pandemic started, healthcare experts worldwide have been actively investigating the new infection, its epidemiological, clinical and immune-pathological features; they have been developing and implementing new drugs to effectively prevent and treat it [3–8]. This research is especially significant when it comes down to people from occupational groups with higher risks of SARS-CoV-2, healthcare workers included. The latter were the first to face this new disease and became a population group that has suffered the greatest damage. The COVID-19 incidence among healthcare workers has been substantially higher against any other occupational group during all the periods in the pandemic [9–11].

Some studies showed that healthcare workers had several leading risk factors of getting infected with the new coronavirus infection due to their occupational activity. These factors included contacts with infected patients, how close and how long these contacts were, necessity to work with infected biomaterials, insufficient provision with personal protective equipment (PPE) and PPE defects, absence of qualitative instructions prior to work with patients infected with SARS-CoV-2 etc. It is noteworthy that clinical forms with lung involvements were just as frequent in this occupational group as in population in general [11–13].

We should bear in mind that healthcare workers have a specific sex, age, somatic and behavioral 'profile' just as employees of any other organization. Therefore, it is necessary to examine not only occupational risk factors of getting infected but also non-occupational ones in order to identify those able to cause infection and severe clinical course of the disease in future.

Without any doubt, priority tasks the public healthcare has to tackle nowadays are to provide safety for healthcare workers, to develop the most effective prevention programs and new treatment and rehabilitation protocols for them.

Given all the aforementioned, today there is a need in studies that address both clinical COVID-19 symptoms in healthcare workers and non-occupational risk factors of severe clinical forms of the disease as well as studies aimed at identifying laboratory indicators that can be used in clinical practice as markers of the infection clinical course and its outcome.

In this study, our aim was to identify non-occupational risk factors and laboratory markers indicating that severe clinical forms of new coronavirus infection would probably develop in healthcare workers in the initial period of the pandemic.

[1] Statistika koronavirusa v mire [World coronavirus statistics]. *GOGOV*. Available at: https://gogov.ru/covid-19/world (January 01, 2023) (in Russian).

Materials and methods. The study was accomplished in 2020–2021 during the first and second epidemic rises in the COVID-19 incidence in the Russian Federation. The study design was approved by the Local Committee on Ethics of the European medical center 'UMMC-Health' (The Meeting Report No. 1e dated June 02, 2020). Participation in the study was voluntary and each participating healthcare worker gave a written informed consent to it.

The study included 366 healthcare workers who lived in the Sverdlovsk region and was diagnosed with COVID-19. Among the participants, there were 110 doctors (30.0 %), 93 nurses and 28 hospital attendants (25.4 and 7.7 %, accordingly), 40 administrative and managerial staff (10.9 %), as well as 95 utility workers and technicians (25.9 %). The participants' age varied between 18 and 70 years (the median age was 38 years). Most respondents were women (305 or 83.3 %).

Eighty-five healthcare workers (23.2 %) who had the diseases as pneumonia were included into the test group; the remaining 281 (76.8 %) who had it as an acute respiratory infection (ARI) were included into the reference group. There were no deaths among the participants. The COVID-19 was diagnosed in accordance with the Temporary Methodical Guidelines on Prevention, Diagnostics and Treatment of the New Coronavirus Infection (COVID-19) (Versions 6–8).

To confirm the diagnosis and to later estimate how long the SARS-CoV-2 virus persisted in the body, each healthcare worker had several PCR-tests with a 3–5 day interval between them to detect the virus RNA in smears from the pharynx and nose (2356 samples were tested overall). The tests were performed in the PCR laboratory of the European medical center 'UMMC-Health' using test-systems produced by Saint Petersburg Pasteur Institute, MEDIPALTECH LLC, DNK-Tekhnologia TS LLC and Vector-Best JSC. We analyzed a correlation between the threshold cycle value (Ct) that described a viral load and the disease severity as well as a period in the disease progression. Samples with their Ct value being lower than 30 were sent to Smorodintsev Research Institute of Influenza of the RF Public Healthcare Ministry (the laboratory for molecular virology) where the whole genome sequencing of SARS-CoV-2 was performed (58 samples). These tests were performed using the new-generation genome sequencing (NGS) with Illumina MiSeq device and ARTIC Network modified sequencing protocol. The obtained sequences were aligned with MAFFT v7.453 and deposited in an international (EpiCov GISAID[2]) and Russian (VGARus[3]) virus genome aggregator.

In an acute period in the disease, several healthcare workers underwent additional laboratory tests, 168 people overall including 67 with pneumonia (the test group) and 119 with acute respiratory infection (the reference group). The laboratory tests included total blood count, immune phenotyping of leucocyte sub-populations with flow cytometry (T-lymphocytes including T-helpers and cytotoxic T-lymphocytes, CD-index, B-lymphocytes, NK-cells and TNK-cells), some chemical indicators (amylase, alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinine kinase (CK), glucose, total protein, creatinine, cholesterol, total bilirubin, urea, C-reactive protein (CRP)), homeostasis indicators (D-dimer) and some cytokines (interferons IFN-α, IFN-β, IFN-γ, C9-components in the complement system, TNF-α, interleukin IL-1β, IL-6, IL-10) identified with ELISA. All the healthcare workers had several points of laboratory control (from one to four). The total number of tests equaled 304 laboratory units (205 in the test group and 99 in the reference one) for total blood count and biochemical blood test; 286 units (195 in the test and 91 in the reference group) for immune phenotyping of lymphocytes; 101 units (49 in the test and 52 in the

[2] GISAID: database. Available at: https://www.gisaid.org (December 01, 2022).

[3] VGARus (Virus Genome Aggregator of Russia): the Russian platform for aggregating information about virus genomes. Available at: https://genome.crie.ru/app/index (December 19, 2022).

reference group) to estimate D-dimer levels; 288 units (190 in the test and 98 in the reference group) to estimate IL-6 levels in the cytokine profile and 84 units (43 in the test and 41 in the reference group) to estimate other indicators in it (IL-1β, IL-10, TNF-α, IFN-α, IFN-β, IFN-γ). Units of measurement and reference values of the analyzed indicators are provided in Table 2. Laboratory tests were accomplished in the clinical and diagnostic laboratory of the European medical center "UMMC-Health" using Sysmex XN 1000 and Roller 20 PN / ALIFAX hematology analyzers, Beckman Coulter AU680 clinical chemistry analyzer, STA Compact Max homeostasis analyzer (DIAGNOSTICA STAGO S.A.S., France), BD FACSCanto II flow cytometer; all the tests relied on using original reagents provided by the manufacturers of the equipment. Cytokines were analyzed with Cobas e411 (Roche, Switzerland) with original reagents Elecsys IL6, Thermo Scientific Well-Wash automated microplate washer, IEMS incubator / shaker, Multiskan Ascent microplate reader and the following reagents: Human Complement C9 ELISA Kit, VeriKine Human IFN Beta ELISA Kit, Human IFN gamma ELISA Kit, Human IFNα ELISA Kit, Human TNFα ELISA Kit, Human IL-1β ELISA Kit, Human IL-10 ELISA Kit.

To examine clinical symptoms and identify non-occupational risk factors of severe COVID-19, all the participants were offered to fill in a Goggle form developed by the authors to clarify some clinical data and medical records. This Google form was made of 66 questions combined into several information blocks, namely personal details, potential risk factors (anthropometric parameters, blood group and Rh factor, intake of antiviral drugs, smoking, alcohol intake, physical activity, doing sports, chronic diseases, vaccination against several communicable diseases), clinical COVID-19 symptoms, therapy-related issues, consequences of the disease and rehabilitation. When analyzing vaccination records (hepatitis B, diphtheria, tetanus, measles, rubella, flu, and pneumococcal infection), we additionally took data from the participants' vaccination certificates.

The study involved using epidemiological, clinical, immunological, molecular-genetic and statistical methods. We estimated how data were distributed based on Shapiro – Wilk test and Kolmogorov – Smirnov test. Quantitative data were given by median (*Me*), the first and third quartiles (Q1–Q3), minimum and maximum values (Min–Max); categorical data were given by a share and frequency in percent (%). When comparing quantitative indicators, we estimated statistical significance of differences with Mann – Whitney test; categorical indicators were compared with chi-square test (χ^2). Correlations between indicators were analyzed as per Spearman's coefficient and estimated as per the Chaddock scale. Differences were considered statistically significant at $p \leq 0.05$. Probability of an outcome depending on impacts of various risk factors was estimated by drawing up a fourfold contingency table and calculating odds ratio (*OR*) with 95 % confidence interval (95 % CI). We created ROC-curves to identify laboratory markers of severe COVID-19 forms and their threshold values. Only prognostic models with statistical significance ($p < 0.05$) as well as those with specificity and sensitivity higher than 50 % were considered in the study. All the data were statistically analyzed with Microsoft Office 2016 and IBM SPSS Statistics, Version 26.

Results. The respondents most often mentioned the following clinical COVID-19 symptoms typical for any ARI: running nose (211 or 57.7 %); cough (189 or 50.3 %), which was non-productive (dry) in most cases (up to 85 %), as well as sore throat (133 or 36.3 %); 'tightness' and pain in the chest (101 or 27.6 %); shortness of breath (80 or 21.9 %). Among general infection symptoms, many healthcare workers mentioned apparent weakness and elevated fatigability (289 or 79.0 %), muscle and joint pains (188 or 51.3 %) and fever (279 or 76.2 %) which did not exceed 37.5 °C in 47.7 % of the cases, varied between 37.5–38.5 °C in 33.7 % and was higher than 38.5 °C in 18.6 % of the cases. Some healthcare workers had neurological symptoms including anosmia (265 or 72.4 %), headache (210 or 57.4 %) and not so frequent dizziness (83 or 22.7 %) and eyeball pains (97 or 26.5 %). In other cases, gastrointestinal tract was involved since the participants had some dyspeptic symptoms including qualms or retching (37 or 10.1 %), diarrhea (66 or 18.0 %), changes or losses of taste (176 or 48.0 %). In some rare cases, the participants mentioned some skin symptoms including skin rush with various morphological elements, loss of coordination, excessive sweating, heart rhythm disorders, acute sense of smell, metallic taste in the mouth, cramps in lower extremities and sleeping disorders. In singleton cases, the disease involved panic attacks, elevated anxiety and irritability or apathy.

Clinical symptoms of the coronavirus infection could persist for 1–28 days in the infected healthcare workers (*Me* = 10 days). Together with investigating clinical symptoms of the disease, this study involved estimating a viral load by analyzing the threshold cycle value in PCR in different periods of the disease and in different clinical forms as well as duration of the SARS-CoV-2 virus persistence in patients' bodies. We established that the Ct value did not have any statistically significant differences in workers with different clinical forms of COVID-19: the median Ct value equaled 24.8 in the participants who had COVID-19 as ARI and 26.6 in those who had pneumonia (p = 0.136). It was noted that an increase in the threshold cycle value (a decline in a viral load) occurred simultaneously with progression of the disease; Spearman's correlation coefficient between the Ct value and a day in the disease equaled 0.410 (the direct moderate correlation according to the Chaddock scale), $p < 0.001$.

After major COVID-19 symptoms disappeared, most healthcare workers still had the virus RNA in their smears from the pharynx and nose detected by PCR-tests. This indicated they were still 'epidemiologically dangerous' as potential sources of the virus and they could not be allowed to return to their workplaces since they could still spread the infection. Bearing in mind that the SARS-CoV-2 virus persisted in the infected healthcare workers for a long time, their absence from a workplace varied between 13 and 45 days (the median absence equaled 22 days). Different periods during which the virus was excreted into the environment were established for different clinical forms of the disease. Thus, when COVID-19 progressed as ARI, this period varied between 13 and 34 days (*Me* = 21 days); when the lungs were involved, between 14 and 45 days (*Me* = 24 days). Whole-genome sequencing of the SARS-CoV-2 virus from the infected healthcare workers established B.1.1 to be the prevailing strain in the first and second epidemic rises in the incidence (up to 50 % of the analyzed samples); such genetic variants as B.1, B.1.1.397, B.1.1.317, B.1.1.387, B.1.1.409, B.1.1.141, B.1.1.274 were identified in most remaining cases and several others were identified in singleton cases.

Next, we analyzed non-occupational risk factors that could cause severe forms of the coronavirus infection in the examined healthcare workers (Table 1). We established a statistically significantly higher risk of the severe disease for healthcare workers older than 40 years, with BMI indicating overweight (higher than 25.0) and with low physical activity due to absence of any regular training. Sex, blood group, Rh-factor, smoking and alcohol intake, intake of various antiviral drugs and vitamins were not identified as potential risk factors of pneumonia for the examined healthcare workers. Among chronic diseases, genitourinary pathology and diabetes mellitus had certain influence on a probability that the disease would progress in its severe form with the lung involvement. Our analysis of vaccination records did not establish any effects produced by previous vaccinations against hepatitis B, diphtheria, tetanus, measles, rubella, flu, and pneumococcal infection on a risk of pneumonia in patients infected with SARS-CoV-2.

The next stage in the study involved laboratory tests; the examined healthcare workers gave their consent to them. The test results are given in Table 2.

Table 1

Risk factors causing COVID-19 pneumonia in healthcare workers

No.	Risk factor	COVID-19 clinical form				*OR*	95 % CI	χ^2	*p*
		Pneumonia (test group)		ARI (reference group)					
		abs	%	abs	%				
1	2	3	4	5	6	7	8	9	10
1	**Age, years**								
1.1	18–19	0	0.0	2	0.7	–	–	–	–
1.2	20–29	10	11.8	61	21.7	0.48	0.23–0.99	4.13	**0.043**
1.3	30–39	22	25.9	105	37.4	0.59	0.34–1.01	3.79	0.052
1.4	40–49	31	36.5	66	23.5	1.87	1.11–3.15	5.65	**0.018**
1.5	50–59	16	18.8	35	12.4	1.63	0.85–3.12	2.21	0.138
1.6	Older than 60 years	6	7.0	12	4.3	1.70	0.62–4.68	1.09	0.298
1.7	*Older than 40 years	53	62.4	113	40.2	2.46	1.49–4.01	12.91	**<0.001**
2	**Sex**								
2.1.	Men	17	20	43	15.3	1.38	0.74–2.58	1.05	0.306
2.2	Women	68	80	238	84.7	0.72	0.39–1.35	–	–
3	**Body mass index**								
3.1	Below 18.5	2	2.4	18	6.4	0.35	0.08–1.55	2.08	0.150
3.2	18.5–24.9	37	43.5	147	52.3	0.70	0.43–1.15	2.01	0.156
3.3	25–29.9	28	32.9	79	28.1	1.26	0.75–2.17	0.74	0.392
3.4	30–34.9	12	14.1	25	8.9	1.68	0.81–3.51	1.96	0.162
3.5	35–39.9	6	7.1	11	3.9	1.86	0.67–5.20	1.46	0.228
3.6	Above 40	0	0.0	1	0.4	–	–	0.30	0.582
3.7	*Above 25	116	41.3	46	54.1	1.678	1.030–2.734	4.36	**0.037**
4	**Blood group**								
4.1	0	28	32.9	95	33.8	0.96	0.57–1.61	0.02	0.883
4.2	A	28	32.9	109	38.8	0.78	0.46–1.29	0.95	0.329
4.3	B	20	23.5	52	18.5	1.36	0.76–2.43	1.04	0.308
4.4	AB	9	10.6	25	8.9	1.21	0.54–2.71	0.22	0.638
5	**Rh-factor**								
5.1	Rh+	72	84.7	236	84.0	1.06	0.54–2.07	0.03	0.874
5.2	Rh-	13	15.3	45	16.0	0.95	0.48–1.85		
6	**Blood group and Rh-factor**								
6.1	0, Rh+	24	28.2	80	28.5	0.99	0.58–1.69	0.002	0.967
6.2	0, Rh-	4	4.7	15	5.3	0.88	0.28–2.71	0.05	0.818
6.3	A, Rh+	25	29.4	91	32.4	0.87	0.51–1.48	0.27	0.606
6.4	A, Rh-	3	3.5	18	6.4	0.65	0.18–2.29	0.99	0.318
6.5	B, Rh+	16	18.8	44	15.7	1.25	0.66–2.35	0.48	0.490
6.6	B, Rh-	4	4.7	8	2.8	1.69	0.49–5.74	0.71	0.400
6.7	AB, Rh+	7	8.2	21	7.5	1.11	0.46–2.71	0.05	0.817
6.8	AB, Rh-	2	2.4	4	1.4	1.67	0.30–9.27	0.35	0.555
7	**Intake of various antiviral (prevention) drugs**								
7.1	No regular intake of polyvitamins	54	63.5	183	65.1	0.22	0.13–0.36	0.07	0.788
7.2	No regular intake of vitamin C	77	90.6	255	90.7	0.98	0.43–2.26	0.002	0.965
7.3	No regular intake of vitamin D	72	84.7	228	81.1	1.29	0.66–2.49	0.56	0.454
7.4	No regular intake of zinc	82	96.5	267	95.0	1.43	0.40–5.11	0.31	0.578
8	**Bad habits**								
8.1	Smoking	15	17.6	61	21.4	0.77	0.41–1.45	0.65	0.419
8.2	Alcohol intake	67	78.8	233	82.9	0.77	0.42–1.41	0.74	0.390

End of the Table 1

1	2	3	4	5	6	7	8	9	10
9	**Doing sports and overall physical activity**								
9.1	No sports or training	58	68.2	153	54.5	1.79	1.08–3.01	5.08	**0.025**
9.2	Insufficient physical activity (less than 5000 steps a day)	23	32.9	53	72.6	1.56	0.87–2.81	2.27	0.132
10	**Chronic diseases**								
10.1	Cardiovascular pathology	14	16.5	35	12.5	1.39	0.71–2.72	0.91	0.341
10.2	Bronchopulmonary pathology	7	8.2	17	6.0	1.39	0.56–3.48	0.51	0.476
10.3	Diseases of the nervous system	7	8.2	14	5.0	1.71	0.67–4.39	1.28	0.259
10.4	Gastrointestinal pathology	24	28.2	64	22.8	1.33	0.77–2.31	1.07	0.303
10.5	Genitourinary pathology	14	16.5	16	5.7	3.27	1.52–7.01	10.07	**0.002**
10.6	Diabetes mellitus	5	5.9	1	0.4	17.5	2.02–151.96	12.36	**<0.001**
10.7	Oncological diseases	1	1.2	3	1.1	1.10	0.11–10.75	0.01	0.933
10.8	Autoimmune diseases	2	2.4	7	2.5	0.94	0.19–4.63	0.01	0.943
10.9	Allergic diseases	7	8.2	34	12.1	0.65	0.29–1.53	0.98	0.323
10.10	Herpesviral infection	7	8.2	20	7.1	1.17	0.48–2.82	0.12	0.730
10.11	Pregnancy	0	0.0	6	2.1	–	–	1.845	0.175
11	**Vaccinations against**								
11.1	Viral hepatitis B	85	100.0	279	99.3	–	–	0.61	0.436
11.2	Diphtheria	85	100.0	279	99.3	–	–	0.61	0.436
11.3	Tetanus	85	100.0	279	99.3	–	–	0.61	0.436
11.4	Measles	85	100.0	280	99.6	–	–	0.30	0.582
11.5	Rubella	63	74.1	230	81.6	0.64	0.36–1.13	2.44	0.118
11.6	Pneumococcal infection	7	8.2	45	16.0	0.47	0.20–1.09	3.24	0.072
12	**Flu vaccination**								
12.1	Regular	40	47.1	142	50.5	0.87	0.54–1.41	0.32	0.575
12.2	Periodical	34	40.0	95	33.8	1.31	0.79–2.15	1.09	0.296
12.3	No vaccination	11	12.9	44	15.7	0.80	0.39–1.63	0.377	0.540

Table 2

The results of laboratory tests obtained for healthcare workers with different clinical forms of COVID-19

№	Indicator	Units of measurement	Reference levels	COVID-19 clinical form						*p*
				Pneumonia (test group)			ARI (reference group)			
				Me	Q1–Q3	Min–Max	*Me*	Q1–Q3	Min–Max	
1	2	3	4	5	6	7	8	9	10	11
1.	**Total blood count**									
1.1	Leucocytes	10^9/l	4.5–10.2	5.81	4.63–7.26	1.84–37.73	5.42	4.56–6.81	2.66–9.62	0.104
1.2	Lymphocytes	10^9/l	1–6.5	1.49	0.99–2.15	0.15–4.61	1.97	1.58–2.37	0.68–3.94	**<0.001**
1.3	Neutrophils	10^9/l	1.8–7.7	3.23	2.27–4.73	0.54–34.07	2.74	1.85–3.65	1.03–5.44	**0.001**
1.4	Eosinophils	10^9/l	0–0.7	0.04	0.01–0.14	0.0–0.5	0.07	0.03–0.13	0.0–1.29	**0.014**
1.5	Basophils	10^9/l	0–0.2	0.02	0.01–0.03	0.0–0.53	0.03	0.02–0.04	0.01–0.3	0.099
1.6	Monocytes	10^9/l	0–0.95	0.50	0.35–0.69	0.12–1.45	0.51	0.41–0.68	0.25–1.49	0.243
1.7	Red blood cells	10^{12}/l	3.8–5.3	4.49	4.11–4.84	2.47–5.62	4.59	4.34–4.89	3.73–5.54	**0.010**
1.8	Hematocrit	%	34–47	39.5	36.5–42.4	23.5–52.2	39.5	37.0–43.3	30.8–47.9	0.332
1.9	Hemoglobin	g/l	115–155	137	126–147	74–175	137.5	128–152	96–166	0.21
1.10	Mean corpuscular hemoglobin concentration	g/l	310–370	347	338–354	279–379	348	341–354	303–372	0.497
1.11	Mean corpuscular hemoglobin contents	pg	26–34	30.6	29.7–31.4	22.6–35.1	30.3	29.1–31.1	20.8–31.0	0.055

End of the Table 2

1	2	3	4	5	6	7	8	9	10	11
1.12	Mean corpuscular volume	fl	73–101	87.7	85.5–90.2	72.6–107.1	86.7	83.7–89.4	68.6–97.3	**0.004**
1.13	Corpuscular anisocytosis	fl	37–54	40.9	39.2–44.6	34.2–71.0	40.0	38.5–42.2	33.2–47.0	**0.001**
1.14	Corpuscular anisocytosis, %	%	11.6–14.8	12.9	12.3–13.8	11.0–21.2	12.6	12.1–13.4	11.3–18.0	**0.044**
1.15	Normoblasts	10^9/l	0.03	0.0	0.0–0.003	0.0–0.3	0.0	0.0–0.0	0.0–0.01	**0.001**
1.16	Platelets	10^9/l	142–424	226	184–280	25–540	253	198–301	112–584	0.054
1.17	Mean platelet volume	fl	7–13	10.8	10.1–11.5	8.7–14.6	10.3	9.8–10.7	9.1–12.2	**<0.001**
1.18	Giant platelet count	%	13–43	30.5	25.3–36.5	15.0–56.8	27.3	22.3–30.8	16.9–42.6	**<0.001**
1.19	Platelet distribution width	fl	9–17	12.6	11.2–13.9	9.1–25.3	11.7	10.6–12.8	9.0–16.2	**<0.001**
1.20	Thrombocrit	%	0.17–0.35	0.24	0.19–0.29	0.06–0.59	0.25	0.20–0.30	0.12–0.56	0.162
1.21	ESR	mm/h	0–20	23	10–37	2–108	7	4–13	2–41	<**0.001**
2.	**CD-typing of lymphocyte sub-populations**									
2.1	T-lymphocytes	10^9/l	0.80–2.20	1.13	0.70–1.64	0.09–4.11	1.47	1.15–1.91	0.64–2.97	**<0.001**
2.2	T-helpers	10^9/l	0.60–1.60	0.69	0.41–0.97	0.01–1.93	0.92	0.71–1.12	0.36–1.98	**<0.001**
2.3	Cytotoxic T-lymphocytes	10^9/l	0.19–0.65	0.41	0.24–0.58	0.03–2.44	0.49	0.37–0.62	0.03–1.66	**<0.001**
2.4	CD-index	a.u.	1.0–2.5	1.70	1.20–2.50	0.0–4.80	1.85	1.40–2.40	0.30–4.10	0.203
2.5	NK-cells	10^9/l	0.15–0.60	0.15	0.09–0.24	0.02–1.57	0.24	0.17–0.34	0.06–0.97	**<0.001**
2.6	B-lymphocytes	10^9/l	0.10–0.50	0.16	0.11–0.25	0.0–0.83	0.19	0.13–0.26	0.07–0.53	**0.019**
2.7	TNK - cells	10^9/l	0.01–0.85	0.03	0.01–0.06	0.0–1.0	0.03	0.01–0.07	0.0–0.57	0.590
3	**Biochemical blood test**									
3.1	Alkaline phosphatase	U/l	30–120	62	51–78	25–243	63	52–76	29–174	0.942
3.2	Amylase	U/l	28–100	57	50–73	15–260	62	51–78	21–139	0.277
3.3	AST	U/l	6–36	27	21–35	13–109	21	19–27	11–66	**<0.001**
3.4	ALT	U/l	7–55	26	17–37	7–198	18	14–30	4–162	**<0.001**
3.5	CK	U/l л	0–171	81.5	51–148	11–3152	70	50–93	15–661	**0.016**
3.6	LDH	U/l	0–247	233	188–317	79–752	184.5	161–200	125–286	**<0.001**
3.7	Total protein	g/l	66–83	68.9	63.2–73.5	50.0–83.5	73.2	69.8–75.5	64.2–92.3	**<0.001**
3.8	Urea	mmol/l	2.8–7.2	5.0	3.9–7.9	2.5–69.4	4.4	3.7–5.0	2.1–8.9	**<0.001**
3.9	Total bilirubin	µmol/l	5–21	9.7	6.9–13.5	2.6–128.4	8.2	6.0–10.9	2.5–28.2	**0.002**
3.10	Cholesterol	mmol/l	1.8–5.2	4.1	3.4–4.9	1.7–9.1	4.4	3.8–5.1	2.7–7.7	0.058
3.11	Glucose	mmol/l	4.1–5.9	5.2	4.5–6.3	1.2–21.9	5.1	4.5–4.7	2.5–15.7	0.336
3.12	Creatinine	µmol/l	53–97	86	73–101	45.0–311.0	78	70–86	9.4–116.0	**<0.001**
3.13	CRP	mg/l	0–5	9.12	3.1–24.1	0.29–257.1	1.98	0.7–4.8	0.16–39.7	**0.001**
4	**Homeostasis indicators**									
4.1	D-dimer	µg/ml	0–0.5	0.40	0.27–0.59	0.10–1.80	0.29	0.21–0.37	0.06–2.0	**<0.001**
5	**Cytokine profile**									
5.1	IL-6	pg/ml	0–7	6.32	2.48–16.14	1.50–339.3	2.86	1.5–6.27	1.50–81.0	**<0.001**
5.2	IL-1β	pg/ml	0	0.0	0.0–0.0	0.0–15.0	0.0	0.0–0.0	0.0–0.49	0.721
5.3	IL-10	pg/ml	7.9–12.9	2.68	1.78–3.59	1.22–155.0	1.72	1.58–2.23	1.15–109.0	**0.018**
5.4	TNF-α	pg/ml	0	0.0	0.0–0.166	0.0–19.8	0.0	0.0–0.063	0.0–0.66	0.327
5.5	IFN-α	pg/ml	0	0.0	0.0–0.20	0.0–35.1	0.0	0.0–0.35	0.0–48.4	0.961
5.6	IFN-β	pg/ml	1.2–150	5.33	0.0–17.42	0.0–5645	2.29	0.0–19.76	0.0–7839	0.634
5.7	IFN-γ	pg/ml	0–188.9	0.0	0.0–0.0	0.0–35.6	0.0	0.0–0.0	0.0–1.0	0.052
5.8	C9-component in the complement system	µg/ml	43.7–53.9	67.1	37.8–125.0	5.86–300.0	40.9	26.6–59.4	10.24–123.4	**<0.001**

Having analyzed the laboratory test results, we established statistically significant differences in some indicators but not all of them had prognostic value. To identify prognostic laboratory markers, we performed ROC-analysis with creating ROC-curves, calculating areas under them (AUC) and identifying optimal classification thresholds (cut-off points) considering maximum sensitivity and specificity of the created models.

Creating ROC-curves for total blood count indicators revealed that erythrocyte sedimentation rate, levels of platelets and lymphocytes had prognostic value as regards predicting more severe COVID-19 forms.

The ROC-curve for ESR had the AUC equal to 0.759 ± 0.029 (95 % CI: 0.702–0.816), $p < 0.001$. ESR at the cut-off points was determined as equal to 11.5 mm/hour. The healthcare workers with ESR higher than 11.5 mm/hour were at a higher risk of pneumonia; in case ESR was below 11.5 mm/hour, this risk was low. The model sensitivity equaled 70.0 %; the model specificity, 72.4 %.

The ROC-curve built for the platelet level had the AUC = 0.566 ± 0.033 (95 % CI: 0.491–0.621), $p = 0.054$. The cut-off point for this indicator was determined as equal to $239 \cdot 10^9$/l. The healthcare workers with the platelet level lower than $239 \cdot 10^9$/l were at a higher risk of pneumonia; in case the platelet level was above $239 \cdot 10^9$/l, this risk was low. The model sensitivity equaled 55.5 %; the model specificity, 54.5 %.

The AUC for the ROC-curved built for the lymphocyte level equaled 0.671 ± 0.031 (95 % CI: 0.611–0.731), $p < 0.001$. The threshold value for the lymphocyte level was taken at $1.955 \cdot 10^9$/l. Lymphocyte levels below $1.955 \cdot 10^9$/l indicated elevated risks of pneumonia whereas levels higher than $1.955 \cdot 10^9$/l meant these risks were low. The model sensitivity and specificity equaled 67.0 % and 52.7 % accordingly.

The results obtained by immune phenotyping of lymphocytes made it possible to create statistically significant models ($p < 0.05$) with their specificity and sensitivity being higher than 50 % for relationships between a probability of COVID-19 pneumonia and levels of T-helpers, cytotoxic T-lymphocytes (CTL) and NK-cells.

The ROC-curve for T-helpers had the AUC = 0.675 ± 0.030 (95 % CI: 0.613–0.736), $p < 0.001$. The level of T-helpers at the cut-off point equaled $0.855 \cdot 10^9$/l. The healthcare workers with their level of T-helpers below $0.855 \cdot 10^9$/l were at a higher risk of pneumonia; in case the level was above $0.855 \cdot 10^9$/l, this risk was considered low. The model sensitivity equaled 64.9 %; the model specificity, 58.0 %.

The AUC of the ROC-curve for the relationship between likelihood of pneumonia and the number of cytotoxic T-lymphocytes (CTL) equaled 0.626 ± 0.033 (95 % CI: 0.561–0.690), $p < 0.001$. The threshold CTL value dividing the healthcare workers into groups with low and high likelihood of pneumonia equaled $0.455 \cdot 10^9$/l. If the CTL level was lower than $0.455 \cdot 10^9$/l, a risk of pneumonia was high; if the CTL levels exceeded $0.455 \cdot 10^9$/l, this risk was estimated as low. The model sensitivity equaled 61.1 %; the model specificity, 58.0 %

The AUC of the ROC-curve for NK-cells equaled 0.691 ± 0.031 (95 % CI: 0.630–0.752), $p < 0.001$. The threshold NK-cells level equaled $0.205 \cdot 10^9$/l. The NK-cells level below $0.205 \cdot 10^9$/l allowed estimating a risk of pneumonia as elevated and if this level was higher than $0.205 \cdot 10^9$/l, this risk was low. The model sensitivity equaled 65.4 %; the model specificity, 64.0 %.

Next, we analyzed the results of biochemical blood tests. Prognostic models with sufficient sensitivity, specificity and statistical significance were obtained for a relationship between likelihood of more severe COVID-19 forms and levels of total protein, C-reactive protein (CRP) and lactate dehydrogenase (LDH).

The AUC of the ROC-curve for total protein equaled 0.726 ± 0.029 (95 % CI: 0.618–0.784), $p < 0.001$. The level of total protein at the cut-off point was 71.55 g/l. the healthcare workers with the total protein level equal to or below 71.55 g/l were at an elevated risk of pneumonia; the total protein level higher than 71.55 g/l indicated a low risk. The model sensitivity and specificity equaled 67.6 % and 66.7 % accordingly.

The ROC-curve for C-reactive protein had the AUC = 0.774 ± 0.027 (95 % CI: 0.720–0.827), $p < 0.001$. The threshold CRP level was equal to 4.17 mg/l. The healthcare workers with the CRP level higher than 4.17 mg/l were at an elevated risk of pneumonia; in case the CRP level was lower than 4.17 mg/l, a risk of more severe COVID-19 forms was low. The model sensitivity equaled 67.7 %; the model specificity, 69.5 %.

The ROC-curve for lactate dehydrogenase had the AUC = 0.754 ± 0.029 (95 % CI: 0.697–0.810), $p < 0.001$. The LDH level at the cut-off point equaled 196 U/l. the healthcare workers with the LDH levels higher than 196 U/l had an elevated risk of pneumonia; this risk was low in case the LDH levels was below 196 U/l. The model sensitivity equaled 68.4 %; the model specificity, 67.4 %.

