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

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Research article

AMBIENT AIR QUALITY AND RISK OF CIRCULATORY DISEASES FOR POPULATION OF A LARGE CITY IN THE EUROPEAN NORTH OF RUSSIA

T.N. Rastokina, A.A. Peshkova, T.N. Unguryanu

Northern State Medical University, 51 Troitskii Av., Arkhangelsk, 163000, Russian Federation

Cardiovascular diseases are the most frequent causes of premature mortality associated with ambient air quality. In the Arkhangelsk region, population mortality caused by diseases of the circulatory system is higher than the national average. The aim of this study was to assess ambient air quality in Arkhangelsk and associated health harm in adult population.

The study relied on using data of ambient air monitoring in Arkhangelsk collected over 2011–2022. We analyzed average annual levels of 23 pollutants and primary incidence of diseases of the circulatory system (CVD) and calculated hazard quotients under chronic inhalation exposure (HQ), hazard index (HI) for the cardiovascular system, and the number of attributable deaths associated with exposure to PM_{10} and $PM_{2.5}$.

Over the analyzed period, the average long-term concentrations of most pollutants met hygienic standards. Average levels of formaldehyde, chromium, copper and benzene were found to exceed the MPL by 1.5–2.3 times. Hazard coefficients for formaldehyde (HQ = 2.3), copper (HQ = 1.8) and $PM_{2.5}$ (HQ = 1.7) were established to be above their permissible value. The risk of developing general toxic effects is determined to be high (HI = 6.6) for the cardiovascular system. The main contribution to the risk level is made by copper and $PM_{2.5}$. Attributable outcomes of primary cardiovascular incidence among the adult population of Arkhangelsk associated with exposure to PM_{10} and $PM_{2.5}$ equaled 10.7 and 2.9 ‰ cases per year. The greatest harm under exposure to $PM_{2.5}$ is due to the development of coronary artery disease and amounts to 1.9 ‰ cases per year.

Keywords: *ambient air, pollutants, diseases of the circulatory system, primary incidence, health risk assessment, hazard quotient, hazard index, health harm.*

At present, ambient air pollution remains a global threat for human health. Relying on considerable pool of research data on health harm caused by ambient air pollution, the WHO issued new global air quality guidelines in 2021. The document outlines analytical data according to which approximately 80 % of deaths caused by $PM_{2.5}$ exposure can be prevented by reducing concentrations of this harmful admixture down to their safe levels[1].

In 2022, 40 cities in the Russian Federation with their total population reaching 10.4 million people had very high levels of ambient air pollution with API (air pollution index) ≥ 14; in 2021, only 33 cities were included in this list. Overall, 49 % of the urban population in Russia lives in cities with high and very high ambient air pollution. In 2022, the total pollutant emissions in Russia amounted to 17.174 million tons from station-

Tatiana N. Rastokina – post-graduate student of the Department of Hygiene and Medical Ecology, cardiologist (e-mail: dr.sokurenkotatiana@gmail.com; tel.: +7 (909) 552-79-99; ORCID: https://orcid.org/0000-0002-8074-9075).

Anna A. Peshkova – post-graduate student, cardiologist (e-mail: aqyila@mail.ru; tel.: +7 (964) 291-57-57).

Tatiana N. Unguryanu – Doctor of Medical Sciences, Professor of the Department of Hygiene and Medical Ecology, vice-rector for Scientific and Innovation Work (e-mail: unguryanu_tn@mail.ru; tel.: +7 (8182) 28-57-83; ORCID: https://orcid.org/0000-0001-8936-7324).

[1] WHO global air quality guidelines: Particulate matter ($PM_{2,5}$ and PM_{10}), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide. Geneva, World Health Organization, 2021, 300 p.

nary sources and 4.8 million tons from motor transport[2].