The analysis of the D-dimer level in the healthcare workers with different clinical forms of the coronavirus infection identified the AUC value as equal to 0.711 ± 0.051 (95 % CI: 0.611–0.811) and the model was statistically significant ($p < 0.001$). The threshold D-dimer level equaled 0.325 μg/ml. The healthcare workers with the D-dimer level higher than 0.325 μg/ml were at an elevated risk of pneumonia; in case the D-dimer levels was below 0.325 μg/ml, this risk was considered low. The model sensitivity equaled 63.3 %; the model specificity, 63.5 %.

The next step in the study involved analyzing the cytokine profile of the examined healthcare workers. Statistically significant prognostic models ($p < 0.05$) with satisfactory sensitivity and specificity were obtained only for interleukin-6. The AUC of the ROC-curve for IL-6 equaled 0.658 ± 0.032 (95 % CI: 0.595–0.722). The relationship was statistically significant ($p < 0.001$). The IL-6 level at the cut-off point was equal to 3.63 pg/l. The healthcare workers with the IL-6 level exceeding 3.63 pg/l had an elevated risk of pneumonia and if the IL-6 level was lower than 3.63 pg/l, severe forms of the infection were less likely. The model sensitivity equaled 64.6 %; the model specificity, 64.5 %.

It is worth noting that the outlined threshold levels of the examined laboratory markers are within their reference ranges and it is vital to monitor these markers in dynamics when treating patients with COVID-19. They all have significant prognostic value and in case they tend to grow or decline against their cut-off points, it is necessary to assess risks of more severe COVID-19 forms and make relevant adjustments of a selected therapy.

Discussion. In this study, clinical symptoms of the coronavirus infection were analyzed in healthcare workers as a group with high occupational risks during the first and second COVID-19 pandemic 'wave' in the Russian Federation. Both waves developed predominantly due to B.1.1 SARS-CoV-2 genetic variant. We analyzed viral loads in dynamics during the disease and established how long the virus persisted in patients. The study also involved identifying non-occupational risk factors and prognostic laboratory indicators of more severe COVID-19 forms in healthcare workers.

Among non-occupational risk factors, we highlighted an age older than 40 years, low physical activity, BMI higher than 25 and some comorbidities. In general, our study results correlate well with data reported by some other authors; still, there are some nuances.

Thus, S. Molani and others [14] analyzed data on 6906 hospitalized adults with COVID-19 who were employed by public healthcare institutions in five western states in the USA. The authors reported elevated risks of the severe disease for people older than 50 years with high body mass index and in general this is in line with our findings. Although, our study established greater likelihood of pneumonia for people from occupational risks groups who were older than 40 years.

In their study, L. Kim with colleagues [15] analyzed data on 2491 adults hospitalized with confirmed COVID-19 in a period between March 01 and May 02, 2020. They took data from the COVID-NET, a hospital-based surveillance system aimed to track COVID-19-associated hospitalization. It contains data provided by 154 emergency hospitals located in 74 counties of 13 states. The authors applied multifactorial analysis to estimate relationships between age, sex, and comorbidities and hospitalization in an intensive care unit (ICU) and in-hospital mortality. The following factors were established to be associated with hospitalization in ICU: age groups 50–64, 65–74, 75–84 and ≥ 85 years against an age group 18–39 years (adjusted risk rates (aRR) were 1.53, 1.65, 1.84 and 1.43 accordingly); male sex (aRR was 1.34); obesity (aRR was 1.31); immune suppression (aRR was 1.29) and diabetes mellitus (aRR was 1.13). Factors that made a death more probable included age of 50–64, 65–74, 75–84 and ≥ 85 years against 18–39 years (aRR was 3.11, 5.77, 7.67 and 10.98 accordingly); male sex (aRR was 1.30); immune suppression (aRR was 1.39); renal failure (aRR was 1.33); chronic bronchopulmonary diseases (aRR was 1.31); cardiovascular diseases (aRR was 1,28); neurological disorders (aRR was 1.25) and diabetes mellitus (aRR was 1.19). The data reported in this study correspond to our results as regards influence of age and certain comorbidities on a risk of more severe clinical forms of the coronavirus infection.

Another study was accomplished by J.Y. Ko with colleagues [16] using the COVID-NET database. They analyzed data on 5416 adults with the coronavirus infection and calculated adjusted rates of hospitalization frequency and their 95 % confidence intervals. Hospitalization was shown to be more frequent among people with three or more comorbidities (against their total absence) (5.0 [3.9–6.3]), severe obesity (4.4 [3.4–5.7]), chronic kidney disease (4.0 [3.0–5.2]), diabetes mellitus (3.2 [2.5–4.1]), essential hypertension (2.8 [2.3–3.4]) and bronchial asthma (1.4 [2.3–3.4]). This is interesting as regards complex analysis of simultaneous effects produced by several risk factors, which could be accomplished in future estimations of data on healthcare workers.

F. Zhou with colleagues [17] accomplished their study in Wuhan; it involved analyzing data on 191 patients, 137 of them recovered and 54 died in hospital. Multifactorial regression analysis showed elevated likelihood of severe clinical forms, including fatal ones, for elderly people (OR = 1.10, 95 % CI: 1.03–1.17; $p = 0.004$) with higher scores in the Sequential Organ Failure Assessment (SOFA) (OR = 5.65, 95 % CI: 2.61–12.23; $p < 0.001$) and the D-dimer level higher than 1 µg/l (OR = 18.42, 95 % CI: 2.64–128.55; $p = 0.003$). This corresponds to our data as regards estimating a prognostic value of the D-dimer level in infected people but attention should be paid to the fact that its threshold level is lower for people from occupational risk groups with a certain age, sex and somatic 'profile'.

The systemic review coauthored by Y.-D. Gao with colleagues [18] confirmed several risk factors that could cause COVID-19 progression to its severe and even critical stage. These factors included older age; male sex; such comorbidities as essential hypertension, diabetes mellitus, obesity, chronic pulmonary diseases, heart, liver and kidney diseases, cancer, clinical immune deficiencies, local immune deficiencies such as early ability to secret type I interferon, and pregnancy. This corresponds to our study results as per some indicators (age and chronic diseases) identified for a specific population group with high occupational risks of infection.

It should be noted that male sex was a risk factor identified in all the studies outlined above but we did not have the same finding in our research. This is probably due to the sex-related profile of our participants, which corresponds to common sex structure of healthcare workers; probably, similar research should be accomplished on wider samples made of healthcare workers.

In addition, we were not able to establish any influence of blood groups and Rh-factors on the disease prognosis. Still, the issue has been discussed actively in other literature sources. According to the systemic review by Y. Kim and others [19], many studies report that the blood group (B) can indicate higher susceptibility to the SARS-CoV-2-induced infection and the blood group (0) and negative Rh-factors can act as protectors. The authors also point out that effects produced by a blood group and Rh-factors on clinical outcomes remain unclear and probably there is no relationship between a blood group and the COVID-19 severity or mortality at the moment. Given that, the authors of the review do not recommend to use these indicators as prognostic markers when treating COVID-19 patients.

Analysis of patients' vaccination history was a significant issue in assessing risks of severe clinical forms of the disease. In our study, we did not identify any statistically significant influence of previous vaccinations against several communicable diseases on likelihood of pneumonia in the analyzed healthcare workers infected with the SARS-CoV-2 virus. This concerns vaccinations against viral hepatitis B, diphtheria, tetanus, measles, rubella, pneumococcal infection and flu. However, several studies reported that flu vaccination that was made in an epidemiological season prior to the disease reduced both risks of the infection and its more severe clinical forms. Thus, A. Conlon with colleagues [20] showed in their study that likelihood of getting infected with SARS-CoV-2 was lower among patients who were vaccinated against flu as compared with those who did not get flu vaccination (OR = 0.76, 95 % CI: 0.68–0.86; $p < 0.001$). COVID-19 patients with flu vaccination were less likely to need hospitalization (OR = 0.58, 95 % CI: 0.46–0.73; $p < 0.001$) or artificial ventilation (OR = 0.45, 95 % CI: 0.27–0.78; p = 0.004) and had to spend considerably less time in hospital (OR = 0.76, 95 % CI: 0.65–0.89; $p < 0.001$).

The study by M. Candelli with colleagues [21] established a lower risk of death over 60 days after getting infected with the coronavirus for patients who had previously been vaccinated against flu against those without flu vaccination (p = 0.001). The authors believe flu vaccination can possibly reduce the COVID-19 mortality.

We have come across some articles that estimated a relationship between flu vaccination and the COVID-19 incidence among healthcare workers [22, 23]. The first study was accomplished by N. Massoudi and others [22] in Iran in 2020 and analyzed data on 261 healthcare workers. The authors showed that flu vaccination in 2019 allowed reducing likelihood of the coronavirus infection among healthcare workers in 2020. However, N. Massoudi with colleagues assessed only the risk of getting infected but not the disease progression or risks of its more severe clinical forms. The other study was accomplished in Italy by M. Belingheri with colleagues [23] with analyzing data on 3520 healthcare workers. The authors could not establish any relationship between flu vaccination and the risk of getting infected with SARS-CoV-2.

Some studies focus on investigating a relationship between vaccination against pneumococcal infection and the COVID-19 incidence. The systemic review coauthored by several authors under supervision by H. Im [24] analyzed several studies that reported vaccination against pneumococcal infection to be able to prevent severe COVID-19 clinical forms by preventing incidence and mortality caused by comorbid / secondary infections and superinfections.

Another study [25] involved systemic reviewing and meta-analysis to estimate a relationship between seasonal flu vaccination, vaccination against pneumococcal infection and COVID-19 and its clinical outcomes. Overall, the meta-analysis covered 38 observational studies with significant heterogeneity. Flu vaccination and vaccination against pneumococci were associated with lower risks of getting infected with SARS-CoV-2 (OR = 0.80, 95 % CI: 0.75–0.86 and OR = 0.70, 95 % CI: 0.57–0.88 accordingly). When data on flu vaccination were adjusted as per age, sex, comorbidities and socioeconomic indicators, the aforementioned relationship with a risk of SARS-CoV-2 became weaker. However, this does not concern vaccination against pneumococcal infection, which retained the same association with the risk of infection even after adjustments as per all these confounders. When it comes down to more severe observation points, such as hospitalization in an intensive care unit or death, available data do not confirm any association between such severe

COVID-19 incomes and flu vaccination or vaccination against pneumococcal infection.

Literature data on a role played by flu vaccination and vaccination against pneumococcal infection in COVID-19 progression are so heterogeneous that this requires additional profound analytical investigation. We have not found any open access publications on other vaccinations from the National calendar and therefore have not been able to compare our data with results of other studies.

Our analysis of laboratory markers identified in the analyzed healthcare workers with COVID-19 established several ones with prognostic value including levels of D-dimer, total protein, CRP, LDH, IL-6, ESR, platelets, lymphocytes, T-helpers, CTL and NK-cells. We compared our findings as regards laboratory tests with other research articles and found some interesting points. Some of them were in line with data obtained by other researcher but still there were certain peculiarities.

Thus, Y.-D. Gao with colleagues [18], along with investigating somatic risk factors, gave some attention to analyzing results of laboratory tests obtained for COVID-19 patients. They revealed several laboratory indicators to be important for monitoring of the disease progression. These indicators included lactate dehydrogenase, procalcitonin, C-reactive protein, proinflammatory cytokines such as interleukins IL-6, IL-1β, glycoprotein Krebs von den Lungen-6 (KL-6) and ferritin. Levels of LDH, CRP and interleukin-6 have been highlighted in our study as effective prognostic laboratory markers in healthcare workers able to point at likelihood of lung-involving clinical forms of the coronavirus infection. However, attention should be paid to prognostic value of procalcitonin, ferritin and KL-6 when planning additional investigations on occupational risk groups.

The systemic review accomplished by M. Palladino [26] confirmed that lower levels of platelets, lymphocytes, hemoglobin, eosinophils and basophils and an elevated level of neutrophils and neutrophils to lymphocytes ratio as well as elevated levels of platelets and lymphocytes were associated with unfavorable clinical outcomes in COVID-19 patients.

The meta-analysis by B.M. Henry with colleagues [27] covered 21 research articles. It identified the most effective prognostic indicators of more severe COVID-19 clinical forms, namely, levels of leukocytes, lymphocytes, platelets, IL-6 and ferritin in blood serum.

Other studies established that the D-dimer level in patients with COVID-19 correlated with an unfavorable outcome of the disease and was quite a precise biomarker to predict the clinical course of the infection [28–30]. ROC-analysis established the threshold D-dimer level that allowed identifying whether patients were at a risk of the lung involvement, namely 0.370 μg/l [31]; this is quite close to our threshold level. Another study established an optimal threshold D-dimer level for predicting mortality among COVID-19 patients as equal to 1.5 μg/l [32].

Some studies highlighted the interleukin-6 level as an effective prognostic laboratory indicator and this is well in line with our study results [33, 34].

Data obtained by PCR-based diagnostics were given special attention in analyzing the results of laboratory tests. The threshold cycle value, which is considered to be inversely proportionate to a viral load, was shown to have no associations with severity of clinical symptoms of the infection but still it had a statistically significant relationship with duration of the disease and grew simultaneously with it. However, different opinions on the matter can be found in literature.

Thus, M.E. Brizuela with colleagues [35] analyzed data on 485 patients in their study and established that the viral load with SARS-CoV-2 in smears from the airways, which was identified as per the threshold cycle, correlated authentically with moderate and severe cases and with age.

B. Mishra and others [36] showed in their study that a share of a high viral load ($Ct < 25$) was considerably higher in middle-aged and elderly people against young patients (44.6 % and 43.7 % against 32.2 %, $p < 0.001$).

H.C. Maltezou with colleagues [37] established that patients with a higher viral load tended to have more severe COVID-19 clinical forms and more often needed treatment in

ICU. The authors also detected a higher viral load in elderly patients and those with chronic cardiovascular diseases, essential hypertension, chronic bronchopulmonary diseases, immune suppression, obesity, and neurological pathology. The authors suggest using the Ct value to reveal patients who are at elevated risks of severe infection and death.

However, some other studies yielded contrary results. Thus, A. Karahasan Yagci with colleagues [38] reported that a viral load was not a factor associated with a risk of hospitalization or death. The authors mentioned even lower Ct values in patients with mild clinical COVID-19 variants. Similar data were reported in the study by J.F. Camargo and others [39].

I. Saglik with colleagues [40] did not identify any clear correlation between viral load with SARS-CoV-2 and severe clinical symptoms or deaths among COVID-19 patients either. The authors established that a Ct value in patients grew with time since the moment the disease started and this is in line with our study results. The Ct values were the lowest during the first five days after the first symptoms occurred; then, they grew considerably during the second and third week of the disease. Sex, age, or comorbidities did not have any significant differences in patients with low (≤ 25) and high (> 25) Ct values.

I. Saglik with colleagues also noted that levels of neutrophils, platelets and especially lymphocytes were considerably lower in patients with a high viral load. Estimation of the correlation between the Ct value and levels of prognostic laboratory indicators is a promising research trend and should be considered in future investigations of the issue.

P.P. Salvatore with colleagues [41] reported finding in their study similar to those by I. Saglik et al. as regards the Ct dynamics during the disease. The threshold cycle values were the lowest just after symptoms had occurred and had a significant correlation with a time period since this occurrence ($p < 0.001$); seven days after the first symptoms occurred, the average Ct value equaled 26.5 and 21 days after it was 35.0.

The present study involved whole-genome sequencing of 58 SARS-CoV-2 viruses isolated from the examined healthcare workers. B.1.1. was established to be a prevailing genetic variant; others were identified in singleton cases. SARS-CoV-2 detected in the healthcare workers in this study corresponded to a range of circulating genetic variants of the virus identified in the Sverdlovsk region and the Russian Federation at the initial stage in the pandemic according to the results of SARS-CoV-2 whole-genome sequencing available in the Russian (VGARus)[4] and international (EpiCov GISAID)[5] databases [42]. These results are significant for the complex analysis of the situation and organization of a system for molecular-genetic monitoring of communicable and parasitic diseases in the Russian Federation.

The healthcare workers were examined with the PCR method in dynamics to identify persistence of the SARS-CoV-2 virus in their bodies. It was established to vary between 13 and 45 days (the median was 22 days) and had certain peculiarities for different clinical forms of the infection. Similar data were reported by some other publications by other authors. Thus, Y. Wang with colleagues [43], who accomplished their study during the first pandemic 'wave', showed that duration of the virus persistence correlated with severity of the disease. Patients who had COVID-19 as ARI excreted the virus for 10 days in most cases (81.8 %) whereas patients with severe clinical forms of the disease who needed artificial ventilation excreted the virus for a longer period in 66.7 % of the cases, up to 20–40 days and this is quite similar to our data. X. Zhang and others [44] established in their study that persistent excretion of the virus RNA could be observed in 5.4 % of patients for longer than 45 days. The authors also noted the peak vial

[4] VGARus (Virus Genome Aggregator of Russia): the Russian platform for aggregating information about virus genomes. Available at: https://genome.crie.ru/app/index (December 19, 2022).

[5] GISAID: database. Available at: https://www.gisaid.org (December 01, 2022).

load was higher in patients with the severe disease against those who had it in milder forms.

However, it is noteworthy that the aforementioned publications were predominantly accomplished on general populations without any emphasis on specific cohorts or occupational groups. It is quite difficult to find open-access studies with their focus on investigating various aspects of the COVID-19 incidence among healthcare workers who were at higher risks of getting infected during the COVID-19 pandemic. This makes our study more valuable and offers some areas for further profound investigations among occupational groups with higher risks of infection.

Conclusion. In this study, we analyzed clinical symptoms of COVID-19 in healthcare workers during the initial stage in the pandemic (the first and second epidemic rises in the incidence); described clinical forms of the coronavirus infection and outlined its prevailing symptoms (common ones for communicable diseases, symptoms of acute respiratory infections, damage to the gastrointestinal tract, skin symptoms). The Ct value in PCR tests was shown to have no associations with severity of COVID-19 clinical symptoms; still, it had a direct correlation with a period starting from the moment the disease started. Whole-genome sequencing identified several genetic variants of the SARS-CoV-2 virus in the examined medical workers (predominantly B.1.1 and some others in singleton cases including B.1, B.1.1.397, B.1.1.317, B.1.1.387, B.1.1.409, B.1.1.141, B.1.1.274). The identified genetic variants corresponded to those circulating in the region and the country as a whole at the initial stage of the pandemic. We established how long they persisted in a patient's body (between 13 and 45 days, the median was 22 days).

In addition, we identified non-occupational risk factors of COVID-19 clinical forms with the lung involvement (age older than 40 years, low physical activity, overweight, diabetes mellitus, and diseases of the genitourinary system) as well as laboratory markers with cut-off points that were associated with more severe COVID-19 in the examined healthcare workers (erythrocyte sedimentation rate higher than 11.5 mm/hour; levels of platelets lower than $239 \cdot 10^9$/l, lymphocytes below $1.955 \cdot 10^9$/l, T-helpers below $0.855 \cdot 10^9$/l, cytotoxic T-lymphocytes lower than $0.455 \cdot 10^9$/l, NK-cells below $0.205 \cdot 10^9$/l, D-dimer higher than 0.325 µg/ml, total protein below 71.55 g/l, C-reactive protein higher than 4.17 mg/l, lactate dehydrogenase higher than 196 U/l, interleukin-6 higher than 3.63 pg/l).

It is advisable to use our data on non-occupational risk factors of severe non-communicable diseases when developing recommendations on identifying whether a person is occupationally fit for a specific medical specialty. Laboratory indicators identified in the present study can be widely used in clinical practice including operative adjustment of treatment protocols.

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Research article

STRAINS AND VIRULENCE GENES OF SALMONELLA WITH MULTIDRUG RESISTANCE ISOLATED FROM CHICKEN CARCASSES (HANOI, VIETNAM)

Xuan Da Pham[1], Hao Le Thi Hong[2], Huyen Tran Thi Thanh[3], Long Thanh Le[2], Hoa Vinh Le[2], Ninh Hanh Thi[2], Minh Le Tran[4], Nguyen Thanh Trung[2]

[1]Vietnam National University, Ho Chi Minh City, Vietnam
[2]National Institute for Food Control, 65 Fam Tan Duat Str., Hanoi, Vietnam
[3]Vinmec Research Institute of Stem cell and Gene Technology, Hai Ba Trung, Hanoi, Vietnam
[4] High School for Gifted Students, Hanoi University of Science, 182 Luong The Vinh Str., Hanoi, Vietnam

Salmonella enterica is one of dangerous food-borne pathogens listed by the World Health Organization (WHO). In Vietnam, poultry is one of the most widely eaten meats and is reported as a common source of S. enterica contamination.

The aim of this study was to examine multi-resistant Salmonella strains, to identify susceptibility to antibiotics by using 15 different types of medications and to perform sequencing to analyze antibiotic resistance genes, genotypes, multi-locus sequence-based typing (MLST), and plasmids.

The result of the antibiotic susceptibility test indicated that phenotypic resistance to 9–11 types of antimicrobials was confirmed in all strains. Among 06 sequenced strains, we identified 43 genes associated with antibiotic resistance: strains carrying a range of genes that are associated with aminoglycoside resistance (aac(3), aac(6), ant(3), aph(3), aph(6), aadA); all strains carried blaCTX-M-55 or blaCTX-M-65 gene, which were resistant to the 3rd generation antibiotics; there were also frequently observed sul1, sul2, sul3, tet (A), qnrS1, floR, dfrA14 or dfrA27 genes in sequenced isolates. Besides, the genome sequencing also indicated that all strains carried pathogenicity islands SPI 1, SPI 2, and SPI 3 thereby creating many potential triggers of the disease. Additionally, some carried C63PI, SPI 9, SPI 13, SPI 14, and plus some plasmids such as Col156, IncHI2, IncHI2A, IncFIB, Col (MGD2).

***Keywords**: antimicrobials, Salmonella, multidrug resistance, virulence factor, plasmid, chicken, antibiotic resistance gen, Salmonella pathogenicity island (SPI), beta-lactam.*

Salmonella enterica is the common factor that causes foodborne outbreaks worldwide (Center for Emerging and Infectious Diseases 2016). *Salmonella enterica* is further subdivided into six subspecies, which compose more than 2600 serovars in total. Among these six subspecies, *S. enterica* subsp. *enterica* is the main cause of most human salmonellosis cases [1]. The common source of animal-originated food products where *Salmonella* is generally found in poultry, in particular chicken and egg (FAO and WHO 2002) [2].

In low- and middle-incomes countries like Vietnam, to control the contamination of bac-

Xuan Da Pham – Doctor of Medical Sciences, Associate Professor, director of Center for Genetic and Reproductive Health, Faculty of Medicine (email: drdapro.sc@gmail.com; tel.: (+84) 913 832177; ORCID: https://orcid.org/0000-0002-2262-3028).

Hao Le Thi Hong – Doctor of Medical Sciences, Associate Professor, general director (e-mail: lethihonghao@yahoo.com; tel.: (+84) 904 248167; ORCID: https://orcid.org/0000-0003-3570-8570).

Huyen Tran Thi Thanh – Doctor of Medical Sciences, Director of Genetical Biomedicine Department (e-mail: v.huyenttt47@vinmec.com; tel.: (+84) 243 9753222).

Long Le Thanh – Master of Science, Researcher (e-mail: lethanhlong.ltl@gmail.com; tel.: (+84) 936 450430; ORCID: https://orcid.org/0000-0003-4520-0236).

Vinh Hoa Le – Bachelor of Science, Researcher (e-mail: vinhhoa.lvh@gmail.com; tel.: (+84) 363 059456; ORCID: https://orcid.org/0000-0001-5360-4260).

Ninh Hanh Thi – Bachelor of Science, Researcher (e-mail: ninhhanh891997@gmail.com; tel.: (+84) 338 273077; ORCID: https://orcid.org/0000-0001-9693-3507).

Minh Le Tran – student (e-mail: tranleminhntt@gmail.com; tel.: (+84) 942 472005; ORCID: https://orcid.org/0009-0005-2371-027X).

Nguyen Thanh Trung – Master of Science, Researcher at the Laboratory of Food Microbiology and Genetically Modified Food (e-mail: trungnt@nifc.gov.vn; tel.: (+84) 349 363269; ORCID: https://orcid.org/0000-0002-8732-9911).

teria in livestock, antibiotics have been used widely as an effective solution. Inappropriate usage of antibiotics in agricultural and veterinary practice has led to the rise of new multidrug resistant (MDR) bacteria and transferable genetic loci with this property.

MDR *Salmonella* infection in humans has become a concern to public health agencies. Previous studies reported that the persistence and dissemination of multiple resistant *Salmonella* serovars in the environment are due to the excessive application of antibiotics on land [3]. A recent study of the endemic *Salmonella* distribution in raw meat obtained from traditional markets in Ho Chi Minh city revealed that *Salmonella* isolated from 37.89 % were resistant to at least one antibiotic; 22.98 % were resistant to two to five antibiotics; and 8.70 % were resistant to more than 6 antibiotics [4]. In addition to a high prevalence of *Salmonella* noticed in broiler farms environment, 66.85 % of isolated *Salmonella* exhibited resistance to 2–9 antibiotics. Sixty-two multiple resistance patterns were observed in Mekong Delta, Vietnam [5].

Therefore, it is necessary to study the rate of antibiotic resistance bacterial with all the drug classes, for a better understanding of the link between their phenotype and their genotypes, addressing the mutation that might be responsible for their resistance. This can be done by utilizing different classical molecular typing methods to study the subsequence transmission of antibiotic resistant *Salmonella* in humans, animals and environments. Among other available methods, eligible ones are pulsed-field gel electrophoresis (PFGE) [6] and multi-locus sequence-based typing (MLST) [7].

The impact of antibiotic resistance on human health is a great concern in clinical treatment and agriculture since antibiotics have been used frequently for infection control. However, the limitation of these methods lies in insufficient discriminatory power to separate closely related *Salmonella* isolations in outbreak investigations and to differentiate between the intraserovar isolations from different hosts. The use of whole genome sequencing (WGS) has shown a major impact on the study of molecular epidemiology of AR pathogens [8]. A WGS study in Denmark reported that single nucleotide polymorphisms (SNP), pangenome, k-mer, and nucleotide difference trees were superior to the classical typing method and evaluated the association of the isolates to specific outbreaks of *S. Typhimurium* [9].

The aim of this study was to assess the prevalence of *Salmonella* contamination in chicken and to analyze the antibiotic-resistant genes, genotypes, MLST, virulence factors, and plasmids in WGS of various *Salmonella* serovars isolated from infected samples.

Materials and methods. Six strains in this study were isolated from whole chicken samples, which were collected in Hanoi in September 2019, by following the United States Department of Agriculture (USDA) isolation method MLG 4.10 (USDA 2019)[1].

Antibiotic susceptibility was determined using:

- the Liofilchem discs (Roseto degli Abruzzi (TE), Italy) with the following antibiotics: cefuroxime (CXM, 30 μg), ceftriaxone (CRO, 30 μg), cefoxitin (FOX, 30 μg), cefazoline (CZ, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg);
- the ESBL disc kit according to the recommendations by the Clinical and Laboratory Standards Institute (CLSI)[2]: cefotaxime (CTX, 30 μg); cefotaxime + clavulanic acid (CTL, 30 + 10 μg); ceftazidime (CAZ, 30 μg), ceftazidime + clavulanic acid (CAL, 30 + 10 μg);
- the AmpC disc kit according to the CLSI recommendations[2]: cefotaxime (CTX, 30 μg); cefotaxime 30 μg + cloxacillin (CTC); ceftazidime (CAZ, 30 μg), ceftazidime 30 μg + cloxacillin (CAC), gentamicin (CN, 10 μg), tetracycline (TE, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (C, 10 μg), ampicillin (AMP, 10 μg), meropenem (MRP, 10 μg), imipenem (IMI 10 μg), nalidixic acid (NA, 30 μg), trimethoprim (TM, 5 μg).

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[1] Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges. Laboratory Guidebook. *USDA*, 2019.

[2] Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed. *Clinical and Laboratory Standards Institute (CLSI)*, 2022.

The procedure in brief: prepare *Salmonella* spp. strains suspension ($1.0 \cdot 10^6$ cfu/mL); dip a sterile cotton swab into the standardized bacterial suspension; inoculate the agar by streaking with the swab containing the inoculum; place the antibiotic disk on the surface of the inoculated and dried plate; incubate plates in an inverted position at 37 °C for 16–18 h.

Escherichia coli (ATCC 25922) were used as the quality control standard. *Salmonella* spp. that resisted more than three classes and more than one antibiotic in a single class were designated as a MDR strain.

Genomic DNA was extracted from 1 mL of overnight culture grown in Brain Heart Infusion broth (BHI; BD, USA) using a PureLink™ Genomic DNA Mini Kit (Invitrogen, Thermofisher scientific) according to the manufacturer's protocol. A library was prepared for sequencing and WGS sequencing was performed using the Illumina MiSeq system (Illumina, San Diego, CA, USA), as described by the respective manufacturers.

The raw sequenced reads were analyzed in the *Salmonella* In Silico Typing Resource for serovar identification [10]. ABRicate was applied for screening of the antibiotic resistance genes, plasmid replicon [11]. The antibiotic resistance gene was performed by screening of the draft genome against Resfinder [12], CARD [13] and ARG-ANNOT [14] databases. The search of plasmid replicons was performed by screening of the draft genome against the PlasmidFinder database [15].

The antibiotic resistance profiles of all *Salmonella* isolates are shown in Table 1. These six isolates were multi-resistant to at least 9 of the 15 tested antimicrobials.

The antibiotic susceptibility test indicated that 7 out of 15 antibiotics were 100 % resistant by 6 *Salmonella* strains, including cefuroxime, ceftriaxone, cefazolin, cefotaxime, tetracycline, and ampicillin. Other antibiotics such as trimethoprim, chloramphenicol, and nalidixic acid were also highly resistant by 5 out of 6 isolates.

Four out of six strains were resistant to gentamicin, while half of the total isolates were resistant to ceftazidime. On the other hand, all six strains were susceptible to cefoxitin and ciprofloxacin. Another similar result was obtained in test with two 4th generation antibiotics, imipenem and meropenem, no resistance detected in all 6 strains.

For further genetic analysis, 6 isolates were then sequenced with the next generation sequencing Illumina platform. Quality check showed that sequencing results yield from 441,192 reads of sample 25_S6 to 811,290 reads out of sample 56_S15, with an average read length of around 235–239 bp. After successful assembly, *Salmonella* genome size range from 4.6 million bp to 4.9 million bp with approximately 52 % of GC content as shown in Table 2.

Table 1

Antibiotic resistance profile of *Salmonella* isolates

Samples	CXM	CRO	FOX	CZ	CTX	CAZ	TMP	TE	C	CN	NA	CIP	AMP	IMI	MRP	Resistance, number of drugs
64_S19	R	R	S	R	R	I	S	R	R	R	R	S	R	S	S	9
13_S3	R	R	S	R	R	S	R	R	R	I	R	S	R	S	S	9
25_S6	R	R	S	R	R	R	R	R	R	S	S	S	R	S	S	9
52_S14	R	R	S	R	R	I	R	R	R	R	R	S	R	S	S	10
56_S15	R	R	S	R	R	R	R	R	S	R	R	S	R	S	S	10
21_S5	R	R	S	R	R	R	R	R	R	R	R	S	R	S	S	11
Resistance, number of drugs	6/6	6/6	0/6	6/6	6/6	3/6	5/6	6/6	5/6	4/6	5/6	0/6	6/6	0/6	0/6	

Note: R is resistance, S is sensitivity, I is intermediate state; cefuroxime (CXM), ceftriaxone (CRO), cefoxitin (FOX), cefazolin (CZ), cefotaxime (CTX), ceftazidime (CAZ), trimethoprim (TMP), tetracycline (TE), chloramphenicol (C), gentamicin (CN), nalidixic acid (NA), ciprofloxacin (CIP), ampicillin (AMP), imipenem (IMI), meropenem (MRP).

Table 2

Assembled genome data characteristics

Sample	Readings	Average length	Contigs	Genome length	Average contig length	N50	GS
13_S3	740,518	236	393	4,788,214	116,030	29,823	52.21
21_S5	763,692	239	428	4,931166	146,003	24,548	52.40
25_S6	441,192	235	530	4,878,881	85,034	18,804	52.51
52_S14	676,386	239	506	4,924,654	102,730	22,592	52.54
56_S15	811,290	237	383	4,678,161	262,392	30,011	52.36
64_S19	771,120	237	508	4,918,718	65,335	22,505	52.48

According to *In silico* prediction, the sequenced genomes of MDR isolates were predicted to carry 43 different antimicrobial resistance genes in total (Table 3), which belong to different drug classes (Table 4).

The presence of antimicrobial resistance (AMR) genes in Table 3 demonstrated a close association between genotype and phenotype of six strains analyzed in this study. All of the analyzed strains carried numerously AMR genes, especially genes related to aminoglycoside resistance. There were 17 aminoglycoside resistance genes in total, divided into three resistant mechanisms. All strains carry at least one gene coding for aminoglycoside acetyltransferases, which are *aac(6)-Iaa_1, aac(6)-Ib-cr_1,* and *aadA16_1*. These genes encode aminoglycoside acetyltransferase in *S.* Enteritidis and *S.* Enterica; this enzyme is resistant to aminoglycoside - broad-spectrum antibiotics. Specifically, genes that encode for resistance to aminoglycoside also include *ant(3)-Ia_1* encoding aminoglycoside nucleotidyltransferase (05/06); aph group: *aph(3)-Ib_5, aph(3)-Ia_3, aph(3)-Ia_7, aph(4)-Ia_1,* and *aph(6)-Id_1*, which encode aminoglycoside phosphotransferases (06/06).

The sequenced genome of all six isolates showed the presence of beta-lactam resistance related genes, especially $bla_{\text{CTX-M-55\_1}}$ and $bla_{\text{CTX-M-65\_1}}$. These two genes are involved in resistance of broad-spectrum beta-lactam antibiotic group. Isolate number 56_S15 was predicted to contain gen $bla_{\text{TEM-1B\_1}}$, another gene in beta-lactam resistance gene group. Two out of six strains contained *qnrS1_1* gene. It is considered to be involved in the mechanism of resistance to Fluoroquinolones antibiotics 1 (QnrS1_1 is a plasmid-mediated quinolone resistance protein). These genes were found to be located on mobile genetic elements in those isolates as well.

All six strains carried at least one of two genes (*catA2_1* or *floR-2*) encoding for Chloramphenicol acetyltransferase. Two out of six strains carried *mph(A)_2* gene encoding Macrolide phosphotransferases enzyme. All the sequenced strains carried *tet(A)_6* gene associated with resistance to the tetracycline group. Five out of six strains carried genes (*sul1_5 or sul2_2* or *sul3_2*) related to Sulfonamide resistance by replacing the antibiotic target of Sulfonamide. Two out of six isolates carried the gene *fosA3_1* or *fosA7_1* gene encoding Fosfomycin thiol transferase.

These genes are involved in antibiotic inactivation during resistance to fosfomycin. The genomes of five out of six isolates appeared to carry *dfrA14_5* or *dfrA27_1* gene. These genes are associated with Trimethoprim resistance through the formation of Trimethoprim resistant dihydrofolate reductase Dfr. Three out of six strains were established to have *arr-3_4* or *arr2* gene encoding Rifampin ADP-ribosyltransferase. Two out of six strains turned out to have *lnu(F)_1* gene (equivalent to *lin(F)*), which encodes an integron-mediated nucleotidyltransferase thereby leading to resistance to Lincomycin, and Lindamycin. All strains carried genes associated with multidrug resistance (*golS; mdsA; mdsB; mdsC; mdtK; sdiA; Mrx*).

In silicon serotyping and Multi-Locus Sequence Typing (MLST). The results of MLST analysis showed that the MDR *Salmonella* strains isolated from different areas were clustered into different sequence types and were phenotypically different depending on a serovar, serogroup, and the presence of H and O antigens as well (Table 5).

Table 3

Distribution of antimicrobial resistance genes in *Salmonella* serovars based on *in silico* predictions

Drug classes	Genes	Samples					
		21_S5	25_S6	64_S19	13_S3	52_S14	56_S15
		Number of AMR genes					
		17	18	20	27	27	27
Rifampin	*arr-3_4*	−	−	−	+	+	+
	arr2	−	−	−	+	−	+
	arr3	−	−	−	−	+	−
Aminoglycoside	*aac(3)-IIa*	−	−	−	+	+	+
	aac(3)-IId_1	−	−	−	+	+	+
	aac(3)-IVa_1	+	+	+	−	−	+
	aac(6)-Iaa_1	+	+	+	+	+	+
	aac(6)-Ib-cr_1	−	−	−	−	+	−
	aac(6)-Iy	+	+	+	+	−	+
	aadA1-pm	+	+	+	−	−	−
	aadA16_1	−	−	−	−	+	−
	aadA22	−	−	−	+	−	+
	ant(3)-Ia_1	+	+	+	+	−	+
	aph(3)-Ib_5	−	−	−	−	+	−
	aph(3)-Ia_3	−	−	−	+	−	+
	aph(3)-Ia_7	−	−	+	−	−	−
	aph(4)-Ia_1	+	+	+	−	−	−
	aph(6)-Id_1	−	−	−	+	+	+
Beta-lactam	*bla* $_{CTX-M-55\ 1}$	−	−	−	+	+	+
	bla $_{CTX-M-65\ 1}$	+	+	+	−	−	−
	bla $_{TEM-1B\ 1}$	−	−	−	+	+	+
Diaminopyrimidine	*dfrA14_5*	−	+	+	+	−	+
	dfrA27_1	−	−	−	−	+	−
Chloramphenicol	*catA2_1*	−	−	−	−	+	−
	flor_2	+	+	+	+	+	+
Fosfomycin	*fosA3_1*	−	−	+	−	−	−
	fosA7_1	−	−	−	−	+	−
Lincosamide	*linG*	−	−	−	+	−	+
	Inu(F)_1	−	−	−	+	−	+
Multidrug classes	*golS*	+	+	+	+	+	+
	mdsA	+	+	+	+	+	+
	mdsB	+	+	+	+	+	+
	mdsC	+	+	+	+	+	+
	mdtK	+	+	+	+	+	+
	Mrx	−	−	−	+	+	−
	sdiA	+	+	+	+	+	+
Macrolides	*mph(A)-2*	−	−	−	+	+	−
Quinolone	*qnrS1_1*	−	−	−	+	−	+
Sulfonamides	*sul1_5*	+	+	+	−	+	−
	sul2_2	−	−	−	−	+	−
	sul3_2	−	−	−	−	−	+
Tetracyclin	*tet(A)_6*	+	+	+	+	+	+
	tetR	+	+	+	+	+	+

Note:

Absence (negative) [light blue]

Presence (positive) [red]

Table 4

Antimicrobial resistance genes of *Salmonella* isolates

			Drug Class											
Antibiotic Resistance	Strain code	Strain	Aminoglycoside	Beta-lactam	Chloram-phenicol	Quinolone	Macrolides	Tetra-cycline	Sulfo-namides	Fosfo-mycin	Diamino-pyrimidine	Rifampin	Linco-samide	Multidrug classes
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	13_S3	Newport	*aac(3)-IId_1;* *aac(3)-IIa;* *aadA22;* *ant(3'')-Ia_1;* *aph(3')-Ia_3;* *aph(6)-Id_1;* *aac(6')-Iaa_1;* *aac(6')-Iy;*	$bla_{\text{CTX-M-55\_1;}}$ $bla_{\text{TEM-1B\_1}}$	*floR_2*	*qnrS1_1;*	*mph(A)_2;*	*tet(A)_6;* *TetR*			*dfrA14_5*	*arr-2;* *arr-3_4*	*lnu(F)_1;* *linG;*	*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *Mrx;* *sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-C-AMP	21_S5	Infantis	*aac(3)-IVa_1;* *aac(3)-IV;* *aac(6')-Iaa_1;* *ant(3'')-Ia_1;* *aph(4)-Ia_1;* *aac(6')-Iy;* *aadA1-pm*	$bla_{\text{CTX-M-65\_1}}$	*floR_2*			*tet(A)_6;* *TetR*	*sul1_5;*					*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	25_S6	Infantis	*aac(3)-IVa_1;* *aac(6')-Iaa_1;* *ant(3'')-Ia_1;* *aph(4)-Ia_1;* *aac(6')-Iy;* *aadA1-pm;*	$bla_{\text{CTX-M-65\_1}}$	*floR_2*			*tet(A)_6;* *TetR*	*sul1_5;*		*dfrA14_5*			*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-TE-C-AMP	52_S14	Mele-agridis	*aac(3)-IId_1;* *aac(3)-IIa;* *aac(6')-Iaa_1;* *aac(6')-Ib-cr_1;* *aadA16_1;* *aph(3'')-Ib_5;* *aph(6)-Id_1;*	$bla_{\text{CTX-M-55\_1;}}$ $bla_{\text{TEM-1B\_1}}$	*catA2_1;* *floR_2*		*mph(A)_2*	*tet(A)_6;* *TetR*	*sul1_5;* *sul2_2;*	*fosA7_1;*	*dfrA27_1*	*arr-3_4;* *arr-3;*		*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA;* *Mrx*
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	56_S15	Muenster	*aac(3)-IId_1;* *aac(6')-Iaa_1;* *ant(3'')-Ia_1;* *aph(3')-Ia_3;* *aph(6)-Id_1;* *aac(3)-IIa;* *aac(6')-Iy;* *aadA22;*	$bla_{\text{CTX-M-55\_1;}}$ $bla_{\text{TEM-1B\_1}}$	*floR_2*	*qnrS1_1;*		*tet(A)_6;* *TetR*	*sul3_2*		*dfrA14_5*	*arr-3_4;* *arr-2*	*lnu(F)_1;* *linG;*	*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-CN-C-AMP	64_S19	Infantis	*aac(3)-IVa_1;* *aac(6')-Iaa_1;* *ant(3'')-Ia_1;* *aph(3')-Ia_7;* *aph(4)-Ia_1;* *aac(6')-Iy;* *aadA1-pm;*	$bla_{\text{CTX-M-65\_1}}$	*floR_2*			*tet(A)_6;* *TetR*	*sul1_5*	*fosA3_1*	*dfrA14_5*			*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA;*

Table 5

Serotyping and MLST of *Salmonella* isolates

Sample code	Serovar	Serogroup	H1	H2	antigen O	MLST
13_S3	Newport	C2–C3	e,h	1,2	6,8,20	4157
21_S5	Infantis	-	r	1,5	6,7,14	32
25_S6	Infantis	-	r	1,5	6,7,14	32
52_S14	Meleagridis	-	e,h	l,w	3,{10}{15} {15,34}	463
56_S15	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
64_S19	Infantis	-	r	1,5	6,7,14	32

Within these six isolates, 4 MLST were identified. Three out of 6 strains were classified as sequence type (ST) 32. These 3 isolates were also identified as serovar Infantis, which is the most prevalent serovar in this study. Other serotypes found in this study are Newport, which also classified as serogroup C2–C3 (n = 1); Meleagridis (n = 1); and Muenster (n = 1).

Plasmid replicons and *Salmonella* pathogenicity islands (SPIs). We performed In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. The results are shown Table 6.

Table 6

Plasmid, and *Salmonella* pathogenicity islands (SPIs) of isolates

Strains	Serotype	Plasmid	Number of virulence genes	SPI
13_S3	Newport	Col156 IncHI2 IncHI2A	90	C63PI, S54, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13
21_S5	Infantis	IncF	101	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13
25_S6	Infantis		93	C63PI, S54, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, SPI-14
52_S14	Meleagridis	IncFIB Col(MGD2)	80	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9
56_S15	Muenster		82	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14
64_S19	Infantis		93	C63PI, SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14

The SPIFinder-2.0 prediction findings demonstrated the widespread presence of SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, and SPI-14; all strains turned out to have SPI-1, SPI-2, SPI-3, and SPI-9. Strains 21 S5, 25 S6, and 64_18 are all Infantis serovars; however, they contain distinct pathogenic islands, and virulence genes due to the difference in collecting places.

The mobile element finder revealed a wide range of plasmid and transposons. The plasmids Col156, IncHI2, IncHI2A, IncFIB, Col(MGD2), IncF are among the expected ones (3/6 strains). The CTX-M 55 or CTX-M 65 genes, which are thought to bear responsibility for resistance to cefotaxime and ceftriaxone, were frequently found in Col156 and IncHI2. These plamids were the most significant plasmid lineage involved in the transmission of antibiotic resistance in *Salmonella*, particularly in *S.* Typhimurium strains. β-lactam (blaOXA-1 and blaTEM-1) and quinolons resistant genes (qnrS1_1 and acc(6′)-ib-cr) were horizontally transferred by IncHI2 plasmid.

Results and discussion. The results of our study focus on the situation of MDR Salmonella strains in Hanoi, Vietnam. The growing number of drug classes that Salmonella is capable to resist has become a threat in Vietnam and all over the world. Report for the current multi-resistance was found in 45/46 studies of Salmonella in poultry; Salmonella strains found in the food chain had high rates of resistance to antibiotics such as nalidixic acid (26.8–86.6 %), ampicillin (14.9–68 %), trimethoprim / sulfamethoxazole (16–54.2 %) and were not inducible to carbapenems such as imipenem and meropenem [16].

The fact that all six analyzed strains harbored the gene *bla*$_{\text{CTX-M-65}}$ or *bla*$_{\text{CTX-M-55}}$, demonstrates a very high and widespread level of AmpC and/or ESBL-related gene carrier. The *bla*$_{\text{CTX-M-55}}$ and *bla*$_{\text{CTX-M-65}}$ genes are associated with antibiotic resistance to a variety of essential drugs, including cefotaxime, ceftriaxone, aztreonam, ceftazidime, amoxicillin, ampicillin, ticarcillin, piperacillin, and cefepime. Interestingly, the phenotypic analysis revealed that all of the tested strains were resistant to cefotaxime, ceftriaxone, ceftazidime, and ampicillin. The prevalence of *bla*$_{\text{CTX-M}}$ represents a risk of drug resistance when all of these strains are frequently associated with horizontal transmission between strains of the same species as well as between various species via synaptic plasmids or transposons [17]. Although many studies demonstrated the prevalence of *Salmonella* harboring *bla*$_{\text{CTX-M-55}}$ or *bla*$_{\text{CTX-M-65}}$ worldwide, however, no similar information is obtained from Vietnam. Our study reported identifying *Salmonella* harboring *bla*$_{\text{CTX-M-55}}$ or *bla*$_{\text{CTX-M-65}}$ and co-harboring *bla*$_{\text{CTX-M-55}}$ or *bla*$_{\text{CTX-M-65}}$ with *bla*$_{\text{TEM}}$. On the other hand, Nakayama and others reported extended-spectrum β-lactamase-producing *E. coli* co-harboring *bla*$_{\text{CTX-M-55}}$ or *bla*$_{\text{CTX-M-65}}$ with *bla*$_{\text{TEM}}$ isolates in chicken meat in Vietnam [18].

Thus, the presence of SPI enhanced the survivability of *Salmonella* cells and this became the challenge in the MDR *Salmonella* treatment with antibiotics.

Conclusion. *Salmonella* has been a serious threat to public health for a long time, especially with the spread of its multidrug resistance and virulence genes. Results in this study indicated that *Salmonella* strains were able to resist several important antibiotics, which were commonly used in clinical treatment and agriculture, notably the third generation of cephalosporins (ceftriaxone, cefotaxime, and ceftazidime). Additionally, genomic sequencing of six isolates revealed the identification of 43 genes associated with antibiotic resistance. The presence of genes $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$ (resistant to 3rd generation antibiotics) on *Salmonella* isolated from chickens were confirmed in this study. In addition, the sequenced genomes also demonstrated the variety of SPIs and plasmids in isolated strains.

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META-ANALYSIS OF THE INFLUENCE OF GENDER AND AGE ON THE SEASONAL DYNAMICS OF CEREBRAL STROKES

N.V. Kuzmenko[1, 2], M.G. Pliss[1, 2], V.A. Tsyrlin[1], M.M. Galagudza[1]

[1]Almazov National Medical Research Centre, 2 Akkuratova Str., Saint Petersburg, 197341, Russian Federation
[2] Pavlov First Saint Petersburg State Medical University, 6-8 L'va Tolstogo Str., Saint Petersburg, 197022, Russian Federation

The purpose of this work is to investigate dependence of the seasonal dynamics of HS (hemorrhagic strokes) and IS (ischemic strokes) risk on sex and age using meta-analysis.

In total, 22 publications were selected for this meta-analysis, studying the seasonal dynamics of HS, of which 8 publications presented statistics separately for men and women, and three papers presented statistics for different age groups. Also, 28 publications studying the seasonal dynamics of IS were selected for meta-analysis, of which 11 publications presented statistics separately for men and women, and three papers presented statistics for different age groups.

The meta-analysis of the seasonal dynamics of HS showed that HS risk is less likely in a warmer season compared with a colder one. In men, HS risk was the highest in winter and spring, and in women in winter. Dependence between HS risk and a decrease in air temperature was the same in men and women. According to the results of the meta-analysis (without regard to sex and age), the minimum probability of IS occurs in autumn. In women, IS risk was significantly higher in winter compared to other seasons. In men, the seasonal dynamics of IS was not expressed. In older people, the overall risk of stroke increased, especially IS. In people over 65 years of age, there was a significant dependence of an increase in HS risk on a decrease in air temperature. In people younger than 65 years, HS risk was not associated with cold. A decrease in temperature equally increased IS risk in both age groups.

These results suggest that sex and age may influence the seasonal stroke risk.

Keywords: *hemorrhagic stroke, ischemic stroke, season, gender, age, risk, seasonal dynamics, meta-analysis.*

In 2019, approximately 101 million people had a stroke, and 6.55 million people died from it [1]. Stroke is one of the main causes of disability in the population.

Age-adjusted DALY (Disability Adjusted Life Years) and stroke mortality are significantly higher in men than in women, but the prevalence is higher in women [1]. Almost a third of all strokes occur over the age of 80 [2], but men, on average, have a stroke at a younger age than women [3]. In women of reproductive age, on the one hand, a high level of estrogen serves as protection against cardiovascular events (CVE), on the other hand, pregnancy and contraceptive use increase the risk of stroke. In addition, CVE risk is increased by lifestyle factors (overeating, alcohol abuse, smoking), which are more typical for men than for women [4].

Nataliya V. Kuzmenko – Candidate of Biological Sciences, Senior Researcher at the Department of Experimental Physiology and Pharmacology; Junior Researcher of the Blood Circulation Biophysics Laboratory (e-mail: nat.kuzmencko2011@yandex.ru; tel.: +7 (921) 447-75-81; ORCID: https://orcid.org/0000-0001-6027-7325).

Mikhail G. Pliss – Candidate of Medical Sciences, Head of the Department of Experimental Physiology and Pharmacology; Head of the Biophysics of Blood Circulation Laboratory (e-mail: pliss@niiekf.ru; tel.: +7 (812) 702-37-49 (ext. 003067); ORCID: https://orcid.org/0000-0002-1515-1616).

Vitaly A. Tsyrlin – Doctor of Medical Sciences, Professor, Chief Researcher at the Department of Experimental Physiology and Pharmacology (e-mail: tsyrlinva@mail.ru; tel.: +7 (812) 702-37-49 (ext. 003067); ORCID: https://orcid.org/0000-0002-7767-8560).

Michael M. Galagudza – Corresponding Member and Professor of the RAS, Doctor of Medical Sciences, Director of the Institute of Experimental Medicine (e-mail: galagoudza@mail.ru; tel.: +7 (812) 702-37-49 (ext. 003067); ORCID: https://orcid.org/0000-0001-5129-9944).

The change of seasons is accompanied by both changes in external meteorological conditions (air temperature and humidity, magnitude and variability of atmospheric pressure, partial density of oxygen in the air), and changes in the functioning of the body and lifestyle. In winter, as opposed to summer, people have higher levels of BP (blood pressure), body mass index, thyroid hormone activity, hematocrit, hemoglobin, lipid profile and glucose levels [5–9], which may contribute to an increased CVE risk in winter. Our earlier meta-analyses showed that there are no significant differences in the seasonal dynamics of body functioning between men and women [5–10].

Our studies did not reveal convincing evidence that age could influence the seasonal dynamics of the body functioning in healthy people [5–10]. However, it is known that old age is associated with inhibition of vegetative control mechanisms, and as a result the coherence of the body's response to various stimuli, including meteorological ones, changes [11, 12]. Cooling causes a greater rise in systolic BP and in the activity of the sympathetic nervous system in older people compared with young people, and moderate hyperthermia causes a weakened tachycardia reaction and a tendency to reduce the hypotensive response of diastolic BP [13]. During hypoxia, older volunteers compared with young ones demonstrated a similar or increased rise in BP, but a smaller increase in heart rate [14, 15]. Age-related disorders of cerebral circulation, when brain perfusion changes during heat, cold, or hypoxia, can provoke fainting and strokes. The presence of cardiovascular pathology further exacerbates the imbalance. For example, patients with heart failure have a weakening of the cardiovascular response under hyperthermia compared with healthy people of the same age [12]. In patients with arterial hypertension, there is an increase in the hypertensive response under exposure to cold [16]. Hypertensive patients, compared with healthy people, show a large difference between BP values in winter and summer [17].

The purpose of this work is to investigate dependence of the seasonal dynamics of HS (hemorrhagic strokes) and IS (ischemic strokes) on sex and age using a meta-analysis of publications.

Materials and methods. The meta-analysis was performed in accordance with the PRISMA guidelines[1]. In this meta-analysis, publications selected for the meta-analysis [18] of dependence between HS and IS and climate of a given region were used. The strategy for searching and selecting publications is described in detail in [18]. It was similar for this meta-analysis but the exact geographic location of the study was not important. The search was carried out in PubMed and Scopus databases using the keywords: stroke, ischemic stroke, hemorrhagic stroke, cerebral infarction, cerebral ischemia, and season.

Publications devoted to the study of the seasonal dynamics of events / hospitalizations of HS, IS, but not deaths from them, were selected. The HS and IS data had to be presented separately in absolute terms (or in a form that allows calculating the absolute value for a year and for each season). When selecting publications, the methods of diagnostics were taken into account; due to the imperfection of diagnostics, studies conducted before 1980 were excluded.

In the course of the meta-analysis, the seasonal risk of HS and IS was calculated considering sex and age and without such consideration. If the publication presented statistics on subtypes within HS and IS, then it was combined, as in the meta-analysis [18].

Statistics. Meta-analysis was performed using the statistical program Review Manager 5.3 (Cochrane Library). The Mantel Haenszel (odds ratio – chance coefficient test that allows determining the strength of the connection between events) test was used for analysis. The heterogeneity of the studies included in the meta-analysis was determined by the criterion I^2. The choice of a fixed or randomized effects model was carried out in accordance with the recommendations[2]. Z-test was used to evalu-

[1] PRISMA. Available at: http://www.prisma-statement.org (February 01, 2020).

[2] Borenstein M., Hedges L.V., Higgins J.P.T., Rothstein H.R. Introduction to Meta-analysis. Wiley, Chichester Publ., 2009, 421 p.

ate the statistical significance of the weighted mean effect size. Confidence interval was 95 %. Differences were considered statistically significant at $p < 0.05$. Funnel plots were used to identify bias in the selection of publications.

Results. 746 publications were found on the topic of the meta-analysis, of which 42 were reviews [18]. For this meta-analysis, 22 publications studying the seasonal dynamics of HS were selected, of which 8 publications presented statistics separately for men and women, and three papers presented statistics for different age groups (Table 1). Also, 28 publications studying the seasonal dynamics of IS were selected for meta-analysis, of which 11 publications presented statistics separately for men and women, and three papers presented statistics for different age groups (Table 1). In 16 publications, the seasonal dynamics of HS and IS were simultaneously studied.

Table 1

Publications selected for meta-analysis

Publications	Total number of strokes		Average age (years)	Sex, male (%)		Diagnostics
	HS	IS		HS	IS	
Biller J., 1988 [19]	690	1357	-	43	55.7	HCSR
Cho S., 2018 [20]	-	63,564	≥ 40	-	53	WHOC
Choi Y.I., 2015 [21]	-	968	67.6	-	60.9	MRI
Ding J, 2018 [22]	-	84	39.9	-	52.2	CT, MRI
Evzel'man M.A., 2019 [23]	-	1144	73.5		30	CT, MRI
Feigin V.L., 1998 [24]	64	214	49.5*	36**	38**	CT
Fodor D.M., 2018 [25]	114	969	70.5*	55.3**	52.5**	WHOC
Giroud M., 1989 [26]	45	226	≥ 10	-	-	CT
Hakan T., 2003 [27]	761	-	8–82	45**	-	CT
Huang Q., 2019 [28]	2555	-	55.1	37.5	-	CT
Jakovljević D., 1996 [29]	2493	12,737	≥ 25*	52**	49**	WHOC
Karagiannis A., 2010 [30]	-	1452	72.5	-	50**	-
Khan F.A., 2005 [31]	896	5086	75. 1	48**	49**	CT
Klimaszewska K., 2007 [32]	-	1173	72.4	-	-	-
Knezovic M., 2018 [33]	251	1712	18–104	50	50	-
Kumar P., 2015 [34]	436	663	54	69**	70.6**	-
Liu Y., 2018 [35]	-	961	69.1	-	66.9	CT, MRI
Manfredini R., 2010 [36]	-	43,642	76.8	-	45.5**	WHOC
Mao Y., 2015 [37]	632	2202	71	57.6	55.4	CT
Ogata T., 2004 [38]	-	12,660	71	-	62.7**	-
Ostbye T., 1997 [39]	20,545	-	≥ 15	39**	-	-
Palm F., 2013 [40]	202	1547	71.7	-	-	CT, MRI
Park H., 2008 [41]	1472	1357	59	-	-	-
Passero S., 2000 [42]	1018	-	63.6	62	-	CT
Ricci S., 1992 [43]	52	286	-	-	-	CT
Salam A., 2019 [44]	698	2956	54.4	-	-	WHOC
Simovic S., 2017 [45]	-	415	72.1*	-	50.4	CT, MRI
Soomro M.A., 2011 [46]	46	85	15–88	58.7**	57.6**	CT
Spengos K., 2003 [47]	197	823	22–95	-	-	CT, MRI
Telman G., 2017 [48]	974	-	18–101	59.8	-	CT, MRI
Toyoda K., 2018 [49]	-	2965	74.1	-	60.5**	CT, MRI
van Donkelaar C.E., 2018 [50]	1535	-	56	38	-	CT
Vodonos A., 2017 [51]	-	1174	73.8	-	56.6**	-
Zhong H., 2018 [52]	421	1115	54	-	-	CT, MRI

Note: HI is hemorrhagic stroke, IS is ischemic stroke, HCSR is Harvard Cooperative Stroke Registry; WHOC is World Health Organization Criteria; MRI is Magnetic Resonance Imaging; CT is Computed Tomography; * means statistics are presented separately for groups of different ages, ** means statistics are presented separately for men and women, (-) means no information available.

In total, 36,097 cases of HS were analyzed without considering sex or age, as well as 10,489 cases in men and 14,866 in women; 606 cases in young and middle-aged people; 708 in old people (over 65 years old). Although the results of studies that present HS statistics separately for men and women indicate that HS occurred more often in women, the overall statistics of all selected studies gives evidence that HS occurred with the same frequency in men and women. A meta-analysis of the seasonal dynamics of HS showed that the HS risk is less likely in a warmer season compared to a colder one (Table 2, Fig. 1). On average, the minimum probability of HS was in summer, and the maximum was in winter (Table 2). In men, HS risk was the highest in winter and spring; in women, in winter (Table 2, Fig. 1). Dependence of HS risk on a decrease in air temperature was the same in men and women (Fig. 1). According to the results of three studies, old age increased HS risk on average by 14 % in all seasons (Table 3). In people over 65 years of age, there was a significant dependence of the increase in HS risk on a decrease in air temperature. In the younger group, there was no such dependence; on the contrary, HS risk was higher in spring and autumn than in winter (Table 2, Fig. 2).

In total, 165,196 cases of IS were analyzed without considering sex or age, as well as 40,838 cases in men and 40,809 in women, 3585 cases in young and middle-aged people and 7133 in old people (over 65 years old). Although the publication [45] presents the seasonal dynamics of IS events for sex and age groups, we did not use it due to the obvious error of the authors in the calculations. The conducted meta-analysis showed that, on average, the minimum probability of IS occurs in autumn (Table 3). IS occurred with approximately the same frequency in men and women. A significant increase in the risk of IS in winter compared with other seasons was observed in women, but not in men (Fig. 1). In addition, there was a tendency ($P = 0.08$) for women to have increased IS risk in summer compared with autumn and spring (Fig. 1). According to the results of three studies, IS risk increased with age by approximately 2 times in all seasons. In the group of people older than 65 years, the severity of the seasonal dynamics of IS risk in winter compared to other seasons was slightly higher than in people younger than 65 years (Table 3, Fig. 2). However, dependence of IS risk on a decrease in air temperature did not significantly increase in people with aging (Fig. 2).

Table 2

Dependence of hemorrhagic stroke risk on a season

Compared seasons		Number of studies	Total	Odds ratio	I^2, %	Overall effect test	
Season 1 / total	Season 2 / total					Z	P
Hemorrhagic stroke (all)							
winter / 9611	summer / 8223	22	36,097	1.40 [1.25, 1.56]	81	5.98	0.00001
winter / 9611	spring / 9245	22	36,097	1.10 [1.02, 1.18]	62	2.34	0.02
winter / 9611	autumn / 9018	22	36,097	1.14 [1.04, 1.26]	74	2.82	0.005
autumn / 9018	summer / 8223	22	36,097	1.20 [1.12, 1.27]	38	5.55	0.00001
spring / 9245	summer / 8223	22	36,097	1.26 [1.16, 1.36]	59	5.78	0.00001
spring / 9245	autumn / 9018	22	36,097	1.04 [0.97, 1.12]	49	1.20	0.23
Hemorrhagic stroke (men)							
winter / 2674	summer / 2478	8	10,489	1.29 [0.99, 1.67]	82	1.86	0.06
winter / 2674	spring / 2752	8	10,489	1.04 [0.86, 1.25]	66	0.39	0.70
winter / 2674	autumn / 2585	8	10,489	1.21 [0.97, 1.52]	75	1.67	0.10
autumn / 2585	summer / 2478	8	10,489	1.06 [0.99, 1.13]	0	1.73	0.08
spring / 2752	summer / 2478	8	10,489	1.15 [1.08, 1.22]	0	4.36	0.0001
spring / 2752	autumn / 2585	8	10,489	1.09 [1.02, 1.16]	0	2.64	0.008

End of the Table 2

Compared seasons		Number of studies	Total	Odds ratio	I^2, %	Overall effect test	
Season 1 / total	Season 2 / total					Z	P
Hemorrhagic stroke (women)							
winter / 3967	summer / 3470	8	14,866	1.25 [1.04, 1.49]	60	2.42	0.02
winter / 3967	spring / 3723	8	14,866	1.08 [0.98, 1.18]	13	1.59	0.11
winter / 3967	autumn / 3706	8	14,866	1.17 [0.96, 1.42]	68	1.54	0.12
autumn / 3706	summer / 3470	8	14,866	1.10 [0.99, 1.22]	18	1.82	0.07
spring / 3723	summer / 3470	8	14,866	1.15 [0.99, 1.35]	48	1.82	0.07
spring / 3723	autumn / 3706	8	14,866	1.04 [0.94, 1.15]	20	0.71	0.48
Hemorrhagic stroke (people under 65 y.o.)							
winter / 134	summer / 134	3	606	1.00 [0.75, 1.32]	2	0.01	0.99
winter / 134	spring / 170	3	606	0.73 [0.55, 0.98]	4	2.13	0.03
winter / 134	autumn / 168	3	606	0.74 [0.57, 0.96]	0	2.25	0.02
autumn / 168	summer / 134	3	606	1.19 [0.73, 1.96]	43	0.71	0.48
spring / 170	summer / 134	3	606	1.19 [0.75, 1.89]	37	0.73	0.47
spring / 170	autumn / 168	3	606	1.02 [0.79, 1.31]	0	0.13	0.89
Hemorrhagic stroke (people over 65 y.o.)							
winter / 202	summer / 138	3	708	1.65 [1.29, 2.11]	0	3.96	0.0001
winter / 202	spring / 179	3	708	1.18 [0.93, 1.49]	0	1.37	0.17
winter / 202	autumn / 189	3	708	1.23 [0.79, 1.94]	28	0.91	0.36
autumn / 189	summer / 138	3	708	1.50 [1.17, 1.93]	0	3.20	0.001
spring / 179	summer / 138	3	708	1.40 [1.09, 1.80]	0	2.60	0.009
spring / 179	autumn / 189	3	708	0.93 [0.73, 1.18]	0	0.61	0.54

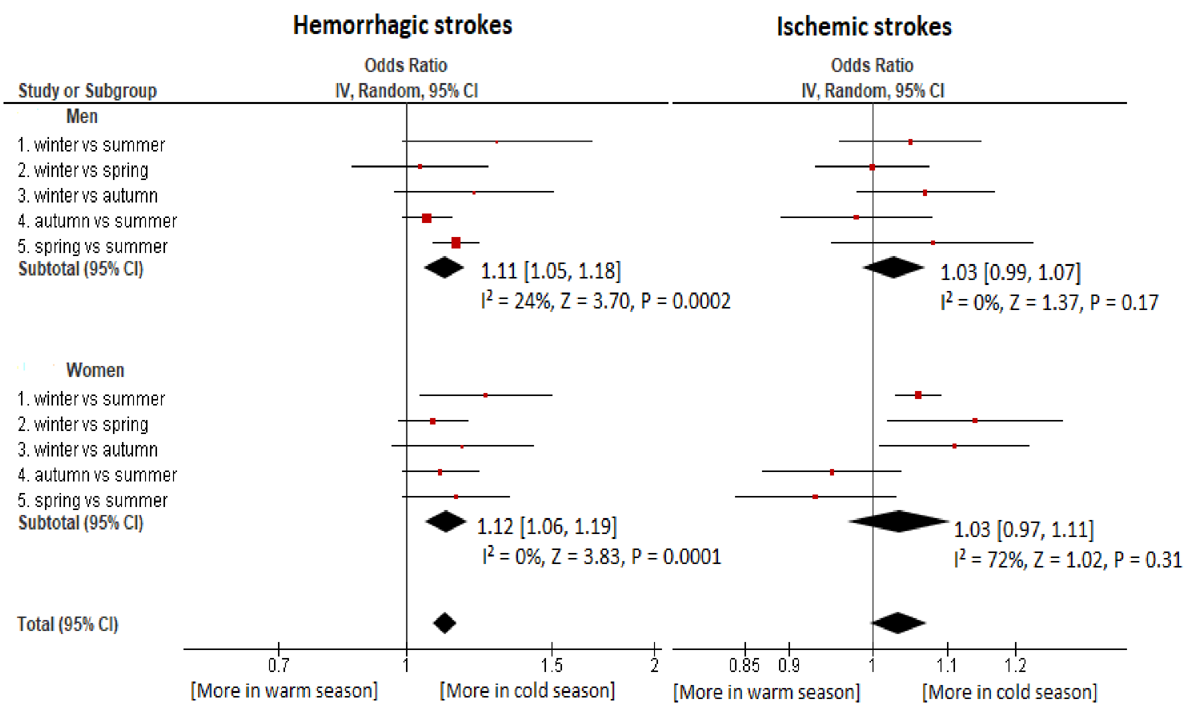

Figure 1. Dependence of strokes risk on a decrease in air temperature for men and women

Table 3

Dependence of ischemic stroke risk on a season

Compared seasons		Number of studies	Total	Odds ratio	I^2, %	Overall effect test	
Season 1 / total	Season 2 / total					Z	P
Ischemic stroke (all)							
winter / 40080	summer / 42247	28	165,196	0.97 [0.90, 1.04]	93	0.81	0.42
winter / 40080	spring / 42467	28	165,196	1.01 [0.94, 1.10]	94	0.35	0.73
winter / 40080	autumn / 40402	28	165,196	1.05 [1.00, 1.11]	82	2.01	0.04
autumn / 40402	summer / 42247	28	165,196	0.91 [0.85, 0.98]	91	2.65	0.008
spring / 42467	summer / 42247	28	165,196	0.96 [0.89, 1.03]	92	1.11	0.27
spring / 42467	autumn / 40402	28	165,196	1.06 [0.97, 1.15]	94	1.32	0.19
Ischemic stroke (men)							
winter / 10284	summer / 10096	11	40,838	1.05 [0.96, 1.15]	78	1.07	0.29
winter / 10284	spring / 10049	11	40,838	1.00 [0.93, 1.08]	66	0.08	0.94
winter / 10284	autumn / 10409	11	40,838	1.07 [0.98, 1.18]	77	1.53	0.13
autumn / 10409	summer / 10096	11	40,838	0.98 [0.89, 1.08]	81	0.43	0.67
spring / 10049	summer / 10096	11	40,838	1.08 [0.95, 1.23]	90	1.15	0.25
spring / 10049	autumn / 10409	11	40,838	1.11 [0.98, 1.25]	88	1.64	0.10
Ischemic stroke (women)							
winter / 10501	summer / 10047	11	40,809	1.06 [1.03, 1.09]	0	3.66	0.0003
winter / 10501	spring / 9961	11	40,809	1.14 [1.02, 1.26]	80	2.40	0.02
winter / 10501	autumn / 10300	11	40,809	1.11 [1.01, 1.21]	73	2.16	0.03
autumn / 10300	summer / 10047	11	40,809	0.95 [0.87, 1.05]	76	0.96	0.34
spring / 9961	summer / 10047	11	40,809	0.93 [0.84, 1.04]	82	1.26	0.21
spring / 9961	autumn / 10300	11	40,809	0.98 [0.89, 1.08]	78	0.41	0.68
Ischemic stroke (people under 65 y.o.)							
winter / 937	summer / 830	3	3585	1.04 [0.69, 1.58]	77	0.21	0.84
winter / 937	spring / 945	3	3585	0.94 [0.76, 1.17]	36	0.55	0.58
winter / 937	autumn / 873	3	3585	1.10 [0.99, 1.22]	0	1.74	0.08
autumn / 873	summer / 830	3	3585	1.01 [0.73, 1.40]	64	0.07	0.95
spring / 945	summer / 830	3	3585	1.20 [0.74, 1.95]	84	0.73	0.46
spring / 945	autumn / 873	3	3585	1.11 [1.00, 1.24]	1	1.89	0.06
Ischemic stroke (people over 65 y.o.)							
winter / 1828	summer / 1721	3	7133	1.30 [0.95, 1.77]	78	1.66	0.10
winter / 1828	spring / 1788	3	7133	1.21 [0.91, 1.61]	75	1.29	0.20
winter / 1828	autumn / 1796	3	7133	1.57 [0.89, 2.76]	93	1.56	0.12
autumn / 1796	summer / 1721	3	7133	0.92 [0.69, 1.23]	71	0.55	0.58
spring / 1788	summer / 1721	3	7133	1.05 [0.97, 1.14]	0	1.30	0.19
spring / 1788	autumn / 1796	3	7133	1.16 [0.85, 1.58]	75	0.95	0.34

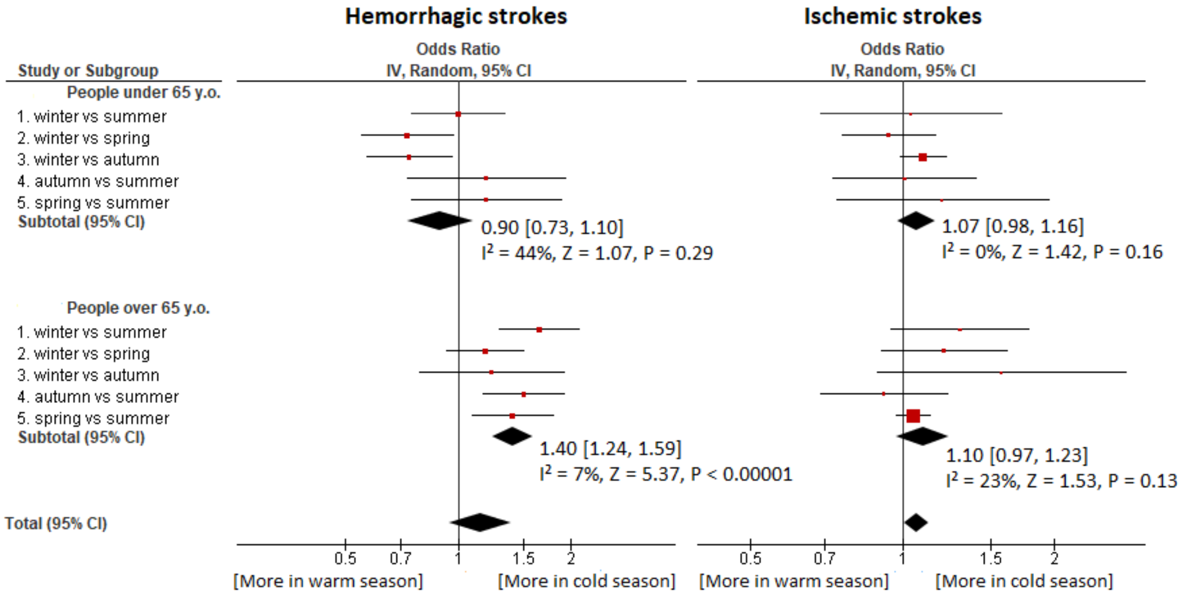

Figure 2. Dependence of strokes risk on a decrease in air temperature for people of different age groups

Age dependence of seasonal stroke risk needs further research due to the small number of studies included in the meta-analysis. Although formally a meta-analysis can be carried out if there are two studies, but, with an increase in the number of studies, the statistical power of the meta-analysis increases, which is especially important when the results tend to be significantly heterogenic [53].

Discussion. High BP is a well-known major factor that can provoke stroke. The seasonal dynamics of HS risk completely coincides with the seasonal dynamics of BP. HS risk and BP level are higher in the cold season compared with the warmer one [5]. In addition to high BP, IS are associated with narrowing and blockage of the arteries, usually as a result of thrombosis or atherosclerosis. Therefore, factors that enhance thrombosis and ischemia will also provoke IS. It has been established that hemoconcentration and lipid profile indicators are maximum in winter [8, 9]. On the other hand, according to the results of our previous meta-analysis, the seasonal dynamics of IS depends on climate of a region [18]. In regions where there was a significant decrease in atmospheric pressure and partial oxygen density in the air and an increase in relative humidity in summer, IS risk at this season increased significantly compared with winter, despite low values of BP, hematocrit and cholesterol in summer. In a climate without significant annual fluctuations in atmospheric pressure and with wet winter, the seasonal dynamics of IS was not pronounced or slightly shifted to winter [18]. According to the results of all publications included in the meta-analysis, the minimum risk of IS was on average in autumn.

Our meta-analysis showed that stroke occurs with approximately the same frequency in men and women, but sex brings nuances to the seasonal dynamics of stroke risk. HS risk was the highest in winter and spring for men, and in winter for women. In addition, the seasonal dynamics of IS (with a maximum risk in winter and a minimum in summer) was expressed in women, but not in men. Seasonal dynamics of levels of sex hormones, which have a protective effect on CVE [54], can also affect the seasonal CVE risk. Testosterone levels in men have been found to be at their lowest in spring [10], which may explain the increased HS risk in men during this season. It is known that there is an inverse relationship between testosterone levels and BP [55]. In women of reproductive age, estrogen levels decrease with a short day and increase with a long day [56, 57]. Melatonin is known to produce an anti-estrogenic effect by inhibiting the aromatase enzyme, which is involved in the synthesis of estrogens from androgenic precursors [58]. In addition, women show a greater dependence between a risk of stroke and abdominal obesity, elevated BP and glucose levels [59–61]. Also, women have been shown to cool faster than men [62]. These reasons may be responsible for the greater winter risk of strokes in women. At the same time, there are observations that women tolerate heat worse than men, in particular, due to a lower level of sweating [63]. Also, women have more pronounced physiological responses to hypoxia [64]. According to our meta-analysis, a trend towards an increased IS risk in summer compared with spring and autumn was observed in women, but not in men.

The meta-analysis has shown that the overall risk of stroke increases in older people, especially the incidence of IS. In addition, HS risk in the group of people older than 65 years increased with decreasing air temperature. In people younger than 65 years, HS risk was not associated with cold. The increase in IS risk in winter compared with other seasons was also slightly greater in the group of older people; however, a decrease in temperature equally increased IS risk in both age groups. Old age is known to be associated with increased BP reactivity to fluctuations in air temperature [13]. Studies have shown that fluctuations in air temperature are more likely to cause strokes in elderly than in young people [65, 66]. On the other hand, according to A.H. Nave et al. (2015) [67], an increase in the level of lipoproteins, as a risk factor for IS, is most relevant for young people.

Prevention of the seasonal risk of stroke should be aimed at minimizing, on the one hand, uncomfortable external conditions (heating, air conditioning, clothing, etc.), and, on the other hand, seasonal changes in the functioning of the body. For the latter, both adherence to a proper lifestyle (moderate nutrition, sufficient fluid intake, physical activity) and drug correction of blood pressure, lipid profile, glucose, and blood viscosity are important. The prevention of seasonal risk of IS is especially in need of research, since IS constitutes the bulk of all strokes. In addition, the seasonal IS risk is often not associated with seasonal increase in BP, lipid profile, and hematocrit, but is associated with hypoxic conditions in summer, which are typical, for example, for East Asia [5, 8, 9, 18]. In this case, an excessive medical decrease in BP in summer may not improve the condition of patients, but, on the contrary, worsen it. It is known that IS can provoke both rise and fall in BP [68]. Many researchers note that the regimen and dosage of antihypertensive drugs need seasonal adjustment [69, 70]. According to the results of our meta-analysis, fluctuations in air temperature are the most dangerous in relation to the risk of stroke for women and people over 65.

Conclusions:

1. HS risk is less likely in a warmer season compared with a colder one. In men, HS risk was the highest in winter and spring; in women, in winter. Dependence of HS risk on a decrease in air temperature is the same in men and women.

2. On average, the minimum probability of IS occurs in autumn. In winter, IS risk in women was significantly higher compared to other seasons. In men, the seasonal dynamics of IS was not expressed.

3. In older people, the overall risk of stroke increased, especially IS. In people over 65 years of age, there was a significant dependence of an increase in HS risk on a decrease in air temperature. In people younger than 65 years, HS risk was not associated with cold. A decrease in temperature equally increased IS risk in both age groups.

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Research article

MARKERS OF COMBINED AEROGENIC EXPOSURE TO METAL OXIDES AND TRANSFORMED PLASMA PROTEOMIC PROFILES IN CHILDREN

M.A. Zemlyanova[1,2,3], N.V. Zaitseva[1,4], Yu.V. Koldibekova[1], E.V. Peskova[1], N.I. Bulatova[1], M.S. Stepankov[1]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation
[2] Perm State University, 15 Bukireva Str., Perm, 614990, Russian Federation
[3]Perm National Research Polytechnic University, 29 Komsomolskii Ave., Perm, 614990, Russian Federation
[4]Russian Academy of Sciences, Medical Sciences Division (Preventive Medicine Section), 14 Leninskii Ave., Moscow, 119071, Russian Federation

Changes in homeostatic balance of the body, primarily at the cellular-molecular level, are a relevant research object in fundamental and applied studies. They can be eligible indicators for predicting negative effects under exposure to chemical risk factors.

The aim of this study was to substantiate markers of a transformed plasma proteomic profile in children. These markers should have prognostic value and an evidence-based association with combined aerogenic exposure to metal oxides (copper and nickel oxides used as an example). We propose an innovative methodical approach based on plasma proteomic profiling that includes the following: identification of identical proteins and genes encoding their expression; quantification of indicators within the 'identical protein – a chemical concentration in blood' system; prediction of negative effects as per indicators of homeostasis destabilization at the cellular-molecular level under chronic aerogenic exposure to chemicals. The proposed algorithm was tested by comparing changed proteins and peptides identified in plasma proteomic profiles of children exposed simultaneously to nickel and copper oxides in ambient air in actual conditions and small rodents under experimental combined and isolated exposure to the analyzed chemicals in levels equal to real ones.

Long-term aerogenic exposure simultaneously to copper and nickel oxides was established to create elevated nickel and copper levels in blood of exposed children substantiated as markers of exposure. They were up to 2.4 times higher against the same indicators in unexposed children and reference levels as well. The results of field observations were verified by elevated levels of the same chemicals in blood under experimental modeling of an equivalent combined exposure performed on biological models. APOBEC1 complement factor (the A1CF gene) was substantiated as an identical proteomic marker based on plasma proteomic profiling in experimental and field investigations. It has an evidence-based association with markers of exposure (nickel and copper simultaneously identified in blood). Lower expression of this protein under persistent combined aerogenic exposure to nickel and copper oxides makes it possible to predict such a negative effect as modification of low density lipoproteins with further induction of atherosclerotic changes in vessels, the latter being a risk factor of cardiovascular diseases.

Keywords: *proteomic markers, markers of exposure, children, biological model, the A1CF gene expression, prediction of negative effects.*

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

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

Yulia V. Koldibekova – Candidate of Biological Sciences, Senior Researcher, Head of the Laboratory for Metabolism and Pharmacokinetics at the Department for Biochemical and Cytogenetic Diagnostic Techniques (e-mail: koldibekova@fcrisk.ru; tel.: +7 (342) 237-18-15; ORCID: http://orcid.org/0000-0002-3924-4526).

Ekaterina V. Peskova – Junior Researcher at the Laboratory of Biochemical and Nanosensory Diagnostics of the Department for Biochemical and Cytogenetic Methods of Diagnostics (e-mail: peskova@fcrisk.ru; tel.: +7 (342) 237-18-15; ORCID: https://orcid.org/0000-0002-8050-3059).

Natalya I. Bulatova – Researcher at the Laboratory of Biochemical and Nanosensory Diagnostics (e-mail: 1179815@mail.ru; tel.: +7 (342) 236-80-18; ORCID: https://orcid.org/0000-0003-3392-9097).

Mark S. Stepankov – Junior Researcher at the Laboratory of Biochemical and Nanosensor Diagnostics (e-mail: stepankov@fcrisk.ru; tel.: +7 (342) 237-18-15; ORCID: https://orcid .org/0000-0002-7226-7682).

It is important to investigate changes in homeostatic balance of the body at the earliest stages in their development, primarily at the cellular-molecular level. This is a promising area in both fundamental and applied research [1]. Proteomic analysis enriches theoretical knowledge on cellular-molecular mechanisms of negative effects thereby increasing the predictive potential of diagnostics when it comes down to some non-communicable diseases. Searching for informative molecular markers is a priority trend in fundamental research in the Russian Federation[1]. New knowledge on modification of etiopathogenesis of diseases caused by exposure to risk-inducing factors provides solid scientific grounds for identifying conditions, causes, occurrence, prevention and reduction of health risks and health harm [2].

Mechanisms of negative effects can be modified due to interaction between chemical risk factors and some genes that modulate expression of certain proteins responsible for providing functional activity of molecular-biological processes [3]. Homeostasis transforms at the cellular-molecular level against the persisting modifying effects produced by chemical factors and this leads to negative health outcomes thereby increasing a risk of a disease[2]. Analysis of genes coding for proteins that are influenced by chemical factors gives an insight into changes in biological functions and molecular networks occurring under chemical exposures. Investigation of such genetic-chemical interactions is a powerful information resource eligible for obtaining and developing knowledge about etiology and molecular mechanisms that underlie modification of processes associated with exposure to chemical risk factors [4, 5].

Proteins have a key role in providing vital activity of cells and the body as a whole. So, identified qualitative and quantitative changes in them can be potentially informative for the earliest detection of negative effects able to induce further substantial functional disorders of critical organs and systems.

Therefore, at present the most promising studies focus on searching for proteomic markers and their combinations as potential molecular targets. They reflect the functional state and characteristics of mechanisms with etiopathogenetic motivation as a response to chemical exposures. Identification of genetic-chemical interactions between expression of a protein marker and exposure factors makes it possible to predict negative health outcomes. This gave grounds for formulating the aim of the present study, which continues the research cycle performed by the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. The cycle concentrates on using 'omic' technologies for substantiating informative molecular-cellular markers of negative effects [6, 7].

The aim of the present study was to identify markers of a transformed plasma proteomic profile in children. These markers should have prognostic value and an evidenced association with combined aerogenic exposure to metal oxides (copper and nickel oxides used as an example)

Materials and methods. We applied an innovative methodical approach to identify and substantiate molecular protein markers changes in which were associated

[1] Programma fundamental'nykh nauchnykh issledovanii v Rossiiskoi Federatsii na dolgosrochnyi period (2021–2030 gody): Rasporyazhenie Pravitel'stva Rossiiskoi Federatsii ot 31.12.2020 № 3684-r [The program of long-term fundamental research in the Russian Federation (2021–2030): the RF Government Order issued on December 31, 2020 No. 3684-r]. *KonsultantPlus*. Available at: https://www.consultant.ru/document/cons_doc_LAW_373604/ (January 21, 2023) (in Russian).

[2] Drozdova E.V., Dudchik N.V., Sychik S.I., Shevlyakov V.V. Integrated toxicity assessment of environmental factors and objects using alternative biological test models: methodology and technology. Minsk, Transtekhnika Belorussian Scientific Research Institute of Transport Publ., 2017, 216 p. (in Russian).

with exposure to airborne copper and nickel oxides. The approach included the following stages:

– confirming actual aerogenic exposure based on identifying the relevant indicators within the ‘chemical levels in the air – chemical levels in biological media’ system;

– comparing protein stains with statistically significantly different intensities based on proteomic plasma profiling and identification of distinguished proteins;

– identifying proteins and peptides that were identical as per the results of experimental and field studies under combined exposure to the analyzed chemicals;

– quantifying parameters of cause-effect relations between identical proteins and peptides and chemical levels in biological media;

– predicting negative health outcomes based on building and analyzing a biotransformation molecular matrix of identical proteins together with identifying their functions, biological processes and expression in tissues.

The suggested algorithm was tested by comparing changed proteins and peptides identified in proteomic plasma profiles of children under actual combined exposure to airborne nickel and copper oxides and small rodents (a biological model) under experimental combined and isolated exposure equivalent to actual levels of the analyzed chemicals in the air.

The experimental studies were performed on female Wistar rats. Twenty four animals were divided into 4 groups, 6 rats in each: the test group No. 1 was exposed to isolated standard nickel oxide in a dose equal to 0.38 mg/kg; the test group No. 2 was exposed to isolated copper oxide in a dose equal to 1.23 mg/kg; the test group No. 3 was exposed to a combination of copper and nickel oxides in the aforementioned doses; the group No. 4 was a reference one kept under the same conditions as the test groups but without any exposure to the analyzed chemicals. The animals in the experiment were exposed to doses of chemicals equivalent to the actual chronic aerogenic exposure considering animals’ body weight and species peculiarities. Blood samples were collected from the rats 24 hours after the exposure. Blood was taken from the sublingual vein in a volume of 3 cm^3.

The experiments were performed in conformity with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123)[3] and the requirements fixed by the Ethics Committee of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

Plasma proteomic profiles were identified for 45 children aged 4–7 years. Twenty-five of them lived under long-term exposure to airborne nickel oxide (0.0034 mg/(kg·day)) and copper oxide (0.0016 mg/(kg·day)) and were included in the test group; the remaining 20 children were not exposed to the analyzed chemicals (the reference group). Children were included into the test group in case they had elevated (≥ 1.2 Rfl) cooper and nickel levels in blood; levels of the analyzed chemicals equaled minimal or reference values in blood of the children from the reference group (nickel Rfl = 0.01 mg/dm^3, copper Rfl = 0.9 mg/dm^3)[4].

The children were examined in conformity with the ethical principles stated in the

[3] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123); the edition of the CED Protocol No. 170 dated December 02, 2005. Strasbourg, 1986, 11 p. Available at: https://www.biosoil.ru/files/docs/bioethics/info/European%20Convention%20for%20the%20Protection%20of%20Vertebrate%20Animals%20used%20for%20Experimental%20and%20Other%20Scientific%20Purposes.pdf (February 01, 2023).

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Declaration of Helsinki (64th WMA General Assembly, 2013[5]) provided that their legal representatives gave their voluntary informed consent to their participation in the study. All the studies conformed to the requirements fixed by the Ethics Committee of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (the Meeting Report No. 1 dated February 04, 2021).

Copper and nickel levels in blood were identified in conformity with the Methodical guidelines MUK 4.1.3230-14[6] using Agilent 7500cx mass spectrometer (Agilent Technologies, USA).

Analysis of plasma proteomic profile included sampling, two-dimensional electrophoresis in polyacrylamide gel[7], analysis of two-dimensional electrophoretogram, spotting out significant protein stains as per their intensity, mass-spectrometric analysis performed with UltiMate 3000 chromatographer (Germany) and ABSciex 4000 QTRAP tandem mass spectrometer with Nanospray 3 ionization source (Canada). Proteins were identified based on the UniProt[8] database with the selection as per *Homo Sapience* taxon and *Rattus norvegicus* taxon. Genes coding for identified proteins were established using the HUGO Gene Nomenclature Committee database (HGNC)[9] and The Rat Genome Database (RGD)[10]. Biological functions of proteins were described using The Gene Ontology[11]; data on phylogenetics and functional genomics were taken from the PhyloGenes[12]; the obtained data on protein expression in tissues were analyzed using Tissue expression database[13] and The Human Protein Atlas[14]. Data about probable etiopathogenetic mechanisms of predicted negative health outcomes associated with chemical exposures were obtained and analyzed by using Comparative Toxicogenomics[15] and DisGeNET[16].

Indicator values identified in the exposed children were compared with the same indicators in the unexposed ones; in the animal experiments, the test groups were compared with the reference one. Descriptive statistics of quantitative variables was given with the mean value (M) and error of mean (m). Statistical significance of differences between compared groups was determined with the Mann – Whitney test ($U \leq Ucr$), the levels of significance being $p \leq 0.05$. All the data were statistically analyzed with Statistica 10.

Molecular markers of negative effects were substantiated based on created models of 'chemical levels in blood – statistically significant intensity of a protein stain' relation-

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ships. The latter were described with a multiple liner regression as per the formula (2):

$$y_j = b_{0j} + \sum_i b_{ij} x_i \qquad (2)$$

where y_j is a dependent variable (intensity of the j[th] protein stain, int);

x_i is an independent variable, i[th] influencing factor (chemical level in blood, mg/dm^3);

b_{0j}, b_{ij} are the model coefficients.

Validity and relevance of the created models were compared with dispersion analysis using the Fisher's F-test, determination coefficient (R^2), and the Student's t-test, the statistical significance being $p \leq 0.05$.

Results and discussion. Comparative analysis of copper and nickel levels in blood of the experimental animals established authentic statistical differences between the groups. Isolated exposure to nickel created its levels in blood equal to 0.014 ± 0.002 mg/dm^3; this level was 0.008 ± 0.001 mg/dm^3 under combined exposure to both analyzed chemicals. These concentrations were 2.9 times and 1.7 times higher accordingly than in the reference group (p = 0.001–0.012). Copper level in blood equaled 2.323 ± 0.060 mg/dm^3 under isolated exposure and 2.006 ± 0.047 mg/dm^3 under combined exposure, being 1.5 times and 1.3 times higher accordingly than the same indicator in the reference group (p = 0.0001–0.002). Nickel and copper levels in blood were 1.2 times and 1.8 times higher accordingly under isolated exposure than under combined one (p = 0.002–0.022).

Actual simultaneous exposure to copper and nickel oxides created elevated blood levels of the analyzed chemicals in the exposed children; these levels were 1.2–2.4 times higher against the same indicators in the unexposed children (p = 0.032–0.033) and 1.2–1.3 times higher than the reference levels. The established and parameterized association of copper and nickel in blood and levels of these chemicals in ambient air (copper a_0 = 0.515, a_1 = 752.32; nickel a_0 = 0.005, a_1 = 145.36, p = 0.05) substantiates copper and nickel levels in blood as relevant markers of aerogenic exposure.

We compared the results obtained by densitometry of plasma proteomic profiles in the experimental animals under isolated and combined exposure to the analyzed chemicals. The comparison identified 8 proteins identical in all the experimental groups (Table 1).

We performed densitometry to study and compare proteins in plasma proteomic profiles of the exposed and unexposed children. As a result, we identified 20 stains with authentically different intensity and a proven association with elevated nickel and copper levels in blood (Table 2).

Table 1

Statistically significant proteins proven to be associated with experimental combined exposure

No.	Statistically significant proteins			Validity of the model for 'chemical – intensity of a protein stain under combined exposure' relationship ($p \leq 0.05$)
	Isolated exposure		Combined exposure	
	copper	nickel	copper + nickel	
1	2	3	4	5
1.	Telomerase protein component 1	-	Telomerase protein component 1	0.002
2.	Keratin, type II cytoskeletal 75	-	Keratin, type II cytoskeletal 75	0.0001
3.	**Peroxisomal 2,4-dienoyl-CoA reductase**	-	**Peroxisomal 2,4-dienoyl-CoA reductase**	0.002
4.	Advillin	-	Advillin	0.019

End of the Table 1

1	2	3	4	5
5.	Cytosolic aldehyde dehydrogenase 1	-	Cytosolic aldehyde dehydrogenase 1	0.008
6.	-	Elongation factor 1-γ	Elongation factor 1-γ	0.001
7.	-	Styrene carrier protein 2	Styrene carrier protein 2	0.002
8.	-	Myosin-6	Myosin-6	0.002
9.	-	Calcium-binding protein 7	Calcium-binding protein 7	0.002
10.	-	Vesicular transporter SEC22B	Vesicular transporter SEC22B	0.001
Statistically significant proteins identified under both isolated and combined experimental exposure				
11.	Hemoglobin subunit β-2			0.001
12.	Glycogen synthase, muscular			0.001
13.	**CAP-GLY** domain containing **linker 4**			0.002
14.	Vesicular transporter SEC22A			0.001
15.	Protein SDA1 homolog			0.003
16.	Transcription activator BRG1			0.001
17.	Ig kappa chain C region, allele			0.001
18.	APOBEC1 complement factor			0.006

Table 2

The parameters of the multi-factor relationship between changes in intensity of a protein stain and simultaneous copper and nickel levels in blood of the exposed children

No.	Protein in a stain	A change in intensity of a protein stain	The parameters of the 'exposure marker (nickel and copper in blood) – proteomic marker (intensity of a protein stain)' relationship				Validity of differences ($p \leq 0.05$)
			b_0	Copper (b_1)	Nickel (b_2)	Determination coefficient (R^2)	
1.	Sodium/hydrogen exchanger 2	Decline	5291.9	-1458	-54,723	0.24	0.013
2.	Protein 33 with a spiral coil	Decline	5245.2	-1202.9	-63,777.4	0.21	0.025
3.	Myotubularin	Decline	1803	-1065.8	-39,425.8	0.23	0.016
4.	Coagulation factor V	Decline	439.2	-270.6	-7449.8	0.19	0.032
5.	Ornithine decarboxylase antizyme 2	Decline	1112.9	-616.7	-28,418.7	0.25	0.011
6.	RING finger protein unkempt homolog	Decline	2656.4	-821.9	-39,891.8	0.19	0.031
7.	Vitronectin	Decline	3092.7	-1860.8	-40,674.4	0.16	0.059
8.	Centrosomal protein 290kDa	Decline	2242.6	-830.3	-49,443.7	0.30	0.003
9.	Zinc finger protein 221 protein	Decline	2989.8	-1496.5	-49,250.8	0.24	0.013
10.	Apolipoprotein A-I	Decline	936.8	-660.6	1007.4	0.19	0.032
11.	ADAM-like, decysin 1	Decline	3739.1	-1020.1	-62,371.9	0.25	0.010
12.	Nuclear protein MDM1	Growth	1596.3	935.8	38,990	0.17	0.048
13.	APOBEC1 complement factor	Decline	3581.2	-384.5	-11,137.7	0.13	0.017
14.	DNAJ homolog subfamily C member 3	Growth	2231.2	1747.2	63,004.9	0.24	0.013
15.	WD repeat protein 64	Growth	1014.9	768.6	44,043.7	0.22	0.019
16.	L-type beta-4 voltage-gated calcium channel subunit	Decline	1391.3	-861.5	-22,471.4	0.25	0.010
17.	Leucine-rich repeats and immunoglobulin-0like domains, protein 3	Decline	2531.7	-1032.3	-40,311.9	0.19	0.033
18.	Protein-glutamine gamma-glutamyltransferase e	Growth	394.6	-35.5	32,116.8	0.19	0.032
19.	Pyroglutamyl-peptidase 1-like protein	Growth	1657.4	666.3	39,792.4	0.17	0.048
20.	Clathrin heavy chain 2	Decline	2605.8	-1031.1	-95,362.5	0.36	0.001

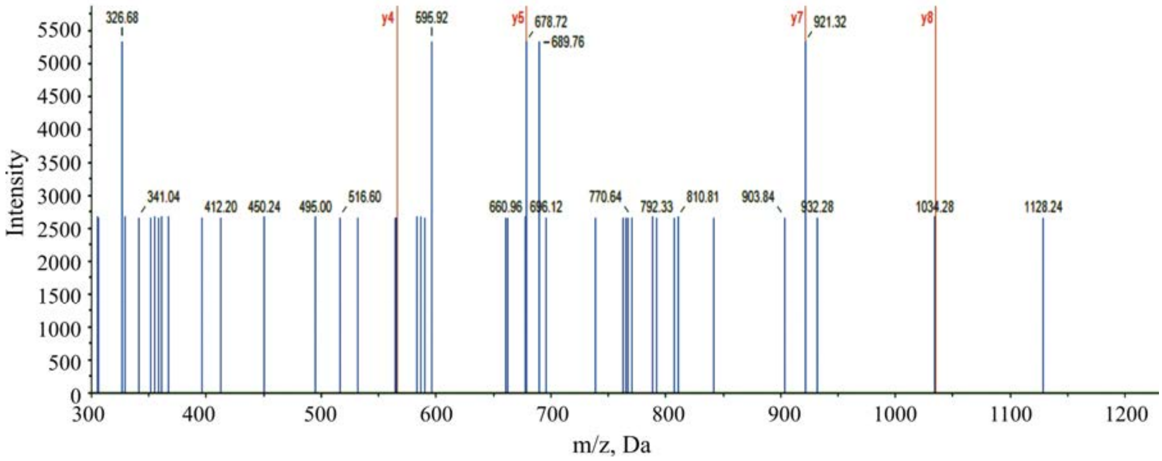

Figure. The spectrum of the SGPGLSGTQK peptide (APOBEC-1 complement factor) (SwissProt database) in a child's plasma

We performed comparative biotransformation analysis of phylogenetics of the identified proteins and genes coding for their expression under experimental and actual combined exposure. As a result, we established one identical protein, APOBEC1 complement factor, and the orthologous A1CF gene encoding it in the human body. The Figure provides the spectrum of the identical protein peptide.

APOBEC1 complement factor is a vital component in the enzymatic complex. It modifies mRNA of apolipoprotein B (ApoB), which is necessary for collecting low density lipoproteins (LDL) from lipids. In rats (Rattus norvegicus), APOBEC1 complement factor is widely spread in the liver [8], kidneys [9], intestines [10], thyroid gland, and the nervous system [11]. In the human body, this factor is expressed solely in GIT epithelial cells and small intestines [12, 13]. Several experimental studies have established that APOBEC-1 super-expression in the liver reduces ApoB levels effectively thereby regulating cholesterol metabolism [14]. Low A1CF gene expression is a reason why consumption of fats in high quantities is potentially hazardous for human health. As exogenous lipids are being absorbed, the ApoB level grows thereby inducing increased synthesis of low density lipoproteins [15], which may lead to atherosclerotic changes in the vessels [14, 16].

We identified and estimated a multi-factor relationship ($R^2 = 0.19$; $b_0 = 3581.2$; $b_1 = -384.5$; $b_2 = -11{,}137.7$; $p = 0.017$) between a decline in intensity of the APOBEC-1 complement factor in plasma and elevated nickel and copper levels as markers of exposure. This relationship is consistent with the results of research focusing on genetic-chemical interactions between the protein and analyzed chemicals. Exposure to copper and nickel has been shown to reduce the A1CF gene expression. This indicates the analyzed chemicals are able to modify molecular functions and biological processes of this protein [17]. Some experimental research established that exposure to nickel caused elevated triglycerides and LDL levels in blood serum and this can have some negative effects on lipid metabolism as a whole [18]. Exposure to airborne copper can induce higher production of reactive oxygen species and oxidative stress. As a result, LDL oxidative modification occurs; this induces a local immune response in vessel walls with subsequent atherosclerotic changes developing in them as a risk factor of cardiovascular diseases [19–21].

Conclusion. Our research has established that long-term combined exposure to airborne copper and nickel oxides creates elevated copper and nickel levels in blood of the exposed children. These levels are 1.2–2.4 times higher than the same indicators in the unexposed children and are also higher than the reference levels for these chemicals. Therefore, they are substantiated as marker of exposure. Results of the field observations have been verified by 1.7–2.9 times higher levels of the same chemicals (against the reference group) established in experimental modeling of the equivalent combined exposure on a biological model (small rodents). We have substantiated APOBEC1 complement factor (the A1CF gene) as a proteomic marker identical under both experimental and actual exposure. It has a proven association with markers of exposure (simultaneous nickel and copper levels in blood). Declining expression of this protein under persistent exposure to airborne copper and nickel oxides makes it possible to predict developing negative health outcomes such as modification of low density lipoproteins with subsequent induction of atherosclerotic changes in the vessels, which are a risk factor of cardiovascular diseases.

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Research article

EFFECTS OF SELENIUM OXIDE NANOPARTICLES ON THE MORPHOFUNCTIONAL STATE OF THE LIVER: EXPERIMENTAL DATA

Yu.V. Ryabova, M.P. Sutunkova, A.I. Chemezov, I.A. Minigalieva, T.V. Bushueva, I.G. Shelomentsev, S.V. Klinova, R.R. Sakhautdinova

Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, 30 Popov Str., Yekaterinburg, 620014, Russian Federation

Copper smelters are the sources of emission of complex aerosols containing, inter alia, selenium-containing nanoparticles (NPs). It is very difficult to adequately estimate the hazard posed by such particles since available data on them are scarce and have been obtained in comparatively few experimental studies with rather contradicting results.

The aim of our study was to determine toxic health effects of selenium-containing nanoparticles more precisely with a focus on liver as a target organ.

Liver toxicity following exposure to suspended selenium oxide nanoparticles was investigated in a sub-chronic experiment on outbred male albino rats. The suspension was prepared by laser ablation of 99%-pure selenium plates. We examined ultrastructural changes by electron microscopy, did cytological and histological analyses of the liver, biochemical blood testing and metabolomic blood screening.

We observed lesions in the liver and inhibited secretory functions at various levels, from molecular to organismic, in the exposed animals. The microscopic examination showed that the number of normal and normal-vesicular mitochondria in liver cells went down by 7.78 %, $p < 0.05$; the metabolomic screening established lower levels of glycocholic acid in blood serum, $p < 0.001$; levels of alanine aminotransferase in blood serum grew by 30 %, $p < 0.05$; the number of acaryotic hepatocytes demonstrated a 3.1-fold increase, $p < 0.05$, according to the results of histological assessment of liver specimens. The touch smears of the liver examined showed a 2.2-fold increase in the number of degenerated hepatocytes ($p < 0.05$).

These experimental data can be used to estimate a potential hazard of selenium-containing nanoparticles within social-hygienic monitoring and biomedical predictions of health damage caused by exposure to such NPs. Altered levels of lysophosphatidylinositol can be a marker of exposure to the examined NPs and necessitate the search for early diagnostic predictors of associated health disorders.

Keywords: *toxicity, hazard assessment, nanoparticles, selenium, liver, mechanism of action, in vivo, experiment.*

Yuliya V. Ryabova – Junior Researcher at the Department of Toxicology and Bioprophylaxis (e-mail: ryabovaiuvl@gmail.com; tel.: +7 (343)253-87-54; ORCID: https://orcid.org/0000-0003-2677-0479).

Marina P. Sutunkova – Doctor of Medical Sciences, director (e-mail: sutunkova@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-1743-7642).

Aleksei I. Chemezov – Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: chemezov@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0001-6167-7347).

Ilzira A. Minigalieva – Doctor of Biological Sciences, Head of the Department of Toxicology and Bioprophylaxis (e-mail: ilzira-minigalieva@yandex.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-0097-7845).

Tatiana V. Bushueva – Candidate of Medical Sciences, Head of the Laboratory Diagnostics Department (e-mail: bushueva@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-5872-2001).

Ivan G. Shelomentsev – Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: shelomencev@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-8795-8777).

Svetlana V. Klinova – Researcher at the Department of Toxicology and Bioprophylaxis (e-mail: klinova.svetlana@gmail.com; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-0927-4062).

Renata R. Sakhautdinova – Candidate of Medical Sciences, Head of the Department of Laboratory and Diagnostic Technologies (e-mail: sahautdinova@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-2726-9259).

Copper smelters are sources of emission of complex aerosols containing, *inter alia,* selenium-containing nanoparticles (NPs). The latter become airborne when copper is refined either by electrolysis or in the melt flow. Workers of copper smelting, electrolysis or chemical metallurgy workshops are exposed to these aerosols.

It is very difficult to adequately estimate the hazard posed by selenium-containing nanoparticles since available data on them are scarce and have been obtained in comparatively few experimental studies with rather contradicting results. Toxic effects produced by nanoparticles are determined not only by their physical properties but also specific features of the basic chemical element [1]; obviously, consideration of data on biological activity of selenium should not be limited to its nano-sized form.

Adverse health effects of selenium are widely known. Since this trace element is biologically close to sulfur, it is able to replace the latter in chemical compounds [2, 3]. Selenium is a known pro-oxidant [4]. Hydrogen selenide, a selenium metabolite, is able to inactivate metal-containing enzymes, primarily oxidases [5]. Some studies report a negative impact of selenium on carbohydrate metabolism, its potential role in the development of type 2 diabetes mellitus [6], and contribution to cognitive impairments [7, 8]. Selenium affects multiple organs, with liver being its major target [9, 10].

Harmful effects of selenium-containing NPs have been described elsewhere [11] but the state of the liver has been addressed only in few experiments and *in vivo* studies showing inconsistent findings. Thus, authors describe rather ambiguous changes in activity of so-called 'liver' enzymes in blood serum: alanine transaminase became either more [12–14] or less active [15, 16] while aspartate transaminase became more active [17], less active [16], or its activity did not change [12, 15]. As for alkaline phosphatase, its activity in blood serum grew unambiguously [15, 17] following the exposure to selenium NPs. Histological assessment of the liver did not establish any apparent dystrophic changes though it was noted that exposure to high doses of selenium-containing NPs (5 [15] and 8 [17] mg Se/kg body weight) induced destruction of hepatocytes [15, 17]. Besides, accumulation of Se NPs in the body led to a significant increase in the selenium content in the liver [15, 16, 18, 19]. It is noteworthy that both selenium as a trace element and selenium-containing NPs in higher concentrations are pro-oxidants able to induce production of reactive oxygen species [20], and the process may be aggravated by their bioaccumulation in the liver [11].

The aim of our study was to determine toxic effects of selenium oxide nanoparticles on liver in experimental animals.

Materials and methods. Toxic effects of selenium nanoparticles (NPs) were investigated using selenium oxide nanoparticles (SeO NPs). The suspension of SeO NPs was prepared at the Ural Center for Shared Use "Modern Nanotechnologies" of the Ural Federal University, Yekaterinburg, Russian Federation, by pulsed laser ablation in sterile deionized water using thin selenium plates (99.99 %). Shapes and sizes of the analyzed particles were identified using scanning electron microscopy and described with a graph showing particle size distribution, the mean diameter equaling 51 ± 14 nanometers (Figure 1).

Stability of the suspensions was estimated by identifying their zeta potential and appeared to be quite high (up to 42 mV). This allowed us to raise particle concentrations in these suspensions to 0.25 mg/ml by partial water evaporation at 50 °C affecting neither size distribution nor chemical identity of NPs.

Health effects of SeO NPs were estimated in an experiment on 12 outbred male albino rats aged 4 months at the beginning of the experiment. The control group also included 12 rats, with their initial body weight of all animals varying between 200 and 270 grams (± 20 % of the mean). Subchronic poisoning was simulated by repeated intraperitoneal injections, thrice a week for 6 weeks (18 injections in total). Doses were selected based on the results of previous experimental studies. Solutions of the stable NP suspension were introduced as follows: 1 ml of the suspension in a single dose of 0.2 mg/kg and 1 ml of deionized water (SeO NPs 0.1 group);

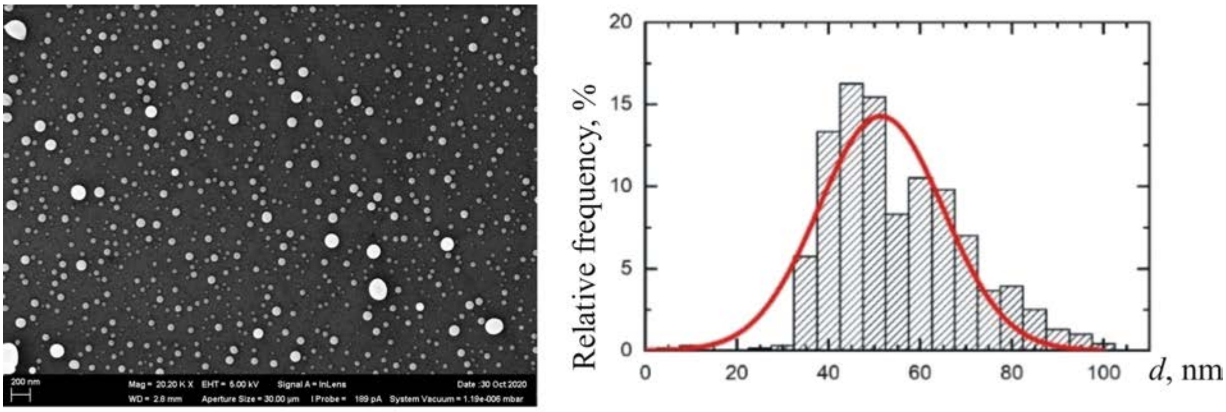


Figure 1. SEM-image of SeO NPs in a suspension prepared for the experiments (scanning electron microscopy, 20,200 × magnification) and a graph showing particle size distribution

2 ml of the suspension in a single dose 1 mg/kg (SeO NPs 0.5 group); 2 ml of the suspension in a single dose 2 mg/kg (SeO NPs 1 group); 2 ml of deionized water (Control). Animals were kept, bred and taken out of the experiment in accordance with conventional requirements. The study was approved by the Local Ethics Committee of the Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, Protocol No. 2 of April 20, 2021.

Following the exposure cessation, we did serum biochemistry using Cobas Integra® 400 plus analyzer (Roche Diagnostics GmbH, Germany) and relevant diagnostic sets. Activity of succinate dehydrogenase (SDH) in blood lymphocytes was identified and then used as an indicator of bioenergetic metabolism [21].

High performance liquid chromatography – mass spectrometry (HPLC-MS) was used for metabolomic screening. Chromatographic separation was accomplished using a liquid chromatographer with C18 column in gradient elution mode and detection was performed with a quadrupole time-of-flight mass spectrometer. A set of value pairs 'm/z – signal intensity' was obtained for each experimental group. These values corresponded to individual metabolites in blood. A mean or a median (in case of a non-normal distribution) value of a signal was established for each substance in the experimental group. The data obtained for each group were then compared to establish statistical differences. The ultimate results included the values demonstrating more than a two-fold change before/after comparison of the experimental results. Generalization as per an exact mass and fragmentation spectra was then performed for the selected m/z values; these spectra were obtained by repeated analysis using a mass spectrometer in tandem mode with different energy levels. The resulting spectra were analyzed using free-access databases (HMDB, MoNA, METLIN, MassBank EU).

Cell ultrastructure was estimated using a Hitachi REGULUS SU8220 scanning electron microscope in the STEM mode. Mitochondria were classified in accordance with Sun et al. [22] based on the topology of mitochondrial inner membrane (matrix density and homogeneity, the number of cristae) [22]. In calculations, type A mitochondria (normal) and type B mitochondria (normal-vesicular) were considered normal whereas type C (vesicular), type D (vesicular-swollen), and type E (swollen) were considered pathologically altered.

For imprint cytology, we did touch smears of cross-sections of the liver, kidneys, spleen, and mesenteric lymph nodes, let them dry at room temperature, and then stained using Leishman's staining method. Cell composition and signs of cell damage were estimated using a Carl Zeiss Primo Star light binocular microscope with USCMOS video camera at 100× and 1000× magnifications.

We examined histological changes in the liver of rats from the SeO NP exposure and control groups. Acaryotic hepatocytes and Kupffer cells were quantified by morphometric analysis using Avtandilov mesh.

The statistical significance of differences in mean values of indicators describing toxic effects was estimated by using Student's t-test with a correction for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Results and discussion. The data obtained by metabolomic blood screening were analyzed using the principal component technique. The analysis revealed certain clusterization of samples in the test groups before/after comparison thereby indicating substantial changes in blood of the experimental animals (Figure 2). Samples of the animals from the control group created one cluster excluding one animal; obviously, it had certain deviations in its blood composition.

Only some of the selected substances had enough intensity of an analytical signal to obtain informative fragment spectra; therefore, generalization was not possible for all the metabolites in the groups. The metabolites identified belonged to lipids and phospholipids (Table 1).

Levels of acylcarnitines and their derivatives (decanoylcartinine, hydroxydecenoylcarnitine, hydroxyhexadecanoylcarnitine, tetradecadienoylcarnitine, see Table 1, Nos. 3–6) increased in all the groups.

On the one hand, these metabolites transport fatty acids through the carnitine shuttle in mitochondrial inner membranes for further beta-oxidation; on the other hand, they transport excessive products of this process from mitochondria to the extracellular space since excessive quantities of oxy-Acyl-CoA derivatives disrupt mitochondria functions up to initiation of apoptosis [23–25].

Variations in contents of the aforementioned substances indicate ongoing changes in beta-oxidation of fatty acids that takes place in mitochondria; this was previously shown for exposure to selenium salts [26] and for the first time has been demonstrated in our study for exposure to selenium NPs.

The assumed damage to mitochondria mediated by their functional disorders was confirmed microscopically. Ultrastructural investigation established a decrease in the proportion of normal mitochondria (type A and B as per Mei G. Sun [22], 87.44 ± 1.14 % in the SeO NPs 1 group against 94.82 ± 0.95 % in the control group, $p < 0.05$) in liver cells.

Pathological changes in mitochondria, so-called 'energy stations' within a cell, were identified by metabolomic analysis and visualized with electron microscopy. These changes led to a decrease in their energy potential. The latter was indirectly evidenced by a statistically significant, monotonic and dose-dependent decline in activity of succinate dehydrogenase in blood lymphocytes following the exposure to SeO NPs (517.50 ± 2.58 formazan granules in 50 lymphocytes in the SeO NPs 0.1 group against 575.78 ± 6.10 formazan granules in 50 lymphocytes in the control group, 495.14 ± 6.91 formazan granules in 50 lymphocytes in the SeO NPs 0.5 group and 484.00 ± 7.14 formazan granules in 50 lymphocytes in the SeO NPs 1 group against the control group and the SeO NPs 0.1 group, $p < 0.05$). This decline in the activity of succinate dehydrogenase can be attributed to the ability of selenium to replace sulfur in compounds as described elsewhere [3].

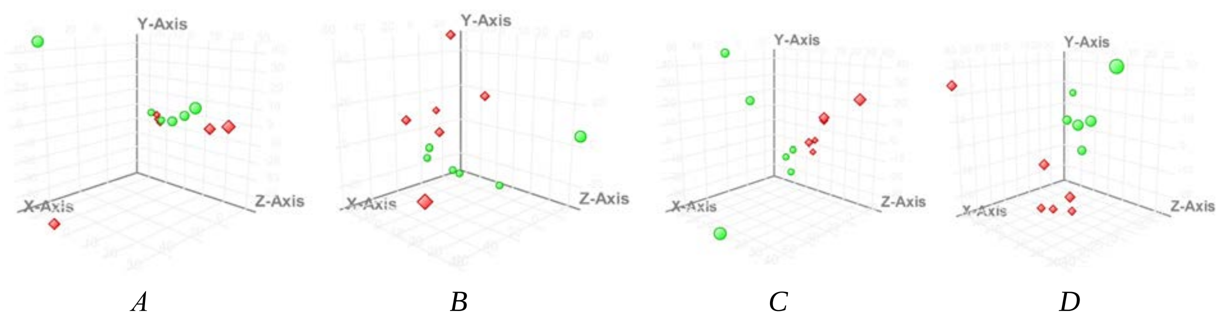


Figure 2. Results obtained by Principal Component Analysis of HPLC-MS spectra of blood samples (● pre-exposure, □ post-exposure): A is the control, B is SeO NPs 0.1 group, C is SeO NPs 0.5 group, C is SeO NPs 1 group

Table 1

Metabolomic screening of rats' blood following sub-chronic exposure to selenium oxide nanoparticles

No.	Substance	Groups and changes in metabolite levels, before/after comparison					
		SeO NPs 0.1	*p*	SeO NPs 0.5	*p*	SeO NPs 1	*p*
1	Bile acid	-		↓	0.029	-	
2	Glycocholic acid	-		↓	< 0.001	-	
3	Decanoylcartinine	-		↓	0.008	-	
4	Hydroxydecenoylcarnitine	-		↓	< 0.001	↓	0.006
5	Hydroxyhexadecanoylcarnitine	↓	0.041	↓	0.005	↓	0.048
6	Tetradecadienoylcarnitine	-		↑	< 0.001	↑	0.007
7	Tetracosahexaenoic acid	-		↓	0.043	-	
8	Methyl arachidonate	-		↑	0.003	↑	0.001
9	Methyl hexadecanoic acid	-		↑	0.004	↑	< 0.001
10	Methyl linoleate	↑	< 0.001	↑	< 0.001	↑	< 0.001
11	Methyl-[10]-gingerol	↑	0.004	↑	0.002	-	
12	13'-hydroxy-alpha-tocopherol	↑	0.026	-		↑	0.020
13	Phosphorylcholine	↑	0.008	-		↑	0.006
14	PC(16:1/2:0)	-		-		↓	0.029
15	PC(3:0/2:0)	-		↑	< 0.001	-	
16	LPC(9:0)	↓	0.008	-		-	
17	LPC(18:2)	↑	0.008	-		-	
18	LPC(18:3)	-		↑	0.008	-	
19	LPC(20:4)	↑	0.007	↑	0.046	-	
20	LPC(28:6)	-		↓	0.006	-	
21	2-(9-Oxononanoyl)-glicero-3-phosphocholine	-		-		↓	0.006
22	LPE(18:2)	-		↑	0.045	-	
23	PS(3:0/2:0)	-		↑	0.046	-	
24	LPI(18:0)	-		-		↑	0.040
25	LPI(20:4)	↑	< 0.001	-		-	

Notes: PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; LPI, lysophosphatidylinositol. The first figure in brackets stands for a carbon chain length of a fatty acid fragment in a compound and the second one stands for the number of double bonds in it. The symbol '↑' means growing intensity of an analytical signal of a metabolite mass in before/after comparison; the symbol '↓', declining intensity; '-' means no significant changes in contents were identified for this substance in the given experimental group.

NPs are easily transported along the bloodstream and penetrate into cells of various organs [27]; this capability, together with the fundamental role of succinate dehydrogenase in the chain for mitochondria electron transportation, determines functional changes in vital organs and systems. The liver is the first organ to be affected, which is quite logical with its barrier function borne in mind. Besides, toxic effects of nanoparticles depend on their chemical essence [1] and the liver is the major depot [10] and target organ for toxic effects of selenium [9].

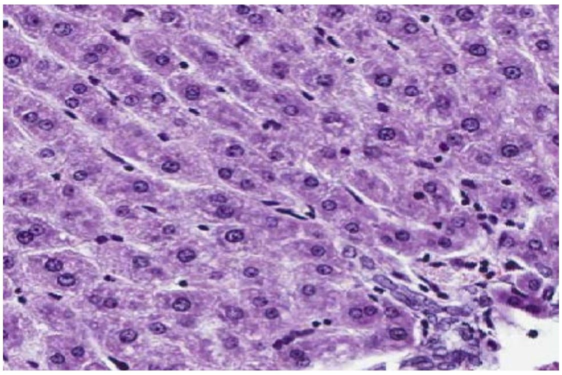
Morphofunctional changes in the liver were established by cytological examination of touch smears of the liver, histomorphological analysis of liver tissues, biochemical testing of blood serum, and metabolomic analysis.

The cytological examination of touch smears of liver showed an increase in the proportion of degenerated hepatocytes (16.33 ± 0.92 % in the SeO NPs 0.5 group and 17.33 ± 0.99 % in the SeO NPs 1 group against 6.33 ± 0.49 % in the control group and 6.83 ± 0.79 % in the SeO NPs 0.1 group).

Histomorphology revealed a drastic growth in the number of acaryotic hepatocytes (27.60 ± 1.46 cells in the SeO NPs 1 group against 8.90 ± 0.56 cells in the control group, $p < 0.05$) and Kupffer cells (20.50 ± 0.71 cells in the SeO NPs 1 group against 11.50 ± 0.58 cells in the control group, $p < 0.05$). The liver structure of the rats from the control group corresponded to its histological standard: hepatocytes forming liver plates and portal tracts were intact; there were apparent dystrophic changes in hepatocytes and acaryotic hepatocytes in the liver of the rats from the SeO NPs 1 group (Figure 3).

Alanine transaminase (ALT) became more active in blood serum of the rats from the SeO NPs 1 group against the controls and the difference was statistically significant. Levels of alkaline phosphatase (AP) went down in all the animals exposed to selenium nanoparticles and this decrease was dose-dependent but statistical significance was established only for the difference between the SeO NPs 1 group and the controls (Table 2).

All these trends combined, including an increase in ALT activity, lower AP levels (Table 2), degenerative changes in hepatocytes (Figure 3), lower levels of bile and glycocholic acid (Table 1, Nos. 1–2), that were identified in the rats exposed to SeO NPs can indicate that exposure to SeO NPs damages the liver and impairs its secretory functions [26, 28]. The latter is consistent with the less intensive analytical signal of lysophosphatidylcholines LPC (9:0) and LPC (28:6) (Table 1, Nos. 16, 20) with short and very long fatty acids in their structure since these substances are synthesized predominantly in the liver, participate in transportation of fatty acids, and are precursors of membrane phospholipids [29]. On the other hand, an increase in the levels of lysophosphatidylcholines LPC (18:2), LPC (18:3) and LPC (20:4) (Table 1, Nos. 17–19) can indicate that inflammatory processes have intensified in the experimental animals [30]. The aforementioned substances, being transporters of linoleic, linolenic and arachidonic acids, are precursors of eicosanoids, or inflammation mediators, and can be synthesized directly from membrane phospholipids due to effects of phospholipase A [31].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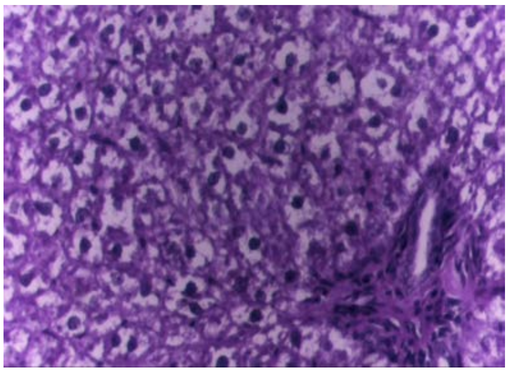
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Figure 3. A rat's liver (stained with hematoxylin – eosin, 100× magnification): A is the control group, B is the SeO NPs 1 group

Table 2

Indicators describing the state of the liver of rats following subchronic exposure to selenium oxide nanoparticles

Indicators	Control	SeO NPs 0.01	SeO NPs 0.5	SeO NPs 1
ALT in blood serum, U/l	42.96 ± 2.55	**56.64 ± 3.47 ***	56.44 ± 4.95	46.58 ± 3.76
AP in blood serum, U/l	199.28 ± 9.45	189.87 ± 15.45	175.05 ± 10.08	**127.76 ± 12.37 *♦●**

Notes: * statistically different from the control groups; ♦ from the SeO NPs 0.1 group; ● from the SeO NPs 0.5 group ($p < 0.05$, Student's t-test).

Inhibition of liver secretory functions is also evidenced by accumulation of fatty acid esters in blood. These esters are decomposed into very low density lipoproteins by liver phospholipases with a simultaneous decrease in levels of tetracosahexaenoic acid, which may occur when sulfur is replaced with selenium in lipases with a relevant decline in their activity [3].

In addition, we should note that lysophosphatidylethanolamines are known to be able to inhibit phospholipase synthesis in the liver but the mechanism of action has not been clarified yet [32]. Elevated LPE (18:2) levels in the SeO NPs 0.5 group are well in line with the highest concentrations of fatty acid esters identified in blood of the rats from this group.

A statistically significant increase in the levels of lysophosphatidylinositols, which are primarily synthesized in the liver (80 % of the total pool) [33], was observed in the SeO NPs 0.1 group and SeO NPs 1 group (Table 1, Nos. 24, 25). We did not identify it in the SeO NPs 0.5 group probably because the liver functions were the most inhibited in this group due to changes in levels of bile acid and fatty acid esters described above. Lysophosphatidylinositols act as precursors for synthesis of phosphatidylinositol di – and triphosphates, cell membrane modifiers able to change its fluidity and thereby promote changes in activity of membrane channels [34]. Fusion and decomposition of phospholipid membranes are known to be associated with the latter being enriched with LPI derivatives [35].

On the other hand, these substances are ligands for GPR55 [36], a receptor conjugated with G-protein, which mediates intracellular signal cascades and stimulates several processes including exocytosis–secretion of insulin and neuromediators; production of pro- and anti-inflammatory interleukins, phospholipase–synthesis of pro- and anti-inflammatory eicosanoids; cell proliferation and migration; stimulation of angiogenesis under artificial LPI introduction was shown in tumor cells [35, 37–39].

Changes in the levels of lysophosphatidylinositols have never been established previously in studies addressing metabolome responses of the body to effects produced by selenium compounds including salts.

Conclusion. Barrier functions performed by the liver as well as the liver being the major depot and target organ for toxic effects of selenium determine a cascade of impairments at all levels. At organismic level, contents of alanine transaminase and alkaline phosphatase in blood serum were established to have changed by 30 % and 57 %, respectively, $p < 0.05$. At cellular and tissue levels, the number of degenerated hepatocytes was established to have grown by 2.2 times, $p < 0.05$; at sub-cellular level, the proportion of normal mitochondria (types A and B as per Mei G. Sun) went down by 7.78 % in the liver of exposed animals, $p < 0.05$. For the first time, changes were identified at molecular level following the exposure to SeO NPs; specifically, there were changes in beta-oxidation of fatty acids occurring in mitochondria due to effects produced by selenium-containing nanoparticles. They were estimated by changes in levels of acylcarnitines and their derivatives in rats' blood. We also established a decrease in cell energy potential, which was indirectly evidenced by a 16 % decrease in activity of succinate dehydrogenase, $p < 0.05$. Besides, damage to the liver and inhibition of its secretory functions can be further confirmed by accumulation of fatty acid esters in blood, elevated levels of lysophosphatidylethanolamines, lower levels of bile and glycocholic acid.

These experimental data can be used to estimate a potential hazard of selenium-containing nanoparticles as a chemical risk factor, both environmental and occupational one, within socio-hygienic monitoring and biomedical predictions of health damage related to exposure to such NPs. Such assessment should consider the established capability of selenium-containing nanoparticles to affect the metabolome profile and produce subchronic toxic effects on warm-blooded animals.

Changes in the levels of lysophosphatidylinositol that have been established in our study for the first time in response to selenium exposure can be used as a starting point in searching for early diagnostic predictors of health disorders induced by exposure to the examined NPs.

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Research article

POLYMORPHISMS OF XENOBIOTIC METABOLISM ENZYME GENES CYP2E1, GSTM1, GSTT1, EPHX1 AS BIOMARKERS OF SENSITIVITY TO EXPOSURE TO WATER DISINFECTION BYPRODUCTS (USING CHLOROFORM AS AN EXAMPLE)

E.V. Drozdova[1], K.V. Kaliasniova[1], V.E. Syakhovich[2], N.A. Dalhina[1]

[1]Scientific and Practical Center for Hygiene, 8 Akademicheskaya Str., Minsk, 220012, Republic of Belarus
[2]National Anti-Doping Laboratory, 31 ag. Lesnoi, Minsk region, 223040, Republic of Belarus

Chloroform accumulation in the body and the increase in its steady-state concentrations in blood of exposed people have been established to be associated with polymorphisms in enzyme genes involved in metabolism of water disinfection byproducts (A415G of EPHX1 gene, C1091T of CYP2E1 gene, deletions of GSTT1 and GSTM1) ($p < 0.000001$). These genes polymorphisms correlate with higher chloroform levels in blood of people consuming chlorinated drinking water: by 43.8 % and higher for GSTM1 null genotype; by 68.2 % and higher for GSTT1; by 80.4 % and higher for EPHX1 ($p < 0.01$). Polymorphism in EPHX1 gene makes chloroform accumulation much more probable (blood levels ≥ P75), which is the most pronounced when combined with GSTT1 gene polymorphism.

The study results allow us to consider hetero- and homozygous polymorphic genotypes AG/GG for the EPHX1 gene, CT/TT for the CYP2E1 gene, and the null allele in the GSTT1 and GSTM1 genes as genetic predisposition factors for chloroform accumulation in the body. This increases the probability of health outcomes associated with chronic exposure to this disinfection by-product.

The A415G polymorphism of the EPHX1 gene and GSTT1 deletion, their combinations including the combination with the GSTM1 null and/or the C1091T polymorphism of the CYP2E1 gene can be used as the most informative biomarkers of sensitivity when assessing risks associated with exposure to trihalomethanes (chloroform) at levels not exceeding MPC in water.

Keywords: *CYP2E1, GSTM1, GSTT1, EPHX1 genes, disinfection by-products, drinking water, gene polymorphism, biomonitoring, health risk assessment, biomarkers of susceptibility.*

Disinfection is an integral stage in water treatment. It guarantees that population is provided with epidemiologically safe drinking water and this is especially vital for surface water sources as less protected from any external impacts. At the same time, use of reagents for water disinfection creates up to 400 disinfection by-products (DBPs); some of them are able to produce long-term effects. DBPs composition and levels in treated drinking water vary significantly depending on levels of natural organic compounds in water prior to treatment and an applied method of disinfection [1, 2]. Tap water supply systems widely rely on various chlorination methods with trihalomethanes (THMs) as typical indicator DBPs (chloroform, dichlorobromomethane, chlorodibromomethane and bromoform). Since all of them are volatile organic compounds, they can enter the body not only orally but also by inhalation and dermal absorption. The latter make a substantial contribution to the total daily DBPs load due to intensive household water use (showering, bathing, cleaning, washing, doing the dishes, etc.) [3–5]. Although these compounds occur in water in very low and even trace concentrations, simultaneous exposure to several of them creates public health risks associated with water use, especially under long-term exposure [1–11]. Use of water containing several trihalomethanes creates elevated risks of pregnancy complications (intrauterine growth retardation, low birth weight,

Alena V. Drozdova – Candidate of Medical Sciences, Associate Professor, Deputy Director for Science (e-mail: drozdovaev@mail.ru; tel.: +7 (017) 370-50-15; ORCID: https://orcid.org/0000-0002-3032-0895).

Ekaterina V. Kolesneva – Senior Researcher at the Laboratory of Industrial Toxicology (e-mail: kolesneva_kate@mail.ru; tel.: +375-17-388-48-22; ORCID: https://orcid.org/0000-0003-1605-4786).

Vitaly E. Syakhovich – Head of the Research Laboratory (e-mail: rlab.head@antidoping.by; tel.: +375-17-265-58-88; ORCID: https://orcid.org/0000-0002-2558-6824).

Natallia A. Dalhina – Researcher (e-mail: dlginan@rambler.ru; tel.: +375-17-351-23-84; ORCID: http://orcid.org/0000-0003-4012-2507).

premature birth, and congenital anomalies) [1, 6]. Established carcinogenic effects of chloroform, bromoform and dichlorobromomethane allowed the International Agency on Cancer Research (IARC) to rank them as 2B carcinogens (possibly carcinogenic for humans); the evidence was provided by animal experiments [1, 2, 7–9]. Epidemiological studies established a correlation between exposure to trihalomethanes and bladder cancer and colorectal cancer [1, 10, 11]. Considering all the latest data on hazards posed by trihalomethanes, there is a trend to develop stricter standards for these DBPs levels in drinking water in international regulatory documents. However, any revision of national standards should be based on reliable evidence considering water use in the Republic and peculiar sensitivity of the population to exogenous pollutants. This may be achieved by applying certain methodical approaches [12].

It is very difficult to estimate actual effects of trihalomethanes on the human body due to their complex chemical composition, especially when it comes down to a mixture of DBPs, and multiple ways of introduction. More precise and objective quantification of health risks is achieved, among other things, by using data on internal exposure, that is, levels of pollutants (their metabolites) in biological media [13–16]. Chloroform levels in blood have been proven to be reliable biomarkers of exposure to trihalomethanes in water. Chloroform concentration in blood grows just after showering, bathing, doing the dishes by hand, or drinking hot drinks made of tap water (the highest levels are detected after showering / bathing) and then declines rapidly [17–20]. However, low chloroform levels can be detected in blood even 8 hours after water use. Its slower partitioning out of adipose tissues anв relatively high (e.g., daily) frequency of exposure events such as showering / bathing are thought to produce steady-state blood chloroform concentrations. Examining a blood sample independently of water use establishes such levels and allows more precise estimation of internal exposure for subsequent health risk assessment [21].

So, THMs blood levels depend on intensity of exposure and body weight. But they are also affected by some other factors, activity of xenobiotic metabolism being the most significant one. Inter-individual variations in xenobiotic metabolism capacities may be due to polymorphisms of the genes coding for the enzymes themselves or of the genes coding for the receptors or transcription factors which regulate the expression of the enzymes. Also, polymorphisms in several regions of genes may cause altered ligand affinity, transactivation activity or expression levels of the receptor subsequently influencing the expression of the downstream target genes [22–24]. As a result, slower toxicant excretion and / or greater production of toxic metabolites and their accumulation in the body make adverse biological effects much more probable. This, in its turn, leads to greater probability of chronic non-communicable diseases caused by environmental exposures. Therefore, polymorphisms of xenobiotic metabolism genes in a genotype can underlie differences in individual susceptibility to chemical environmental exposures. Abnormal alleles (polymorphisms) of enzyme genes participating in xenobiotic metabolism are valuable predictors of aforementioned diseases and can therefore be used as markers of body susceptibility to effects produced by toxicants [24–27].

Cytochrome P450 2E1 (CYP2E1) is a basic enzyme that catalyzes trihalomethanes metabolism in human and rat bodies. Secondary metabolism of destruction products (phosgene) is also important but its relative significance depends on availability of glutathione, other thiols and other nucleophile compounds (hystidine and cysteine). Reduced glutathione is able to eliminate practically all chloroform metabolites that occur under relatively low chloroform levels. A balance between oxidative and reduced trichloromethane metabolism (chloroform in particular), depends on a substance, tissue, dose and oxidative stress intensity. For the CYP2E1 gene, the focus is most often on closely linked polymorphisms as per PstI/RsaI restriction endonucleases when a mutant allele makes for elevated transcription and enzymatic activity. Frequency of this allele is different in different populations; the homozygous allele occurs in 6 % of Asians and 35 % have the heterozygous one whereas the latter occurs only in 6 % of Europeans.

Polymorphism of the GSTM1 gene localized in the chromosome 1 (glutathione-S-transferase of μ class) is associated with two alleles: functional GSTM(+) and nonfunctional null GSTM(−) with substantial deletion due to which protein product is not synthesized at all. The GSTT1 gene localized on the chromosome 22 (glutathione-S-transferase (GST) teta-1) also has two alleles: functional GSTT1(+) and nonfunctional null GSTT1(−) corresponding to partial or complete deletion that results in lower activity of a protein or its complete absence. L.C. Backer with colleagues (2008) established that research participants with GSTT1(−) had higher chloroform levels in blood after showering than GSTT1(+)-participants [20]. Kenneth P. Cantor and others (2010) confirmed a hypothesis in their study that there was a correlation between bladder cancer and genetic polymorphisms of the GSTM1 and GSTZ1 genes [28]. Some studies provide evidence of intrauterine growth retardation in case a mother has polymorphisms of the following genes: CYP2E1, MTHFR (F.C. Infante-Rivard, 2004) [29], CYP2E1 and GSTZ1 (B. Zhou with colleagues, 2018) [30], CYP2E1 (S.G. Bonou, 2017) [31]. The cross-sectional study performed by P. Yang with colleagues (2016) revealed an association between semen quality in a given population under exposure to THMs in water and polymorphisms of the CYP2E1, GSTZ1 and GSTT1 genes [32]. In Caucasians, prevalence of GSTM1(−) and GSTT1(−) genotypes equaled 40–50 % and 10–20 % accordingly [33].

The microsomal epoxide hydrolase is a vital component in xenobiotic metabolism. The gene that codes for it (EPHX1) has two well-known functionally significant polymorphisms able to change the enzyme properties. The first is in the 3rd exon (T337C or tyrosine being replaced with hystidine in position 113 (Tyr113His)) and in the 4th exon (A415G or hystidine being replaced with arginine in position 139 (His139Arg)). The T337C polymorphism reduces the enzyme activity by 50 % (a 'slowing down' allele) and the A415G polymorphism increases it by approximately 25 % (an 'accelerating' allele). Accelerated transformation of natural epoxides into highly active metabolites damages DNA thereby increasing the quantity of chromosome aberrations and causing development of several pathologies.

In the Republic of Belarus, so far there have been no studies investigating THMs levels in blood of people provided with tap water from surface water sources as well as studies addressing influence of genetic variability within a population on these levels. In our study, the working hypothesis was that gene polymorphisms of xenobiotic metabolism genes participating in chloroform metabolism could lead to potentially higher chloroform levels in biological media of exposed people under long-term exposure. Ultimately, this may create elevated health risks associated with harmful effects produced by disinfection byproducts on the human body.

The aim of this study was to investigate effects produced by polymorphisms of the CYP2E1, GSTM1, GSTT1, and EPHX1 enzyme genes on disinfection byproducts metabolism and substantiate biomarkers of individual susceptibility to exposure to trihalomethanes (using chloroform as an example) in an exposed population.

Materials and methods. We created two groups of volunteers who lived in Minsk. The test group included exposed people living in Moskovskii and Frunzenskii districts. They were provided with tap water from a surface water source disinfected by chlorination (150 people overall). The reference group included non-exposed people who lived in Pervomaiskii district and were provided with tap water from underground water sources that did not require chlorination (47 people). Both groups were comparable as per age (18–40 years) and sex.

Chloroform levels in tap water were below MPC on the test territory prior to the study and during it; they varied between 0.49 and 0.52 MPC (MPC ≤ 0.2 mg/dm^3). Chloroform was not identified in water distribution networks on the reference territory within the method's sensitivity (< 0.0125 mg/dm^3). Chloroform was measured in water by gas chromatography (LOD for chloroform equals 0.0125 mg/dm^3).

Peripheral blood samples were collected from all the participants in healthcare institutions (polyclinics) in Minsk in spring; sampling was performed in the morning. All the stages in the study conformed to the ethical standards and all the participants gave their informed consent to it; the study also involved questioning them regarding individual water use.

All blood samples were analyzed for chloroform (as biomarkers of exposure) at National Anti-Doping Laboratory using gas chromatography – low resolution mass spectrometry. The method was developed within this research (SOP LM 174-2020 The method for identifying trihalomethanes (chloroform) in biological media) and relied on using AGILENT 7890 gas chromatographer with AGILENT 7000 triple quadrupole GSC/MS system (Thermo Fisher Scientific, USA) [34].

The genotypes of all the participants were examined to identify the A415G polymorphism of the EPHX1 gene, C1091T of the CYP2E1 gene and null mutations of glutathione-S-transferase genes, μ and θ class, (GSTT1(−) и GSTM1(−)). Candidate genes for this study were selected based on pathways of trihalomethane (chloroform) metabolism in the body. Genotype assay was performed in the Scientific and Practical Center for Hygiene. DNA was extracted from blood samples by using Nukleosorb, a commercial reagent kit for DMA extraction, complete set B, produced by Primetech LLC (Belarus) in accordance with the manufacturer instructions. We established genotypes as per target locuses by using the polymerase chain reaction (PCR) in real time mode with the C1000 Touch PCR thermal cycler (BioRad, USA). The obtained results were then analyzed with relevant software. Table 1 provides profiles of polymorphisms, localization of the analyzed genes and used restriction enzymes.

Statistical analysis. We analyzed differences in distribution of genotype and allele frequencies in the test and reference groups by using χ^2. We estimated whether the distribution of the observed genotype and allele frequencies in the analyzed population corresponded to the Hardy – Weinberg equilibrium. Qualitative indicators were given as absolute values and fractions. We applied several conventional indexes to describe qualitative indicators (chloroform levels in blood): Max or maximum values, Min or minimal values; Me or median, upper and lower quartiles (interquartile range) as [P25; P75], 10, 90 and 95 percentiles (P10, P90, P95), and confidence interval 95 % CI. Quantitative data were statistically analyzed considering specific distribution of data: independent samples were compared as per an analyzed indicator with independent t-test and Mann – Whitney U-test. To compare an indicator in several independent groups, we applied the non-parametric Kruskal – Wallis test (H). Odds ratios were calculated for each polymorphism and their combinations considering 95 % confidence interval of identifying higher chloroform levels (≥ P75) in blood of people with certain polymorphisms against those without them.

In this study, the statistical significance was taken at $p < 0.05$. All the data were analyzed with conventional applied statistical software packages Statistica 12.0 and Microsoft Office Excel.

Table 2 shows how genotypes and alleles of the analyzed genetic polymorphisms were distributed in two groups; the distribution corresponded to the Hardy – Weinberg principle.

Table 1

Primer sequences and restriction enzymes used in PCR analysis of target candidate gene polymorphisms

Gene, localization	Polymorphism	Primer sequence, (5’ › 3’)	Product length, bp	Restriction enzyme	Alleles, bp
CYP2E1 10q24.3	5`-regulatory area C1091T	F CCAGTCGAGTCTACATTGTCA R TTCATTCTGTCTTCTAACTGG	411	RsaI	C (412) T (351, 61)
GSTM1 1q13.3	Deletion	F GAACTCCCTGAAAAGCTAAAGC R GTTGGGCTCAAATATACGGTGG	219	-	norm (219) deletion (0)
GSTT1 22q11.2	Deletion	F TCACCGGATCATGGCCAGCA R TTCCTTACTGGTCCTCACATCTC	459	-	norm (459) deletion (0)
EPHX1 1g42.1	4th exon A415G (His139Arg)	F ACATCCACTTCATCCACGT R ATGCCTCTGAGAAGCCAT	210	RsaI	A (210) G (163, 47)

Table 2

The A415G polymorphisms of the EPHX1 gene (His139Arg), the C1091T gene CYP2E1, GSTT1(−) and GSTM1(−): distribution of alleles and genotypes in the test and reference group

Genotypes/ alleles*	Genotype frequency, %		*p* (for different groups)
	The test group	The reference group	
C1091T CYP2E1			
CC	90.0	95.7	$p = 0.225$
CT	10.0	4.3	
TT	0.0	0.0	
C	95.0	97.9	$p = 0.393$
T	5.0	2.1	
GSTT1			
n	76.7	83.0	$p = 0.361$
del	23.3	17.0	
GSTM1			
n	63.3	63.8	$p = 0.951$
del	36.7	36.2	
A415G EPHX1			
AA	78.0	83.0	$p = 0.461$
GA	12.7	12.8	$p = 0.986$
GG	9.3	4.2	$p = 0.263$
A	84.3	91.3	$p = 0.228$
G	15.7	8.7	

Note: *AA, CC is homozygous wild-type genotype; AG, CT is heterozygous genotype; GG, TT is homozygous mutant genotype; A, C is wild-type allele; G, T is mutant allele; n is wild-type gene; del is homozygous deletion.

The results obtained by investigating distribution of xenobiotic metabolism gene polymorphisms in the test and reference groups are in line with literature data on distribution of xenobiotic metabolism gene polymorphisms in the European population. We examined frequency of the C1091T polymorphic locus of the CYP2E1 gene and detected the heterozygous CT genotype in 10 % of the people in the test group and in 4.3 % of the people in the reference group. The remaining genotypes identified in the participants as per this locus corresponded to the homozygous wild-type CC genotype. Shares of the null GSTM(−) and GSTT1(−) equaled 36.7 % and 23.3 % accordingly in the test group and 36.2 % and 17.0 % in the reference group. Frequency of the 4th exon A415G polymorphism of the EPHX1 gene was analyzed and as a result the polymorphic allele G was established in 15.7 % of the people in the test group; the mutant homozygotes GG genotype, in 9.3 %; and the heterozygous AG genotype, in 12.7 %. The same indicators equaled 8.7 %, 4.2 % and 12.8 % accordingly in the reference group.

Fifty-eight point seven percent of the people in the test group and 51.1 % of the people in the reference one had a mutant homo- and heterozygous genotype at least as per one of four genes: GSTM1, GSTT1, EPHX1, and CYP2E1. In the reference group, a polymorphism of one of four genes was identified in 32.9 % of the cases; polymorphisms of two genes, in 18.7 %; polymorphisms of three genes, in 7.3 %. Polymorphisms of several genes were identified simultaneously in the test and reference groups in the following combinations: 6.7 % and 8.5 % accordingly for the GSTM1+EPHX1; the GSTM1+GSTT1, 5.3 % and 4.3 %; the GSTT1+EPHX1, 2.7 % and 0.0 %; the GSTT1+CYP2E1, 2.0 % and 2.1 %; the EPHX1+CYP2E2, 2.7 % and 2.1 %; the GSTM1+GSTT1+EPHX1, 2.7 % and 2.1 %; the GSTM1+GSTT1+CYP2E1, 2.0 % and 2.1 %; the GSTT1+EPHX1+CYP2E1, 2.7 % and 0.0 %.

Statistically significant differences in relative frequencies of the mutant homozygous, heterozygous and normal homozygous genotype were not identified as per the analyzed genes in both groups and all the compared pairs ($p > 0.05$). This indicates that the samples were comparable.

Results and discussion. Chloroform was identified in 100 % of the samples taken in both groups. Its levels varied between 0.03 and 0.54 ng/ml, P95 0.27 ng/ml, in the test group; and between 0.004 and 0.37 ng/ml, P95 0.13 ng/ml, in the reference group. Differences in chloroform levels in blood of unexposed and exposed (through water) people were statistically significant (U = 2336, $p < 0.01$) [34]. Chloroform levels identified in blood were not higher (one sample excluded) than reference concentrations (0.0004 mg/l) proposed by Russian experts and established as per aspartate aminotransferase levels in blood (K.V. Chetverkina with colleagues, 2018) [16].

The next stage involved estimating associations between polymorphisms of the relevant genes encoding for xenobiotic metabolism enzymes and chloroform levels in blood. The research article describes identified associations and regularities only for the test group since we did not establish any statistically significant differences between allele distribution in the test and reference groups, but at the same time we established statistically significant differences for basic associations of the analyzed genes polymorphisms with chloroform blood levels for the test group corresponded to those detected in the reference one.

To analyze the associations, results of chloroform identification in blood of the volunteers from the test group were generalized as per 42 conditional sub-groups (variants) depending on the presence / absence of the analyzed gene polymorphisms: the analyzed gene polymorphisms are absent in a genotype (sub-group No. 2); the analyzed gene polymorphisms are present in a genotype (No. 3); one of the analyzed gene polymorphisms (mono) in a genotype (No. 4–7); a gene polymorphism in a genotype combined with other gene polymorphisms (No. 8–11); polymorphisms of two or three genes in different combinations in a genotype (No. 12–22); polymorphisms of specific genes (No. 23–26) or their specific combinations (No. 27–42) are absent in a genotype. Since the chloroform concentrations were distributed normally only in some sub-groups, further data analysis relied on non-parametric methods. For each sub-group, we calculated a number of samples and their share of the total number of samples in the test group. Chloroform levels were calculated as Max, Min, Me, P25, P75, P10, P90. We also calculated a number of samples in a sub-group (absolute values and their % of the total number of samples in a sub-group) with a chloroform level ≥ P75 and P90 values, ≤ P10, P25, P75 and P90 values for the test group as a whole.

Table 3 provides the data generalized as per sub-groups and chloroform levels in blood of the volunteers from the test group depending on presence (absence) of the analyzed polymorphisms (26 sub-groups that are the most interesting for investigation); the results are visualized as a range chart covering major 19 sub-groups, Figure 1.

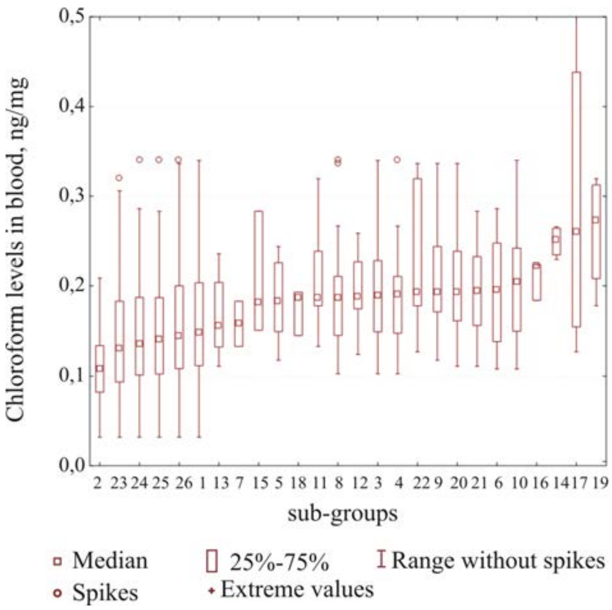

Figure 1. The range chart showing chloroform levels in blood of the volunteers from the test group depending on the presence of polymorphisms of the analyzed xenobiotic metabolism genes CYP2E1, GSTM1, GSTT1, and EPHX1 (the sub-groups are given in the order following the growing median values of chloroform levels)

Table 3

Chloroform levels in blood of the volunteers from the test group, ng/ml

No.	Sub-group as per presence / absence of polymorphisms *(clarification)*	Number of tests	% of the total samples	Me [P25 ÷ P75]	P10	P90	The share of samples (%) with a chloroform levels higher (lower) than the relevant percentile as per the test group as a whole			
							≥ P75	≥ P90	< P10	< P25
1.	The test group as a whole	150	100.0	0.1488 [0.1118 ÷ 0.2035]	0.0819	0.2392	25.3	10.0	10.0	25.3
2.	Polymorphisms are absent	62	41.3	0.1082 [0.0822 ÷ 0.1341]	0.0628	0.1568	4.8	0.0	24.2	56.5
3.	Polymorphisms are present	88	58.7	0.1892 [0.1495 ÷ 0.2281]	0.1310	0.2660	60.2	17.0	0.0	23.9
4.	The GSTM1 polymorphism *(mono)*	30	20.0	0.1903 [0.1480 ÷ 0.2105]	0.1279	0.2351	30.0	6.7	0.0	26.7
5.	The GSTT1 polymorphism *(mono)*	9	6.0	0.1826 [0.1497 ÷ 0.2257]	0.1178	0.2440	44.4	11.1	0.0	22.2
6.	The EPHX1 polymorphism *(mono)*	8	5.3	0.1951 [0.1387 ÷ 0.2478]	0.1081	0.2862	50.0	37.5	0.0	37.5
7.	The CYP2E1 polymorphism *(mono)*	2	1.3	0.1581 [0.1333 ÷ 0.1829]	0.1333	0.1829	0.0	0.0	0.0	50.0
8.	The GSTM1 polymorphism *(not only mono)*	55	36.7	0.1869 [0.1455 ÷ 0.2105]	0.1272	0.2387	30.9	9.1	0.0	27.3
9.	The GSTT1 polymorphism *(not only mono)*	35	23.3	0.1930 [0.1711 ÷ 0.2440]	0.1451	0.3061	48.6	28.6	0.0	14.3
10.	The EPHX1 polymorphism *(not only mono)*	33	22.0	0.2040 [0.1494 ÷ 0.2422]	0.1310	0.3061	54.5	30.3	0.0	24.2
11.	The CYP2E1 polymorphism *(not only mono)*	15	10.0	0.1869 [0.1778 ÷ 0.2385]	0.1455	0.3061	40.0	20.0	0.0	13.3
12.	The GSTM1+GSTT1 polymorphisms	8	5.3	0.1873 [0.1745 ÷ 0.2268]	0.1243	0.2586	37.5	12.5	0.0	12.5
13.	The GSTM1+EPHX1 polymorphisms	10	6.7	0.1556 [0.1326 ÷ 0.2039]	0.1219	0.2230	30.0	0.0	0.0	40.0
14.	The GSTT1+EPHX1 polymorphisms	4	2.7	0.2512 [0.2346 ÷ 0.2643]	0.2294	0.2660	100.0	75.0	0.0	0.0
15.	The GSTT1+CYP2E1 polymorphisms	3	2.0	0.1819 [0.1514 ÷ 0.2832]	0.1514	0.2832	33.3	33.3	0.0	0.0
16.	The EPHX1+CYP2E1 polymorphisms	3	2.0	0.2222 [0.1839 ÷ 0.2237]	0.1839	0.2237	66.7	0.0	0.0	0.0
17.	The GSTM1+GSTT1+EPHX1 polymorphisms	4	2.7	0.2597 [0.1550 ÷ 0.4383]	0.1272	0.5401	50.0	50.0	0.0	25.0
18.	The GSTM1+GSTT1+CYP2E1 polymorphisms	3	2.0	0.1869 [0.1455 ÷ 0.1930]	0.1455	0.1930	0.0	0.0	0.0	33.3
19.	The GSTT1+EPHX1+CYP2E1 polymorphisms	4	2.7	0.2723 [0.2082 ÷ 0.3128]	0.1778	0.3195	75.0	75.0	0.0	0.0
20.	Polymorphisms of more than one gene	39	26.0	0.1930 [0.1617 ÷ 0.2385]	0.1325	0.3061	46.2	23.1	0.0	17.9
21.	Polymorphisms of two genes	28	18.7	0.1944 [0.1565 ÷ 0.2325]	0.1325	0.2626	46.4	17.9	0.0	17.9
22.	Polymorphism of three genes	11	7.3	0.1930 [0.1778 ÷ 0.3195]	0.1455	0.3366	45.5	36.4	0.0	18.2
23.	The GSTM1 polymorphisms are absent	95	63.3	0.1310 [0.0935 ÷ 0.1829]	0.0718	0.2398	22.1	10.5	15.8	63.2
24.	The GSTT1 polymorphism is absent	115	76.7	0.1363 [0.1013 ÷ 0.1872]	0.0773	0.2222	18.3	4.3	13.0	57.4
25.	The EPHX1 polymorphism is absent	117	78.0	0.1414 [0.1025 ÷ 0.1869]	0.0773	0.2225	17.1	4.3	12.8	57.3
26.	The CYP2E1 polymorphism is absent	135	90.0	0.1446 [0.1084 ÷ 0.2000]	0.0812	0.2378	23.7	8.9	11.1	50.4

Chloroform was identified in levels varying between 0.1025 and 0.5401 ng/ml, *Me* = 0.1892 ng/ml [0.1495; 0.2281], P90 = 0.2660 ng/ml, in blood of the exposed volunteers from the test group who had a polymorphism of at least one analyzed gene in their genotype. Chloroform levels were between 0.0321 and 0.2087 ng/ml, *Me* = 0.1082 ng/ml [0.0822; 0.1341], P90 = 0.1568 ng/ml, in blood of the exposed people without the analyzed gene polymorphisms in their genotype. We estimated validity of the established differences in chloroform levels between the sub-groups with gene polymorphisms and their combinations (No. 3–22) and the sub-group without polymorphisms (No. 2) as per the Mann – Whitney test. The revealed differences were statistically significant ($p < 0.01$) for all the sub-groups, except the sub-group No. 7 'CYP2E1 polymorphism (mono)' (two-sided $p > 0.05$). This might be due to a small number of people in this sub-group. Additionally, we estimated significance of differences between the sub-groups with polymorphisms (No. 3–22) and the sub-groups without relevant polymorphisms or their combinations (the sub-groups No. 23–26 and other 16 sub-groups without any combinations of polymorphisms). The differences turned out to be significant for the sub-groups No. 3–6, 8–11, 14, 19–22 ($p < 0.05$) and to be statistically insignificant for the sub-groups No. 7, 12–13, 15–18 ($p > 0.05$).

We compared differences between 12 basic sub-groups (No. 2, 4–7, 12–19) using the Kruskal – Wallis method and the median test; as a result, the differences turned out to be statistically significant: H (12, N+150) = 80.5, $p < 0.01$, $\chi^2 = 63.5$, cs = 12, $p < 0.01$ (with the Bonferroni correction). Pair comparisons between 16 basic sub-groups (No. 4–19) with the Mann – Whitney test (99 pairs compared overall) established statistically significant differences between chloroform levels in blood of the volunteers in the following sub-groups: No. 4 and No. 14 (U = 10, Z = – 2.66, $p < 0.01$), No. 4 and No. 19 (U = 22, Z = – 2.00, $p < 0.05$), No. 5 and No. 14 (U = 3, Z = – 2.24, $p < 0.05$), No. 12 and No. 14 (U = 3, Z = – 2.12, $p < 0.05$), No. 13 and No. 14 (U = 1, Z = – 2.62, $p < 0.01$), No. 13 and No. 19 (U = 4, Z = – 2.19, $p < 0.05$), No. 13 and No. 9 (U = 100, Z = – 2.03, $p < 0.05$), No. 8 and No. 14 (U = 22, Z = – 2.64, $p < 0.01$), No. 8 and No. 19 (U = 44, Z = – 1.97, $p < 0.05$). Chloroform levels (median ones) were 32 % and 43.1 % lower in the sub-group No. 4 (GSTM1 polymorphism (mono)) against the sub-groups No. 14 (GSTT1+EPHX1 polymorphisms) and No. 19 (GSTT1+EPHX1+CYP2E1 polymorphisms) accordingly. They were also 24.1 %, 61.5 % and 75.0 % lower in the sub-group No. 13 (GSTM1+EPHX1 polymorphisms) against the sub-groups No. 9 (GSTT1 polymorphism (not only mono)), No. 14 (GSTT1+EPHX1 polymorphisms) and No. 19 (GSTT1+EPHX1+CYP2E1 polymorphisms) accordingly; chloroform levels were also 34.4 % and 45.7 % lower in the sub-group No. 8 (GSTM1 polymorphism (not only mono)) against the sub-groups No. 14 and No. 19. Chloroform levels were higher in blood of the exposed people in the sub-group No. 14 against the sub-groups No. 4, 5, 12, 13 and 8 with differences varying between 23.2 % and 38.1 %, as well as in the sub-group No. 19 against the sub-groups 4, 8 13 with differences varying between 30.2 % and 42.9 % (Figure 2).

We analyzed differences between chloroform levels in blood depending on the presence / absence of a specific gene polymorphism (the results are visualized in Figure 3). Present polymorphisms of the GSTT1 and EPHX1 genes as well as their simultaneous presence leads to a growth in chloroform levels (the sub-groups No. 10, 14, 17, and 19).

We compared how samples with different chloroform levels in blood (< P10, < P25, ≥ P75, ≥ P90 of the total test group) were distributed in the sub-groups. We established that all the samples with chloroform levels < P10 (0.0819 ng/ml) (24.2 % of the total number of samples in the sub-group) and 92.1 % of the samples with chloroform levels < P25 (0.1118 ng/ml) (56.5 % of the sub-group) belonged to the sub-group No. 2 'Polymorphisms are absent' whereas the share of the samples with chloroform levels < P25 was two times lower (23.9 %) in the sub-group 3 'Polymorphisms are present'.

In the test group, 44.7 % of the samples with chloroform levels ≥ than the upper quartile (P75) (0.2035 ng/ml) were taken from people with the GSTT1 or GSTM1 gene polymorphisms; 47.4 %, with the EPHX1 gene polymorphisms; 15.8 %, the CYP2E1 gene polymorphisms; the analyzed polymorphisms were absent only in 7.9 % of such cases. The highest share of the samples with chloroform levels ≥ P75 was identified in the sub-group No. 5 'GSTT1 polymorphism (mono)' (44.4 %), No. 9 'GSTT1 polymorphism (not only mono)' (48.6 %), No. 6 'EPHX1 polymorphism (mono)' and No.17 'GSTM1+GSTT1+EPHX1 polymorphisms' (50 %), No. 10 'EPHX1 polymorphism (not only mono)' (54.5 %), No. 3 'Polymorphisms are present' (60.2 %), No. 16 'EPHX1+ CYP2E1 polymorphisms' (66.7 %), No. 19 'GSTT1+ EPHX1+CYP2E1 polymorphisms' (75 %), No. 14 'GSTT1+EPHX1 polymorphisms' (100 %). The EPHX1 gene polymorphisms were present in genotypes in all the sub-groups with the share of the samples with chloroform levels being ≥ P75 equal to 50 % and higher.

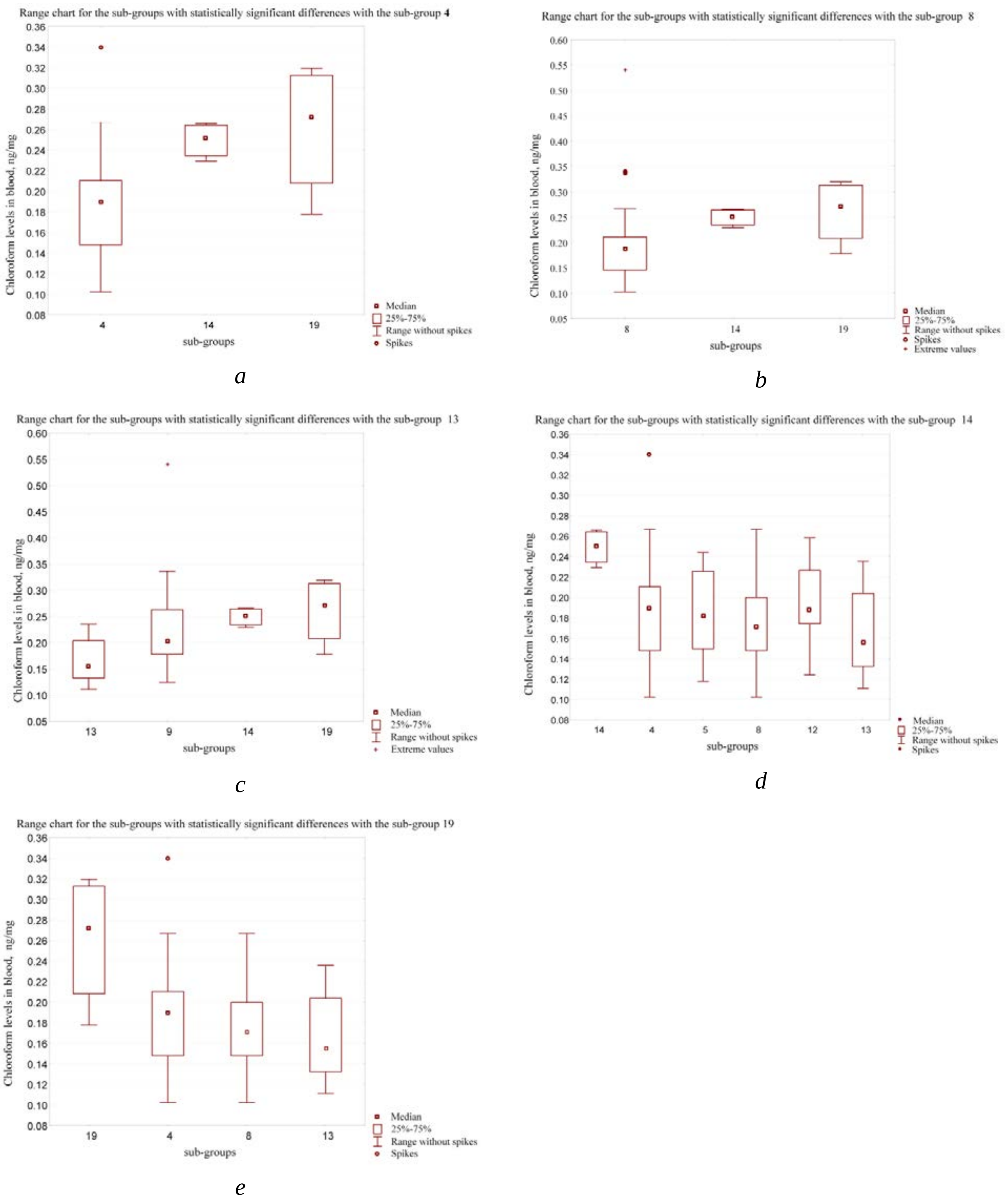

Figure 2. Chloroform levels in blood of the volunteers from the test group: range charts for the sub-groups with statistically significant differences with the sub-groups: *a*, with the sub-group No. 4; *b*, with the sub-group No. 8; *c*, with the sub-group No. 13; *d*, with the sub-group No. 14; *e*, with the sub-group No. 19

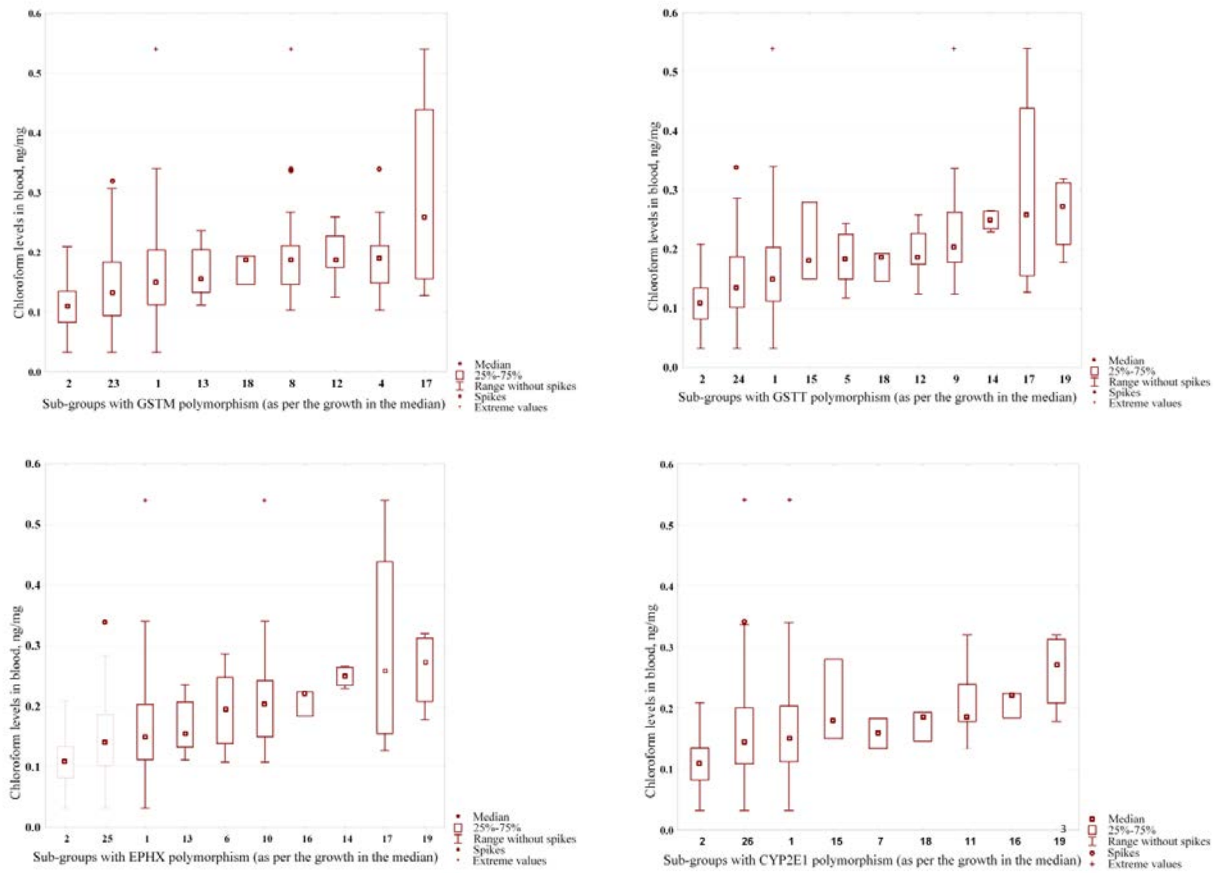

Figure 3. Chloroform levels in blood of the volunteers from the test group: range charts for the sub-groups with present / absent polymorphisms of the CYP2E1, GSTM1, GSTT1, and EPHX1 genes *(the sub-groups are given in a sequence following the growth in the median level)*

All the samples with chloroform levels in blood ≥ P90 (0.2392 ng/ml) belonged to the volunteers with gene polymorphisms in their genotype; 66.7 % of them had the GSTM1 or EPHX1 gene polymorphisms; 33.3 %, the GSTT1 gene polymorphisms; 20.0 %, the CYP2E1 gene polymorphisms (accordingly 75 % of the samples in the sub-groups No. 13 'GSTT1+EPHX1' and No. 19 'GSTT1+EPHX1+CYP2E1', 50 % of the samples in the sub-group No. 17 'GSTM1+GSTT1+EPHX1', 37.5 % of the samples in the sub-group No. 6 'EPHX1 polymorphism (mono)'.

We calculated odds ratios (OR) for the analyzed population; the results are given in Table 4. We established that odds ratios (OR) of chloroform levels in blood being ≥ P75 were significantly higher for the exposed people with the polymorphisms of the analyzed genes (or their combinations) in their genotype than for their counterparts without any polymorphisms (OR = 29.8, 95 % CI: 8.7–102.5). Depending on a specific polymorphism, the differences in the calculated odds ratios varied between 8.4 times (the sub-group No. 4 'GSTM1 polymorphism (mono)') and 59 times (the sub-group No. 19 'GSTT1+EPHX1+CYP2E1') against the sub-group where the analyzed polymorphisms were absent. The sub-group No. 7 'CYP2E1 polymorphism (mono)' and No. 18 'GSTM1+GSTT1+CYP2E1 polymorphisms' were the only exceptions (chloroform levels were < P75). The detected differences were statistically significant ($p < 0.05$) except the sub-group No. 15. The highest probability of a relatively higher chloroform level in blood (≥ P75) was identified for the cases when the EPHX1 gene polymorphism was present in a genotype, especially in a combination with the GSTT1 gene polymorphism (the differences were statistically significant for all the combinations, $p < 0.05$) (Table 4, Figure 1).

Table 4

Odds ratios (OR) for polymorphic genes and their combinations for chloroform levels in blood being ≥ P75

Sub-group as per presence / absence of polymorphisms *(clarification)*	OR (95 % CI) (for the samples with chloroform levels ≥ P75 with polymorphisms and without them)	Relative risk (RR)
3. Polymorphisms are present	29.8 (8.7–102.5)	12.4
4. GSTM1 polymorphism *(mono)*	8.4 (21–34.1)	6.2
5. GSTT1 polymorphism *(mono)*	15.7 (2.7–90.8)	9.2
6. EPHX1 polymorphism *(mono)*	19.7 (3.2–119.9)	10.3
7. CYP2E1 polymorphism *(mono)*	0*	0.0
8. GSTM1 polymorphism *(not only mono)*	8.8 (2.4–32.1)	6.4
9. GSTT1 polymorphism *(not only mono)*	18.6 (4.9–70.7)	10.0
10. EPHX1 polymorphism *(not only mono)*	23.6 (6.1–90.8)	11.3
11. CYP2E1 polymorphism *(not only mono)*	13.1 (2.8–62.0)	8.3
12. GSTM1+GSTT1 polymorphisms	11.8 (1.9–74.5)	7.8
13. GSTM1+EPHX1 polymorphisms	8.4 (1.4–50.1)	6.2
14. GSTT1+EPHX1 polymorphisms	–**	20.7
15. GSTT1+CYP2E1 polymorphisms	9.8 (0.7–141.4)***	6.9
16. EPHX1+CYP2E1 polymorphisms	39.3 (2.7–565.8)	13.8
17. GSTM1+GSTT1+EPHX1 polymorphisms	19.7 (2.0–191.8)	10.3
18. GSTM1+GSTT1+CYP2E1 polymorphisms	0*	0.0
19. GSTT1+EPHX1+CYP2E1 polymorphisms	59.0 (4.6–750.5)	15.5
20. Polymorphisms of more than one gene	16.9 (4.5–63.1)	9.5
21. Polymorphisms of two genes	51.1 (10.8–241.5)	14.9
22. Polymorphisms of three genes	24.6 (4.3–141.9)	11.5

Note: * all the samples in the sub-groups with these polymorphisms contained chloroform in a concentration below P75, ** means it was impossible to calculate OR since all the samples had chloroform levels > P75 in this sub-group, *** means the established differences are not valid, $p > 0.05$.

Conclusions. In the test group, the polymorphic locus C1091T of the CYP2E1 gene was identified in 10 %, of the cases; null genotypes of the GSTM1 and GSTT1 genes, 36.7 % and 23.3 % accordingly; the 4th exon polymorphism A415G of the EPHX1 gene, 15.7 % (the mutant homozygotes GG accounted for 9.3 % and the heterozygous genotype AG accounted for 12.7 %).

The analyzed polymorphisms (A415G of the EPHX1 gene, C1091T of the CYP2E1, null mutations of the glutathione transferase genes GSTT1 and GSTM1) in a genotype are associated with slower chloroform excretion and metabolism, its accumulation in the body and elevated steady-state chloroform levels in blood of people under long-term exposure to disinfection byproducts in water. Chloroform concentrations in water within MPC (0.2 mg/dm^3) created the following chloroform levels in exposed people's blood: Me = 0.1892 ng/ml [0.1495; 0.2281] in people with a polymorphism / polymorphisms and Me = 0.1082 ng/ml [0.0822; 0.1341] in people with normal genotypes of the analyzed genes (U = 492, Z = 8.53, $p < 0.0000001$). Chloroform levels (as per the median value) were 80.4 % (and more) higher in blood of people with the A415G polymorphism of the EPHX1 gene; 68.2 % (and more) higher in people with the null allele of the GSTT1 gene; 43.8 % (and more) higher in people with the null allele of the GSTM1 gene (the differences were statistically significant at $p < 0.01$).

Odds ratios (OR) for chloroform levels in blood being ≥ P75 were significantly higher (29.8 times) for exposed people with the analyzed gene polymorphisms (their combinations) than for people without them in their genotypes. The EPHX1 gene polymorphism makes even higher chloroform levels in blood (≥ P75, ≥ P90) much more probable especially when it is combined with the GSTT1 gene polymorphism.

These established peculiarities make it possible to consider hetero- and homozygous polymorphic AG/GG genotypes as per the EPHX1 gene, CT/TT as per the CYP2E1 gene as well as a null allele in the GSTT1 and GSTM1 genes to be factors of genetic predisposition to chloroform accumulation in the body. The latter stimulates negative health outcomes due to risks associated with chronic exposure to this disinfection byproduct. Given all the above stated, the aforementioned polymorphisms can be used as biomarkers of individual sensitivity when estimating susceptibility to exposure to chloroform that penetrates the body with tap water. They can be also eligible for developing more effective preventive measures.

We suggest using the A415G polymorphism of the EPHX1 gene and deletion of the GSTT1 glutathione transferase gene as well as their combinations, including those with the GSTM1 gene deletion and / or the C1091T polymorphism of the CYP2E1 gene as the most informative biomarkers of sensitivity when assessing health risks associated with exposure to chloroform in tap water when its levels do not exceed maximum permissible ones.

A promising trend in further research would be to investigate associations between exposure to trihalomethanes and diseases with pathogenetic causation considering genetic susceptibility in a given population.

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Competing interests. The authors declare no competing interests.

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Review

HYGIENIC ASPECTS OF ANTI COVID-19 MEASURES

T.S. Isiutina-Fedotkova, Y.V. Zhernov, V.V. Makarova, D.V. Shcherbakov, N.N. Zabroda, N.A. Ermakova, V.A. Sukhov, A.A. Klimova, D.A. Kraskevich

I.M. Sechenov First Moscow State Medical University, 8 Trubetskaya Str., bldg 2, Moscow, 119991, Russian Federation

The aim of this analytical review was to hygienically assess non-specific prevention of the COVID-19 infection. Such measures have been examined profoundly both by Russian and foreign researchers all over the world. The pandemic of this new coronavirus infection has shown that sanitary and preventive measures are among the most significant components in fighting against it, along with anti-epidemic activities and treatment measures, development of new vaccines and medications. All over the world, many countries introduce several sanitary-epidemiological and social measures to prevent spreading of the SARS-CoV-2 virus that causes COVID-19.

The review dwells on the results obtained by investigating effectiveness of non-specific prevention of the new coronavirus infection in different countries. As illustrated in the review, it was important to introduce restrictive measures with their major aim being to prevent (or limit) the infection transmission by airborne droplets or through household contacts. Researchers performed hygienic assessment of personal protective equipment used for protection of respiratory organs and hand skin and developed recommendations on its safe and effective use and utilization.

Self-isolation as a restrictive measure to prevent the COVID-19 pandemic from spreading was a temporary one. Nevertheless, during the pandemic peak billions of people all over the world had to remain at home after the strict self-isolation had been introduced. The review provides some data on estimating the level of commitment among population to follow recommendations on limiting the infection spread in Russia and abroad. In Russia, there is a reliable and effective state infrastructure of public healthcare. It made it possible to keep the pandemic situation under control starting from the early days when cases of pneumonia with unspecified etiology were reported in December 2019 and the first COVID-19 cases were registered in the country. Several measures were introduced including administrative, organizational, technical and sanitary-hygienic ones. However, it was a challenging task to create a relevant response to the COVID-19 pandemic that the public healthcare system in Russia had to tackle.

Keywords: *COVID-19, pandemic, public health, non-specific prevention, risk assessment, face masks, gloves, social distancing, self-isolation.*

Tatiana S. Isiutina-Fedotkova – Candidate of Medical Sciences, Associate Professor at the General Hygiene Department (e-mail: isyutina-fedotkova_t_s@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: https://orcid.org/0000-0001-8423-9243).

Yury V. Zhernov – Doctor of Medical Sciences, Professor at the General Hygiene Department (e-mail: zhernov_yu_v@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: http://orcid.org/0000-0001-8734-5527).

Valentina V. Makarova – Candidate of Medical Sciences, Associate Professor at the General Hygiene Department (e-mail: makarova_v_v@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: http://orcid.org/0000-0002-7213-4265).

Denis V. Shcherbakov – Candidate of Medical Sciences, Associate Professor at the General Hygiene Department (e-mail: shcherbakov_d_v@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: http://orcid.org/0000-0003-0226-9276).

Nadezhda N. Zabroda – Doctor of Medical Sciences, Professor at the General Hygiene Department (e-mail: zabroda_n_n@staff.sechenov.ru; tel.: +7 (499) 248-53-85; ORCID: http://orcid.org/0000-0003-3913-552X).

Nina A. Ermakova – Senior lecturer at the General Hygiene Department (e-mail: ermakova_n_a@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: http://orcid.org/0000-0002-9745-4265).

Vitaly A. Sukhov – Assistant at the General Hygiene Department (e-mail: sukhov_v_a@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: http://orcid.org/0000-0003-2993-0108).

Anna A. Klimova – student at the F.F. Erisman Institute of Public Health (e-mail: klimova_a_a@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: https://orcid.org/0000-0002-8939-9315).

Denis A. Kraskevich – Assistant at the General Hygiene Department (e-mail: kraskevich_d_a@staff.sechenov.ru; tel.: +7 (499) 248-51-55; ORCID: http://orcid.org/0000-0003-1905-874X).

Most countries introduced restrictive sanitary-hygienic measures to prevent the new coronavirus infection from rapid spreading. Such measures included curfew (people being prohibited to visit public places at certain time), 'lockdown' (closing down enterprises, restaurants, bars, etc.), and limitations on mass events; also, social distancing became widely spread when employees had to work remotely, people had to keep a social distance between each other of 1.0–1.5 meters minimum in public transport and public places, and self-isolation was the strictest measure in this respect [1–3]. In addition, wearing face masks became mandatory in public transport and public places[1] [4].

Experts from Cambridge and Oxford investigated the significance of physical distancing as a measure aimed at reducing risks of COVID-19 infection. However, they failed to establish precisely what distance was safe during contacts with an infected person within varied contexts and safe duration of such contacts. Instead of creating some unified fixed rules for a minimal distance, differentiated recommendations were developed; they considered multiple factors, which collectively determined a risk of biological threats posed by COVID-19. This made it possible not only to provide the greatest protection in case risks of infection were high but also to help people keep greater freedom in case risks were not so high. The authors offered to introduce combined measures that included keeping a safe minimal distance, air ventilation, disinfecting surfaces and air in enclosed spaces, as well as wearing face masks. It was also thought necessary to bear in mind duration of contacts with a potential source of infection [5].

Large-scale examinations were performed by Canadian experts; they presented an analytical review and meta-analysis of 172 research articles written by authors from 16 different countries located on six continents including 44 works that addressed issues related to assessing risks of SARS-CoV-2 spread in healthcare organizations and non-medical institutions as well (n = 25,697). The authors analyzed research articles that described patients with confirmed or suspected COVID-19, SARS-CoV-1 or MERS (Middle-East Respiratory Syndrome) and people who had close contacts with them. They analyzed effects produced on a risk of infection by several factors including a distance between healthy people and people infected with COVID-19 (1 meter, more and less than 1 meter); wearing various types of face masks; eye protection; etc. The study aimed to estimate these factors in order to identify what physical distance would provide a reduction in risks of infection when taking care of a person infected with SARS-CoV-2, SARS-CoV-1 or MERS-CoV. Respiratory protective equipment included surgical masks and respirators No. 95; eye protection was provided by visors, face shields and protective glasses. The analysis revealed that virus transmission was lower in case a physical distance between people was 1 meter or more in comparison with a situation when it was less than 1 meter (n = 10,736, combined adjusted odds ratio [aOR] – 0.18, 95 % CI: between 0.09 and 0.38; risk difference [RD] – 10.2 %, 95 % CI: between -11.5 to -7.5; moderate validity); protection became stronger as this distance got longer (a change in relative risk [RR] – 2.02 per 1 meter; $p_{\text{interaction}}$ = 0.041; moderate validity). Wearing a face mask can

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[1] O vvedenii rezhima povyshennoi gotovnosti: Ukaz mera Moskvy ot 5 marta 2020 goda № 12-UM (s izm. na 06.10.2020) [On introducing the increased readiness regime: The Order by the Moscow mayor issued on March 5, 2020 No. 12-UM (last edited on October 06, 2020)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: http://docs.cntd.ru/document/564377628 (August 02, 2022) (in Russian); O dopolnitel'nykh merakh po snizheniyu riskov rasprostraneniya COVID-19 v period sezonnogo pod"ema zabolevaemosti ostrymi respiratornymi virusnymi infektsiyami i grippom: Postanovlenie Glavnogo gosudarstvennogo sanitarnogo vracha RF ot 16 oktyabrya 2020 goda № 31 (s izm. na 20.06.2022) [On additional activities aimed at reducing risks of COVID-19 spread during a seasonal rise in morbidity with acute respiratory virus infections and flu: The Order by the RF Chief Sanitary Inspector issued on October 16, 2020 No. 31 (last edited on June 20, 2022)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: http:// docs.cntd.ru/document/566108530 (August 02, 2022) (in Russian).

lead to a significant decrease in a risk of infection (*n* = 2647; aOR = 0.15, 95 % CI: between 0.07 and 0.34, RD = -14.3 %, between -15.9 and -10.7; low validity), and there was a stronger association with N95 or similar respirators against disposable surgical masks or similar ones (for example, reusable 12–16-layer cotton masks; $p_{\text{interaction}} = 0.090$; posterior probability > 95 %, low validity). Eye protection was also associated with lower risks of infection (*n* = 3713; aOR = 0.22, 95 % CI: between 0.12 and 0.39, RD = -10.6 %, 95 % CI: between -12.5 and -7.7; low validity) [6].

Experts from Singapore assessed risks posed by duration of contacts between healthy people and patients infected with COVID-19, the infection confirmed by laboratory tests. Household contacts were identified as contacts between people who lived together with a COVID-19 patient. Close contacts that could not be considered household ones were those between people who contacted for not less than 30 minutes and a distance between a healthy person and an infected patient was within 2 meters. The authors examined 7700 close contacts (1863 household contacts, 2319 work contacts and 3588 social contacts) associated with 1114 cases confirmed with a PCR-test. Living in the same apartment (multidimensional odds ratio [OR] – 5.38 [95 % CI: 1.82–15.84]; $p = 0.0023$) and a contact with an infected person that lasted 30 minutes or longer (7.86 [3.86–16.02]; $p < 0.0001$) were associated with SARS-CoV-2 transmission in household conditions. As for contacts beyond households, SARS-CoV-2 transmission was associated with the following: a contact with more than one patient (multi-dimensional OR – 3.92 [95 % CI: 2.07–7.40], $p < 0.0001$), talking to an infected patient for 30 minutes or longer (2.67 [1.21–5.88]; $p = 0.015$) and a drive in the same car (3.07 [1.55–6.08]; $p = 0.0013$). Indirect contact, having a meal together or sharing a toilet were associated with SARS-CoV-2 transmission both for household contacts and those beyond it [7].

T. Harweg with colleagues estimated effectiveness of not only a minimal social distance but also a safe square per one person in their study [8]. They created a numeric model to describe the number of pedestrians in cities in dynamics aiming to identify duration of exposure and the overall effectiveness of distancing. The modeling results showed that in case a person kept the minimal social distance of 1.5 meters established by the governmental regulations in Germany, a square equal to 16 m^2 per one person was sufficient for effective prevention of infection.

Experts from France [9] modeled infection by airborne transmission as well as an infecting dose. They highlighted the importance of calculating a pathogen unit that was closely connected with the ‘dose – reaction’ law. New COVID-19 variants with a greater viral burden such as delta (dose) or higher contagiousness such as omicron (contacts) could lead to more intensive airborne transmission. The researchers think that the existing ventilation standards are not sufficient and are not conformed to, especially in public places. This creates higher risks of infection. To prevent airborne transmission, it is necessary to perform multi-indicator analysis considering duration of exposure, a dose of an infectious agent, face mask wearing, as well as a share of infected people in a given population. Therefore, a risk of COVID-19 spread by airborne transmission requires investigating with a focus on duration of exposure and not the minimal distance.

B. Abbas with colleagues assessed risks of infection for healthcare workers in dental clinics [10]. In case a dental procedure lasted longer than 60 minutes, it was given 0.75 score; between 30 and 60 minutes, 0.50 score; in case a procedure lasted for less than 30 minutes, it was given 0.25 score. The total score estimation was calculated for each patient. A risk of SARS-CoV-2 transmission was assessed as low (the score estimation < 4), average (between 4 and 6) or high (the score estimation > 6) depending on the total estimation calculated for each procedure. Therefore, the shorter duration of a contact with a potentially infected person (‘protection by time’), the lower score estimation was given for establishing a risk rank.

Previously, a score estimate was suggested for a risk of the new coronavirus infection COVID-19 based on social-hygienic and behavioral indicators. Score estimation was performed to identify risk categories as regards the new coronavirus infection. Indicators that described adherence to wearing face masks when visiting certain social objects, trips by varied kinds of public transport and their duration, visits to social objects, and keeping a proper social distance were identified as the most significant risk factors.

Also, we suggested a procedure for assessing risks of COVID-19 transmission at social objects and transport infrastructure. An online survey with 1325 respondents from Moscow participating in it revealed that the most significant risk factor was neglecting the requirements to wear face masks and not a failure to keep a social distance in transport. We identified risk categories and suggested a hygienic classification of objects as per high, average, and low risks of COVID-19 transmission.

The COVID-19 pandemic had considerable influence on mass trips by population. Researchers from China examined a risk of the disease transmission between subway commuters by using the SEIR model ('susceptible – exposed – infected – recovered'). The model considered factors that could produce effects on the virus transmission such as effective ventilation, a time spent by a commuter on a trip, the number of commuters in a carriage and at a station, etc. As a result, it was established that a risk of infection grew considerably in case a trip lasted for more than 25 minutes. Physical distances between commuters, effective ventilation as well as quality of disinfection were also significant risk factors. It was recommended to improve ventilation and disinfection inside carriages and impose limitations on duration of trips together with introducing a social distance being not less than 1 meter [11].

Researchers from Argentina reported it was necessary to transform theoretical knowledge on resistance to the new coronavirus infection SARS-CoV-2 into preventive measures for healthcare workers. Regular training provided for both healthcare workers and patients reduced risks of the coronavirus infection spread. As a result, recommendations were developed and implemented with their major purpose being to assess COVID-19 risks in healthcare organizations in Argentina and safety management at the national level [12].

Preventing or limiting airborne transmission or transmission through household contacts is among the most important non-specific preventive measures against the new coronavirus infection COVID-19. Various types of screens including face masks, respirators, face shields and others are applied to reduce risks of airborne transmission. People in Russia were recommended to wear gloves in public places during a period when the COVID-19 incidence grew in order to exclude or limit the virus transmission by contacts.

After 'wearing gloves and masks' introduction, the WHO experts developed many guidelines on how to protect oneself from the coronavirus infection[2]. Wearing gloves is an additional anti-epidemic measure against the COVID-19 spread. Up-to-date medical gloves are made of different materials with different chemical structure, their manufacture relies on various production technologies and processing, and their target functions can also be different. They should conform to requirements securing their protective (barrier) and consumer properties[3], and be manufactured in conformity with the Standard EN 455 that corresponds to the interstate standard GOST EN 455-2014 in the Russian Federation.

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[2] Advice for the public: Coronavirus disease (COVID-19). *WHO*. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public (August 11, 2022).

[3] MR 3.5.1.0113-16. Ispol'zovanie perchatok dlya profilaktiki infektsii, svyazannykh s okazaniem meditsinskoi pomoshchi, v meditsinskikh organizatsiyakh. 3.5.1 Dezinfektologiya (utv. Federal'noi sluzhboi no nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom RF 2 sentyabrya 2016 g.) [MG 3.5.1.0113-16. Use of gloves to prevent healthcare associated infections in healthcare organizations. 3.5.1 Disinfectology (approved by the Head of the Federal Service for Surveillance over Consumer Rights protection and Human Wellbeing, the RF Chief Sanitary Inspector on September 2, 2016)]. *GARANT: information and legal portal*. Available at: https://www.garant.ru/products/ipo/prime/doc/71382342 (August 09, 2022) (in Russian).

It is noteworthy that prior to the COVID-19 pandemic there has never been such mass use of respiratory protective equipment and hand skin protection by both general public and healthcare workers in the history of mankind. The COVID-19 pandemic led to a drastic increase in using personal protective equipment both in healthcare and other organizations all over the world. Wearing a face mask is an economical and affordable way to prevent COVID-19.

Given that, it is becoming urgent to hygienically assess respiratory protective equipment and hand skin protection and to develop recommendations on their safe and effective use and subsequent utilization.

Wearing face masks involved certain changes in skin. Researchers from Serbia assessed short-term effects produced by cotton and medical masks on biophysical skin properties. They measured four biophysical skin properties including transepidermal water loss, the stratum corneum hydration, skin pH changes and the erythema index. Examinations were accomplished prior to and after 3 hours of wearing a mask on a skin covered by it and on open face surface. It was shown that transepidermal water loss increased on open skin after wearing a cotton face mask for three hours and decreased insignificantly after wearing a medical mask. After wearing a mask for three hours, there was an increase in the stratum corneum hydration and pH of mask-covered skin went down. The erythema index grew in both groups (cotton and medical masks); however, those differences were not statistically significant. Therefore, the authors established that skin characteristics changed even after wearing a mask for only 3 hours [13].

Researchers from China described outcomes for face skin caused by long-term wearing a mask, to be exact, during 6 months. Several skin characteristics were estimated in all participants three times a day including transepidermal water loss (TEWL), skin hydration, skin elasticity, skin pore area, skin keratin amount, skin temperature, skin color, and other indicators. As a result, it was established that skin hydration, skin pore area, skin keratin amount and skin color differed greatly on open spots and spots covered by a mask. The experts concluded that long-term daily use of a face mask can change skin characteristics [14].

Other researchers identified bacterial contamination of face masks after use. Their research involved questioning with participating employees of an airport in Moscow and investigating levels of bacterial contamination of face masks. Questioning established that skin sweating under a mask (68.60 %), and feelings of air shortage (66.94 %) were the most frequent and apparent among all the reactions ($p < 0.001$). The more frequent a negative reaction, the more apparent it is ($r = 0.79–0.95$). The authors established a moderate positive correlation between duration of wearing a mask and frequency of face skin sweating ($r = 0.31$). Face skin reactions were more frequent and apparent in people who wore a cotton mask against those who selected a neoprene or a non-woven one: sweating ($p = 0.04$), reddening / peeling / irritation ($p = 0.035$), more apparent pustules, skin rash and inflammation ($p = 0.02$). The experts also identified a correlation between frequency and intensity of skin reactions and bacterial contamination that occurred on an inner surface of a mask after use. There was a moderate positive correlation between the number of colonies and duration of use for a neoprene and cotton mask ($r = 0.33$ and 0.46 accordingly). The number of colonies grows as duration of use becomes longer. There are also several factors that aggravate frequency and intensity of negative reactions including skin problems, young age, work with average and high hardness [15].

Effectiveness of mask protection properties depends on how effectively a material a mask is made from is capable to block drops and aerosol particles that contain viruses. Bacterial filtration as well as permeability of a material can be used as indicators in estimating effectiveness of protection provided by a mask. The authors comparatively assessed effectiveness of a medical, cotton, and neoprene mask. The study revealed that a neoprene mask provided the highest bacterial filtration whereas a cotton one had the highest air permeability. All the examined masks were comparable with a medical one as per a combination of all the analyzed properties and could be used as means to reduce a risk of infection spread [16].

An analytical review of research articles in foreign and Russian editions has established that by now in literature there is not any available conventional terminology for face mask use as well as any unified classification of respiratory protective equipment. Based on this literature review, the authors suggested a new classification of respiratory protective equipment as per effectiveness of protection against airborne diseases. FFP3/KN100/N99/N100 respirators turned out to be the most effective. FFP2/KN95/N95/DS/DL2/KF94 respirators had average effectiveness. Effectiveness of FFP1 respirators and type IIR, II, I medical non-woven masks and gauze masks was below average (the means are mentioned in a descending order as per their effectiveness). Effectiveness of various non-medical masks (non-woven, woven cotton and synthetic ones) and face shields was low and extremely low accordingly[4]. There are also no exact concepts of a 'medical' and 'non-medical' mask.

Experts from China established that most respondents who participated in online survey used face masks correctly during the pandemic. However, certain difficulties occurred in selecting the optimal type of a mask, a possibility to reuse it, as well as a proper way to utilize it. The authors concluded that people should be provided with all the relevant information [17].

Another study aimed to examine personal protection measures during the pandemic in Germany and any potential differences in behavioral patterns depending on an age, sex and education. The total sample included 20,317 respondents who took part in an online survey. As a result, it was established that wearing a face mask was considered the top priority; it was followed by keeping a minimal necessary social distance and hand washing. It was established that more protective measures were usually taken by women, people with higher education and younger people. Risk groups included elderly people, men, and people with low levels of education. The priority prevention activities should be aimed exactly at these population groups [18].

After the Chinese Government introduced mandatory face mask wearing in public places, most people started using them. L. Zhang with colleagues accomplished two online surveys with their aim to identify peculiarities of face mask wearing by urban and rural population as well as people who were under quarantine or self-isolated [19]. As a result, it was established that face masks were worn by more than 90.0 % of the respondents. A share of those who wore masks was higher among educated people, people with high incomes and among elderly people. Face masks were worn rarer by rural population and people under quarantine or self-isolation.

Russian experts established in their studies that most respondents who participated in online surveys in Russia wore masks (96.4 %). This was due to the necessity to conform to the requirements established by the introduced mandatory face mask wearing (72.4 %) as well as due to close contacts with other people (54.0 %). Ninety-one per cent of the respondents wore face masks when visiting food shops, drug stores and medical organizations; 64.0 %, when visiting non-food retail outlets; 76.9 %, in land public transport; and 76.1 %, in underground public transport. The respondents used variable kinds of respiratory protective equipment (RPE). Disposable medical (93.3 % of the respondents) and reusable cotton masks (25.4 % of the respondents) were the most widely spread. One third of the respondents (33.6 %) wore a disposable medical mask strictly for the recommended hours; 35.2 %, for more than 2 hours a day; 28.0 %, during several days. Another online survey

[4] Mask use in the context of COVID-19: interim guidance, 1 December 2020. *WHO*, 2020, 22 p. Available at: https://apps.who.int/iris/bitstream/handle/10665/337199/WHO-2019-nCov-IPC_Masks-2020.5-eng.pdf?sequence=1&isAllowed=y (September 13, 2022).

was performed among personnel employed at public transport (n = 4732); it identified three types of face masks that were used the most frequently: medical (55.6 %), cotton (11.9 %) and neoprene ones (30.4 %). Face mask wearing was uncomfortable for 57.0 % of the respondents. They complained about face hyperhidrosis (65.5 % of the respondents), uncomfortable breathing (48.9 %), skin hyperemia, itching and peeling (26.5 %), headaches (21.3 %), sneezing and lacrimation (13.0 %), pyo-inflammatory diseases of face skin (11.5 %). Frequency and intensity of all the analyzed reactions depended on a material a mask was made from [20].

Taxi and fixed-run bus drivers were recommended to be working in medical masks during the COVID-19 pandemic. A.B. Nevzorova with colleagues established that a face mask produced certain effects on changes in psychophysiological properties of a car driver in city traffic. They revealed that drivers in a face mask had a drastic decline in neural-psychic functions against those who drove without it. Subjective estimations given by the respondents established a considerable (41.7 %) or insignificant (20.4 %) decline in reactions cand 38.0 % of the drivers did not have any deviations due to influence exerted by a mask on driving. Based on these results, the authors concluded that a face mask could be a predictor of a pre-accident situation on the road [21].

Estimation of how well people are aware of proper preventive measures and online training with its focus on the rules for proper use of respiratory protective equipment are important trends in prevention of the new coronavirus infection.

S. Kundu with colleagues estimated knowledge about COVID-19 prevention measures under quarantine among people in Bangladesh. They conducted an online survey in social networks with 1765 adults participating in it. As a result, it was established that 96.6 % of the respondents wore masks when going out to prevent the infection; 98.7 % of the respondents washed their hands with soap after returning home [22]. This estimation indicates that people were highly aware about proper preventive measures.

X. Xue and others believe households contacts to be among major ways of COVID-19 transmission; therefore, wearing gloves reduces a risk of infection when providing cleaning services, delivering foods and socializing with other people [23].

Other authors established that effectiveness of wearing gloves by all the population to prevent COVID-19 was unknown. In their study, the authors made an attempt to identify how effective regular use of gloves by healthy people was in terms of COVID-19 prevention [24].

Iranian experts performed an online survey with 2097 people participating in it. As a result, it was established that 61.9 % of the respondents always washed their hands, 58.2 % wore gloves, and 55.7 % wore masks. The authors detected a significant relation between sex and hand washing (p = 0.006) as well as sex and use of masks and gloves (p < 0.001). The results also revealed that use of gloves had a significant relation with education (p = 0.029) and material welfare (p = 0.011). Mask wearing also had a significant relation with the financial position (p = 0.032). Women were better in taking preventive measures. Overall, almost half of the respondents did not use any non-specific preventive measures against COVID-19 [25].

A study accomplished by Indian experts focused on estimating preventive measures against COVID-19 in treating patients at home. The results established that 15.3 % of the respondents had previously had COVID-19 and 82.2 % of them had been treated at home. Disposable face masks were worn uninterruptedly for 8 hours by 62.2 % of the respondents. A disposable mask was not always thrown away after it had become wet. Only 37.8 % of those who were taking care of COVID-19 patients wore gloves. The experts made a conclusion it was necessary to increase people's awareness about preventive measures. This could be done by introducing training programs for population [26].

The World Health Organization recommended healthcare workers to wear gloves during the COIVID-19 pandemic in case they were taking direct care of patients. Medical gloves are made from variable materials including latex, nitrile rubber, polyvinylchloride, polyurethane, and neoprene. Nitrile and latex gloves are preferable due to their durability. Many negative skin reactions, including irritating contact dermatitis, allergic contact dermatitis and contact urticaria, were registered after using all types of gloves.

Healthcare workers often use latex gloves. Elevated sensitivity to latex made of natural rubber is becoming more and more significant. Between 2.8 and 17 % of healthcare workers were reported to have elevated sensitivity to latex gloves [27].

A study by T. Montero-Vilchez with colleagues aimed to estimate influence exerted by wearing a face mask and nitrile gloves on the epidermis barrier function and skin homeostasis. Thirty-four healthcare workers took part in the study; they all wore nitrile gloves and a face mask uninterruptedly for 2 hours. The experts estimated transepidermal water loss, the stratum corneum hydration, erythema, and skin temperature. As a result, it was established that transepidermal water loss, skin temperature and erythema were significantly higher on a spot covered by gloves than on an uncovered one. Transepidermal water loss, skin temperature and erythema were considerably higher on an area covered by a face mask whereas the stratum corneum hydration was lower. Transepidermal water loss was higher on an area covered by a surgical mask than on one covered by a respiratory mask with a filtrating face window. The experts concluded that skin homeostasis and the epidermis barrier function could be impaired by wearing gloves and face masks. Healthcare workers were recommended to use high-quality personal protective equipment and means for preventing skin diseases [28].

The necessity to use personal protective equipment during the COVID-19 pandemic affected the majority of people worldwide. Mandatory use of masks and gloves by population was introduced in different regions in the Russian Federation depending on an epidemiological situation. Russian experts accomplished sanitary-chemical laboratory tests of masks and gloves to identify chemical contents in them. Levels of analyzed chemicals in samples of all the examined masks did not exceed permissible levels. Cotton gloves and cotton gloves with coating turned out to contain formaldehyde in concentrations that were by 1.48 and 1.16 times higher accordingly than permissible ones. In addition, zinc was identified in cotton gloves with coating in concentrations being by 1.17 times higher than permissible levels. So, cotton gloves with coating had both formaldehyde and zinc in quantities higher than permissible levels. Formaldehyde in gloves can case negative skin reactions. Stricter control over glove production is necessary in order to prevent distribution of low-quality and dangerous items.

Bacterial contamination occurring on the inner surface of gloves used by transport workers was estimated in a study by Russian experts. They established a statistically significant increase in the CFU number after gloves had been used for two hours against the control samples ($p < 0.01$). Statistically significant differences in bacterial contamination of gloves after they had been used for a period between 2 and 12 hours were identified only in the group of workers who used cotton and knitted gloves ($p < 0.01$). The authors did not establish statistically significant differences in bacterial contamination of gloves after 2 and 12 hours of use ($p > 0.05$) [29].

Self-isolation during the COVID-19 pandemic was a temporary measure aimed at preventing the infection from rapid spreading. During the peak in the pandemic, billions of people had to remain at home due to introduced strict self-isolation. In Russia, the total number of people who had to be self-isolated reached 100 million people. All people who came to Russia from abroad had to maintain self-isolation.

In hygienic terms, self-isolation is a forced and long-term (longer than a month) period when a person should remain in an enclosed space keeping low physical activity and spending insufficient time outdoors.

Self-isolation involves hypodynamia, hypoxia, negative changes in eating habits and lifestyle, and psychoemotional burdens. Given that, vital sanitary-hygienic tasks to be tackled include performing sanitary-hygienic assessment of self-isolation and identifying priority risk factors causing non-communicable diseases.

The results of the study [30] made it possible to develop hygiene-based preventive measures aimed at minimizing risks during self-isolation. Low physical activity, hypoxia, nutrient deficiency (an imbalanced diet), and improper work and rest regimes are major sanitary-hygienic risk factors during self-isolation. The authors also developed a hygienic self-isolation index point score (HSIPS) considering the sanitary requirements to diets, work, rest and physical activity in the Russian legislation. Therefore, use of hygienic standards has certain advantages for health risk prevention both under routine conditions and extreme ones including self-isolation.

Experts from Great Britain carried out an online survey among adults (n = 8425; 44.5 ± 14.8 years). The task was to assess physical activity and mental health in the United Kingdom, Ireland, New Zealand and Australia after the governments in these countries introduced either self-isolation or mandatory remote work. Major indicators included a scale showing how mental behavior changed when a person was doing physical exercises. As a result, the authors concluded it was advisable to encourage physical activity during the COVID-19 pandemic and in the post-COVID period since it helps improve mental health and welfare. Men, young people and people with concomitant diseases should pay special attention to exercises [31].

The COVID-19 pandemic affects human health greatly as it changes routine lifestyle due to social distancing and self-isolation at home. This leads to social and economic outcomes including changes in lifestyle and eating habits. Experts from Italy investigated influence exerted by the pandemic on eating habits and lifestyles among people aged between 12 and 86 years (n = 3533). The performed questioning included anthropometric parameters (weight and height); data on eating habits; data on a lifestyle (food purchase, smoking, quality of sleep and physical activity). Weight growth was established in 48.6 % of the respondents; 3.3 % of the smokers decided to quit; there was a slight increase in physical activity, especially with its aim to reduce a person's weight [32].

Nutrition is a major health-determining factor that affects functions of all the mechanisms protecting the body from harmful environmental exposures. Correction of improper nutrition, including vitamin and micronutrient deficiency, has great significance for preventing and treating the new coronavirus infection COVID-19 [33]. Given that, nutrition guidelines have been developed for adults and children who have to stay at home under self-isolation or quarantine due to COVID-19. An information-reference contact-center was opened for timely communication and advice provided for population as regards optimization of nutrition; it has been functioning uninterruptedly since the opening. Activities aimed at preventing COVID-19 infection in food products are of the same importance. Preventive activities have been developed to limit the transmission of the new coronavirus infection through foods.

Z.D. Kifle with colleagues reported that an online survey of 348 Ethiopians revealed certain changes in consumption of some foods, regularity in having meals, duration of sleep, physical activity and psychoemotional strain. There was a significant decline in consumption of food that was not home-made, from 20.4 % to 13.4 % at (p < 0.001), growing food consumption (more than eight cups a day) from 11.5 % to 14.7 % (p < 0.01). Before the pandemic, only 4.9 % of the respondents had psychoemotional strain whereas the indicator grew up to 22.7 % during it. Six point three percent of the respondents had bad sleep before the pandemic but the share grew to 25.9 % during it (p < 0.001) [34].

The system of the sanitary-hygienic standards existing in the Russian Federation establishes certain requirements to people's diets, work and rest regime, and physical activity. It was used for developing hygienic criteria to assess self-isolation. Russian experts developed the hygienic self-isolation index including assessment of physical activity, a diet, psychoemotional burden, and some other indicators [3].

A.A. Antsiferova with colleagues estimated people's commitment to following recommendations to limit the spread of new coronavirus infection in the Russian Federation as a whole and some RF regions in the autumn–winter period 2020–2021. The authors performed an online survey with the total number of participants being 5537 people from 62 RF regions, including Moscow (n = 1157), the Ulyanovsk region (n = 735), the Irkutsk region (n = 595), the Omsk region (n = 452), and the Komi Republic (n = 408). The survey established that 97.3 % of the respondents used masks as a measure to limit the spread of the new coronavirus infection; 97.3 % often washed their hands; 71.1 % kept social distance; use of gloves was the least common measure (42.9 %). The respondents with higher education, both complete and incomplete, more often followed the recommendations on COVID-19 prevention including use of gloves (38.9 %) [35].

Experts from China estimated mental health of healthcare workers during a 4-week quarantine introduced in Hubei. Depression was established to become stronger in 17.9 % of the respondents and stress was identified in 13.7 %. Doctors and nurses were more susceptible to anxiety whereas other healthcare workers and medical students were susceptible to stress [36].

G. Barros [37] presented statistical data on influence exerted by the pandemic on students' mental health in different countries. The results indicated that negative mental outcomes had been growing among students during the COVID-19 pandemic. Most HEI students reported mental disorders that could be associated with lack of direct contacts with people due to switching to distance learning, quarantine and social distancing.

Housing environment is among major health-determining factors and the COVID-19 pandemic again highlighted it was important to analyze housing conditions. Insufficient spacing and absence of confidentiality might result in such mental disorders as anxiety and depression [38].

As people have to spend longer time indoors during the pandemic, they become more and more susceptible to indoor space environmental factors. Color of walls inside a dwelling does not only stimulate the human vision but also influences stress levels. Intense visual irritation changed occupants' mentality and this resulted in depression [39].

It is noteworthy that self-isolation in indoor environment at home can create new habits and lifestyles [30, 40].

Conclusions. In Russia, there is a reliable and effective state infrastructure of public healthcare. It made it possible to keep the pandemic situation under control from the first days. Introduced administrative, organizational, technical and sanitary-hygienic measures gave an opportunity to react adequately to the COVID-19 pandemic. Rospotrebnadzor implemented a set of activities that included there stages:

Stage 1. Preventive and sanitary activities;

Stage 2. Organizational and technical activities;

Stage 3. Organizational and preventive activities [41, 42].

The postulate of protecting by 'time', 'quantity', 'screen' and ' distance' is widely spread in the Russian hygienic science. It is commonly used in radiation hygiene and occupational hygiene and at present is entirely applicable within preventive activities aimed at limiting the coronavirus infection spread. The shorter a contact with an infection source, the lower a dose (the number of infectious agent particles per one cubic meter of air), the greater a distance from an infectious source, the lower is a risk of infection.

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Review

HEALTH DISORDERS IN WORKERS ASSOCIATED WITH HEALTH RISKS AT WORKPLACES IN MINING INDUSTRY IN THE ARCTIC (ANALYTICAL REVIEW)

A.G. Fadeev[1], D.V. Goryaev[1], N.V. Zaitseva[2], P.Z. Shur[2], S.V. Red'ko[2], V.A. Fokin[2]

[1]Krasnoyarsk Regional Office of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 21 Karatanova Str., Krasnoyarsk, 660097, Russian Federation
[2]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation

The review analyzes a range and prevalence of health disorders in workers employed at mining enterprises in the Arctic and exposed to heterogeneous occupational factors. We revealed that working processes typical for basic occupations in underground mining involved exposure to a set of heterogeneous harmful and (or) hazardous occupational factors such as intense occupational noise; elevated vibration; aerosols with predominantly fibrogenic effects, dusts and chemicals; high hardness and intensity typical for physical work; non-ionizing electromagnetic radiation. It was shown that diseases of the ear and mastoid, vibration syndrome, diseases of the musculoskeletal system, respiratory diseases and diseases of the nervous system prevailed both in the structure of general morbidity and in occupational one typical for miners. To create proper working conditions and to minimize effects of harmful and hazardous occupational factors as well as occupational health risks, it is advisable to perform comprehensive hygienic assessment of introduced equipment, machinery and mechanisms; to establish levels and doses of occupational factors. Engineering and technical, technological, medical and preventive and treatment and health-improving activities should be developed on this basis with special emphasis on such occupational groups as drift miners, drill-operators, blasters, timbermen, operators of cargo handling machinery, drilling unit operators, miners in mining outputs and faces, repairmen, and electric gas welders. It seems extremely vital to apply risk assessment methodology to assess occupational health risks for workers employed in mining operations in the Norilsk industrial region considering climatic features of the Arctic. This assessment is important for substantiating relevant activities aimed at managing such risks and protecting workers' health.

Keywords: *mining industry, occupational risk factors, occupational morbidity, the Arctic.*

The Basics of the State Policy of the Russian Federation for the period up to 2035[1] outline major national interests of the country. They include providing high quality of life and wellbeing for people living in the Arctic regions in the country; development of these regions as a strategic resource base and their rational use for accelerating eco-

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

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

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

Pavel Z. Shur – Doctor of Medical Sciences, Chief Researcher-Academic Secretary (e-mail: shur@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0001-5171-3105).

Svetlana V. Red'ko – Senior Researcher at the Health Risk Analysis Department (e-mail: redkosv@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: http://orcid.org/0000-0002-2736-5013).

Vladimir A. Fokin – Researcher at the Health Risk Analysis Department (e-mail: fokin@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-0539-7006).

[1] Ob Osnovakh gosudarstvennoi politiki Rossiiskoi Federatsii v Arktike na period do 2035 goda: Ukaz Prezidenta Rossiiskoi Federatsii ot 5 marta 2020 g. № 164 [On the Basics of the State Policy of the Russian federation in the Arctic for the period up to 2035: The RF President Order issued on March 05, 2020 No. 164]. *Prezident Rossii*. Available at: http://www.kremlin.ru/acts/bank/45255 (January 27, 2023) (in Russian); Ob utverzhdenii gosudarstvennoi programmy Rossiiskoi Federatsii «Sotsial'no-ekonomicheskoe razvitie Arkticheskoi zony Rossiiskoi Federatsii: Postanovlenie Pravitel'stva Rossiiskoi Federatsii ot 30 marta 2021 g. № 484 [On Approval of the State Program of the Russian Federation The Socioeconomic Development of the Arctic Zone in the Russian Federation: the RF Government Order issued on March 30, 2021 No. 484]. *Official Internet portal for legal information*. Available at: http://publication.pravo.gov.ru/Document/View/0001202104020037 (January 27, 2023) (in Russian).

nomic growth in Russia; environmental protection.

The Arctic zone has huge deposits of raw materials and it is impossible to provide sustainable development of the country without them. The Norilsk ore area is the most interesting for the country economy since it is the leading region in Russia as regards mining and processing of nickel, copper, silver and platinum group metals. Deposits located in the Norilsk ore area hold more than one third of silver ores, 73 % of nickel ores, 95 % of platinum group metals in Russia; two of them, namely Oktyabrskoye and Talnakhskoye do not have analogs in the world as per their stock. Average copper levels in ores vary between 1.11 and 4.54 % in 'coppery' ores and 4.06 % in 'solid' ones. On average, 41 % of all the copper, 86 % of all the nickel and 83 % of all the cobalt in the country are mined annually in the Norilsk ore area. It is also rich in silver deposits, 37.9 % of all the reserves in Russia. Almost all the country reserves of platinum group metals are also held in the Norilsk ore area, namely 15.2 thousand tons (95.6 %) [1, 2]. Such harmful factors as harsh climate in the Arctic, ambient air pollution due to emissions from metallurgical plants, and underground mining make issues of public health protection in Norilsk truly vital.

Work environment at mining enterprises creates unique working conditions; according to the International Labor Organization, they pose serious threats for workers [3]. Long-term studies have established that working conditions at mining enterprises in Siberia and the Far North involve exposures to many harmful factors. These factors determine levels and structure of work-related morbidity, occupational morbidity included; they are high levels of dusts, noise, local and general vibration, toxic gases and predominantly fibrogenic aerosols in workplace air, elevated exposure to chemicals in workplace air, non-ionizing radiation as well as work hardness [4, 5].

Profound analysis accomplished at mining enterprises allows stating that occupational morbidity is the highest among underground miners and is 3–4 times higher than that among workers employed in open-pit mining. Results obtained by special assessment of working conditions are widely used in practice to assess prior occupational risks caused on workers' bodies by exposure to harmful occupational factors. Some studies reported that overall assessment of working conditions performed in major occupational groups in mining established combined exposure to harmful occupational factors and therefore working conditions were considered hazardous (class 3) and assigned into hazard categories 3.2–3.4 (from average to very high hazard levels). Basic mining occupations with such hazardous working conditions at workplaces include drift miners, drill-operators, blasters, timbermen, operators of cargo handling machinery, drilling unit operators, miners in mining outputs and faces, and repairmen [6, 7]. High occupational health risks for miners are evidenced by high levels of morbidity with temporary loss of working ability. High general morbidity was identified in underground miners (drift miners, drilling unit operators and electric locomotive operators) due to diseases of the musculoskeletal system (arthrosis, diseases of muscles and soft tissues, dorsopathy), diseases of the nervous system (extremity polyneuropathy) and diseases of the ear (sensorineural hearing loss) [8].

The most common diseases with temporary loss of working ability in miners dealing with non-ferrous ore mining are diseases of the musculoskeletal system (spinal osteochondrosis, lumbodynia, arthralgia, and deforming osteoarthrosis), diseases of the digestive system (gastroduodenitis, stomach and duodenum ulcer, diseases of pancreas); diseases of the circulatory system (essential hypertension, varicose veins of lower extremities); diseases of the respiratory system (acute and chronic bronchitis); diseases of the ear and peripheral nervous system (polyneuropathy) [9, 10].

The most widely spread occupational pathologies of miners, similar to general ones, include diseases of the ear and mastoid (sensorineural hearing loss), vibration disease, diseases of the musculoskeletal system (radiculopathy, scapulohumeral periarthritis and deforming osteoarthrosis), respiratory diseases (chronic bronchitis, bronchial asthma and chronic obstructive pulmonary disease), as well as diseases of the nervous system (vegetative-sensory polyneuropathy) [9].

Long-term physiological, hygienic, clinical and functional studies with their focus on specific working conditions in mining indicate that long-term and intensive exposure to noise as well as noise and vibration in general are priority occupational health risk factors for miners. According to some authors, intensity of exposure to occupational noise corresponds to the hazard category 3.3 (harmful working conditions, hazard category 3) for drift miners in the Norilsk ore area [11]. Exposure to noise and vibration tends to grow as new equipment is implemented due to its forced speed, power and load parameters and as the existing technological processes are intensified. Elevated noise levels are detected due to operating scraper winches and loaders[2]. In general, noise holds the leading place among all the occupational factors as regards violations of the existing hygienic standards[3] and produces harmful effects on all the organs and systems in the body, first of all, on the ear. Therefore, occupational sensorineural hearing loss is an outstanding challenge for occupational medicine [12]. Prevention of occupational sensorineural hearing loss has high medical and social significance since hearing disorders develop in people of working age and lead to loss of occupational activity and poorer quality of life. Vibration pathologies and sensorineural hearing loss under exposure to noise occupy leading places in occupational morbidity of miners. This is largely due to characteristics of occupational noise, which is higher than hygienic standards predominantly in medium and high frequency range and produces the most aggressive effects on the ear [5].

Occupational diseases of the ear are diagnosed based on several indicators including lower speech intelligibility, noise in ears, headaches, heartaches, increased fatigability, and overall weakness.

Some authors reported several mandatory indicators for establishing a relationship between the disease and an occupation. They were two-sided lesion of the ear; gradual and slow progress of the disease in case hearing was normal at the recruitment; application for medical aid due to a disease of the ear; exposure to occupational noise exceeding its permissible levels; not less than 10 years of work under exposure to occupational noise [13]. Some studies revealed that average work records equaled 21.9 ± 1.8 years for underground drift miners and 23.8 ± 1.4 years for underground drilling unit operators by the moment sensorineural hearing loss was diagnosed in them[2].

Diseases of the circulatory system are a significant health outcome of exposure to noise that does not concern hearing[4]. Still, it is noteworthy that over the last decade occupational risk factors have been assigned a major role in progression of circulatory diseases along with genetic, somatic, behavioral and environmental ones. Harmful occupational factors make a substantial contribution to progression of cardiovascular pathologies. Health disorders that describe how essential hypertension develops in workers exposed to noise higher than its permissible levels are

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[2] Preobrazhenskaya E.A. Sistema upravleniya riskom razvitiya professional'noi tugoukhosti u rabotnikov gornodobyvayushchei i mashinostroitel'noi promyshlennosti [The system for managing risks of occupational sensorineural hearing loss in miners and workers employed in civil engineering]: the abstract of the thesis … for the Doctor of Medical Sciences degree. Moscow, 2013, 48 p. (in Russian).

[3] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v RF v 2021 g.: Gosudarstvennyi doklad [On sanitary-epidemiological welfare of the population in the Russian Federation in 2021: The State Report]. Moscow, the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2022, 340 p. (in Russian).

[4] Professional'naya patologiya: natsional'noe rukovodstvo [Occupational pathology: the national guide]. In: N.F. Izmerov ed. Moscow, GEOTAR-Media, 2011, 784 p. (in Russian).

considered work-related ones based on identified cause-effect relations between them and workplace exposures[5] [14, 15].

However, health disorders caused by exposure to noise levels higher than 70 dB can have such early signs as dysfunctions of the autonomic nervous system [16–18]. Several studies reported that workers who were exposed to high noise levels for a long time tended to have higher systolic and / or diastolic blood pressure than those who were not exposed to them [12, 19, 20]. According to Russian and foreign researchers, underground miners who had been working for not less than 10 years under long-term exposure to noise had not only sensorineural hearing loss but also essential hypertension and developing hypertensive disease together with background metabolic syndrome and already existing disorders of the autonomic nervous system that aggravated it [21–23]. At the same time, we can observe a close circle of pathological cause-effect reactions of the body as depletion of adaptation mechanisms and functional disorders of the circulatory system are, on one hand, outcomes of non-specific effects produced on the body by noise but on the other hand they facilitate progression of occupational sensorineural hearing loss[6].

Workers employed in mining and metallurgy tend to be exposed to high occupational vibration at workplaces [8, 24]. We should emphasize that mining usually makes workers to be simultaneously exposed to noise and vibration combined with harsh climatic conditions and these exposures exceed their permissible levels. For example, drift miners are exposed to intense local vibration (4–12 dB higher than its maximum permissible level, it means the working conditions are harmful, the hazard category is 3.3); drillers are exposed to overall vibration with its levels 3–12 dB higher than MPL, the hazard category of these working conditions is 3.2[6]. Besides, vibration and work hardness facilitate development of sensorineural hearing loss with almost the same likelihood (risk) due to impaired cerebral hemodynamics [25]. Therefore, scientific research has provided solid evidence that pathologies caused by exposure to vibration are leading ones in workers employed in contemporary mining [26, 27].

Polyneuropathy of lower extremities, radiculopathy of the lumbosacral spine, and dystrophic changes in the musculoskeletal system account for approximately one third (27.4 % of cases) of occupational diseases in all workers with diseases caused by exposure to vibration diagnosed for the first time in them [5]. Vibration pathology caused by exposure to overall vibration is most often diagnosed in operators of various mining machinery and drilling unit operators. Drift miners and face miners were occupations with the maximum risk of developing vibration pathology due to exposure to local vibration.

Vertebrogenic pathology is an interesting research subject for diagnosing and estimating associations between a disease and an occupation since radiculopathies prevail in people exposed to overall vibration; mono- or polyneuropathy and vegetative-sensory polyneuropathy seem to occur less frequently [4, 27]. Abroad, this pathology is considered a work-related one occurring due to many various causes, the contribution made by occupational factors being equal to 37 %. According to Russian researchers, radiculopathy predominantly develops in people who have static or dynamic loads on the spine, work in a forced posture, and are exposed to vibration at their workplaces. Vegetative-sensory polyneuropa-

[5] Shlyapnikov D.M. Gigienicheskaya otsenka riska razvitiya arterial'noi gipertenzii i effekta profilakticheskikh mer po ego minimizatsii u rabotnikov predpriyatii po dobyche kaliinykh solei v usloviyakh podzemnykh rabot [Hygienic assessment of a risk of essential hypertension and effects produced by preventive activities aimed at its minimization in workers employed at underground potassium salt mining]: the abstract of the thesis … for the Candidate of Medical Sciences degree. Perm, 2016, 24 p. (in Russian).

[6] Preobrazhenskaya E.A. Sistema upravleniya riskom razvitiya professional'noi tugoukhosti u rabotnikov gornodobyvayushchei i mashinostroitel'noi promyshlennosti [The system for managing risks of occupational sensorineural hearing loss in miners and workers employed in civil engineering]: the abstract of the thesis … for the Doctor of Medical Sciences degree. Moscow, 2013, 48 p. (in Russian).

thy becomes the most significant health disorder among vibration pathologies under exposure to local vibration; such exposure creates a higher risk of occupational pathology than exposure to overall vibration [4, 27]. Still, experts in occupational medicine pay more and more attention to combined exposure to several harmful occupational factors as a cause for developing neural-orthopedic pathology[7].

Work hardness is a feature of a work process associated with predominant loads on the musculoskeletal system and other functional systems in the body (cardiovascular, respiratory, etc.). It produces negative effects when combined with other occupational risk factors (noise, vibration, and some others). Work hardness is estimated as per several indicators given in ergometric values describing a work process regardless of individual features of a person who participates in it. Several well-known indicators of work hardness include physical dynamic load; a weight of a cargo a person has to lift and move manually; stereotypic work motions; static load; a work posture; body bending; the necessity to move around. In addition, work processes employed at mining enterprises involve apparent work hardness caused not only by the aforementioned factors but also high speed of work and emotional loads (a risk for one's life and responsibility for other people's lives). All basic work operations in a mine face in ore mines, even in machine mining, should be considered hard due to an uncomfortable work posture and the necessity to keep moving all the time. Excessive physical loads and frequent forced and irrational work postures lead to overstrain of the musculoskeletal system in drift miners and drill-operators [28].

Several technological processes employed in contemporary mining are still associated with work in forced and uncomfortable postures, stereotypic motions and local muscle strain. The total energy costs (145–320 kcal/hour) correspond to average and hard physical load. Working conditions at workplaces of most miners belong to the hazard category 3.1 as per work hardness [29]. The significant total amount of time a miner has to spend in an uncomfortable work posture is a factor causing overstrains in the cervical-thoracic and lumbosacral spine and occupational diseases (lumbosacral radiculopathy, plexitis, and myofascitis) [28]. Vegetative-sensory polyneuropathy of upper extremities is another significant occupational pathology developing due to exposure to such harmful occupational factors as local muscle loads, including those with switching between different work postures, and static loads.

Elevated levels of industrial aerosols are also important occupational factors occurring at workplaces in mining. Pathology caused by exposure to industrial aerosols stably accounts for approximately one third (29.3 %) of occupational diseases in Russia (B.T. Velichkovskiy, 2004). Thus, huge amounts of dusts are emitted into workplace air during drilling and blasting operations, in underground and open-pit mining, during loading and transportation of ores, their crushing and grinding, and during welding. Dusts in high concentrations can produce fibrogenic, toxic, irritating, allergenic, or carcinogenic effects depending on their chemical composition. Some industrial dusts induce occupational lung fibrosis or pneumoconiosis as well as dust bronchitis. These types of dusts are assigned into a specific group called 'aerosols with predominantly fibrogenic effects' (APFE).

Nickel and its compounds are known to produce harmful allergenic, toxic and carcinogenic effects on the lungs [30–33]. Fine dusts occurring in workplace air at copper and nickel production are highly toxic since they consist of micro- and nanoparticles with high permeability [31]. Use of diesel driverless mechanisms creates elevated levels of toxic gaseous components in their exhausts, such as nitrogen oxide, carbon oxides, acrolein, formaldehyde, and hydrocarbons. Their average

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[7] Suvorov V.G. Mediko-biologicheskie osnovy otsenki sochetannogo vliyaniya faktorov proizvodstvennoi sredy i trudovogo protsessa na organizm cheloveka [Biomedical grounds for assessment of combined effects produced by occupational factors on the human body]: the abstract of the thesis … for the Doctor of Medical Sciences degree. Moscow, 2004, 48 p. (in Russian).

shift levels do not usually exceed the relevant hygienic standards but maximum ones are 5.5 times higher than MPC for nitrogen oxides and 1.5 – 2.0 times higher for carbon oxide.

Occupational pathology among miners in the Far North is characterized with high prevalence of such respiratory diseases as pneumoconiosis, acute and chronic dust bronchitis; their highest shares of the total occupational diseases are the most frequently detected in underground miners [34, 35]. Clinical and functional examinations and analysis of occupational pathologies among miners have made it possible to conclude that not only there has been a growth in occupational bronchopulmonary diseases in them but also an increase in the number of severe or complicated forms of pneumoconiosis. Besides, workplace air in mines contains toxic dusts affecting the respiratory system and exposure to them leads to asthmatic bronchitis. Studies accomplished by Russian researchers are also confirmed by data reported by foreign authors examining, for example, occupational diseases in underground miners caused by exposure to dusts. Some studies showed that pneumoconiosis was widespread among miners in America, China, and South Africa [36–38]. Some authors mentioned that calculation of occupational health risks for miners caused by exposure to dusts revealed the highest levels of such risks for mine drifters and the lowest ones for repairmen [39].

Along with the aforementioned data, clinical, functional and sanitary-chemical laboratory tests provide evidence that occupational damage to the lungs occurs in welders after 10 years of work [40]. Some up-to-date chemical and physical studies report welding aerosols to have a complex composition that depends on a welding technology, materials and mode. Basically, they contain metals and their oxides (iron, manganese, chromium, nickel, aluminum, wolfram, etc.), gaseous fluorides, as well as silicon, carbon and nitrogen oxides [41]. Welding aerosols produce fibrogenic, toxic, irritating and sensitizing effects. Diseases of the eye are pathology typical for welders. Prophylaxis aimed at preventing diseases of the eye among welders is quite effective and relevant morbidity is lower than prevalence of respiratory diseases. Welders tend to have pneumoconiosis, chronic dust bronchitis and bronchial asthma. Inhalation exposure to welding aerosols and dusts plays the most significant role in pathogenesis of these diseases [40, 42]. Still, some Russian researchers reported that cataract was among few specific health outcomes caused by exposure to non-ionizing electromagnetic radiation typical for welding. This might lead to temporary, particle or even complete vision loss. The vascular system of the eye has a very specific structure and therefore changes in this organ often occur earlier than in other organs or system. The study [39] established a direct authentic correlation between deviations in the eye health and changes in the nervous and cardiovascular systems, which are the most susceptible to occupational exposures. Cataract develops due to intensive thermal exposure on the eye created by infrared waves; they account for 30–70 % of the total energy radiated by a welding arc. Under this exposure, the crystalline lens is heated to temperatures well beyond the physiological range. The ultraviolet part of the spectrum is also significant since ultraviolet radiation causes inflammatory changes in the front structures of the eye, or such diseases as electric ophthalmia and conjunctivitis [43–45].

Therefore, our analysis of foreign and Russian studies identified potential health hazards and risks for miners and established the following. Most underground miners are exposed to a combination of harmful occupational factors and working conditions at their workplaces are estimated as harmful (class 3), hazard category 3.1–3.4; that is, hazards (harmfulness) vary between average and very high.

We have established that working processes employed at workplaces of basic occupations in underground mining involve exposure to diverse harmful and (or) hazardous occupational factors including intense occupational noise; elevated levels of overall and local vibration; inhalation exposure to aerosols with predominantly fibrogenic effects, dusts, and chemicals; high work hardness and intensity; non-ionizing electromagnetic radiation. Occupational morbidity of underground miners employed at ore mines typically has high levels of diseases of the musculoskeletal, respiratory, and peripheral nervous system as well as circulatory diseases and diseases of the ear in its structure. To create proper working conditions and to minimize effects of harmful and hazardous occupational factors as well as occupational health risks, it is advisable to perform comprehensive hygienic assessment of introduced equipment, machinery and mechanisms; to establish safe levels and doses of occupational factors for underground miners. Engineering and technical, technological, medical and preventive measures as well as treatment and health-improving activities should be developed on this basis with special emphasis on such occupational groups as drift miners, drill-operators, blasters, timbermen, operators of cargo handling machinery, drilling unit operators, miners in mining outputs and faces, repairmen, and electric gas welders. It seems extremely vital to apply risk assessment methodology to assess occupational health risks for workers employed in mining operations in the Norilsk industrial region considering climatic features of the Arctic. This assessment is important for substantiating relevant activities aimed at managing such risks and protecting workers' health.

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