Cardiovascular diseases, in particular, coronary heart disease (CHD) and stroke are the most frequent causes of premature death attributable to ambient air pollution. According to the WHO data, 24 % of CHD burden and 25 % of stroke burden are attributable to ambient air pollution [1]. The number of additional deaths among population due to diseases of the circulatory system (CVDs) associated with ambient air pollution amounted to 0.7 per 100 thousand people in 2022 in Russia; the number of CVD cases attributable to ambient air pollution reached 42.9 per 100 thousand people, which corresponds to 1.4 % of the actual incidence[3].

CVDs hold the second rank place in the structure of incidence in the Arkhangelsk region. According to Rosstat, cardiovascular incidence in the Arkhangelsk region is at the same level with the national average (23.7 per 1000 against 26.29 per 1000). Cardiovascular mortality in the Arkhangelsk region (714.5 per 100 thousand) is 20.7 % higher than the national average.

In 2022 in the Arkhangelsk region, the total pollutant emissions were equal to 117 thousand tons (81.1 %) from stationary sources and 27.33 thousand tons (18.9 %) from mobile sources. In Arkhangelsk, ambient air pollution is mostly created by enterprises dealing with production and distribution of electrical power, water and gas, waste treatment and disposal; by automobile, river and railways transport. Most pollutants enter ambient air with automobile exhausts as their contributions to the total emissions amount to 70.5 %[4].

The aim of this study was to assess ambient air quality in Arkhangelsk and associated health harm in adult population.

Materials and methods. We used data on pollutant levels to assess ambient air quality in Arkhangelsk. They were provided by the Northern Office for Hydrometeorology and Environmental Monitoring and the Center for Use of Natural Resources and Environmental Protection. We analyzed average annual levels of total solid particles (TSP), sulfur dioxide, carbon oxide, nitrogen dioxide, nitrogen oxide, hydrogen sulfide, formaldehyde, methyl mercaptan, benzo(a)pyrene, benzene, toluene, ethyl benzene, xylene, and metals (chromium, manganese, iron, nickel, copper, zinc, and lead) over 2011–2022; ozone, PM_{10} and $PM_{2.5}$ over 2021–2022. We calculated the total number of samples that did not conform to safety standards, average long-term levels of the analyzed pollutants and their levels at the upper exposure limit (P_{90}).

Non-carcinogenic health risks upon exposure to pollutants were assessed according to the Guide R 2.1.10.1920-04 Human Health Risk Assessment from Environmental Chemicals[5]. We calculated hazard quotients (HQ) for chronic inhalation exposure to the analyzed pollutants and hazard index (HI) of general toxic effects caused by unidirectional impacts exerted by adverse chemicals on the cardiovascular system as well as contributions made by specific chemicals into the hazard index.

Cardiovascular incidence among the Arkhangelsk population was analyzed using data from the Form No. 12 the Number of

[2] Sostoyanie zagryazneniya atmosfery v gorodakh na territorii Rossii za 2022 god: ezhegodnik [Ambient air pollution in Russian cities in 2022: annual bulletin]. Saint Petersburg, Rosgidromet GGO, 2023, 256 p. (in Russian).

[3] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2022 godu: Gosudarstvennyi doklad [On sanitary-epidemiological welfare of the population in 2022: State report]. Moscow, the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2023, 368 p. (in Russian).

[4] Sostoyanie i okhrana okruzhayushchei sredy Arkhangel'skoi oblasti za 2022 god: doklad [The state and protection of the environment in the Arkhangelsk region in 2022: report]. In: O.V. Perkhurova ed.; The Center for Use of Natural Resources and Environmental Protection. Arkhangelsk, SAFU, 2023, 529 p. (in Russian).

[5] Guide R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals. In: Yu.A. Rakhmanin, S.M. Novikov, T.A. Shashina, S.I. Ivanov, S.L. Avaliani, K.A. Bushtueva, E.N. Belyaev, M.V. Fokin [et al.] eds. Moscow, Rospotrebnadzor, 2004, 143 p. (in Russian).

Diseases Registered in Patients Living in an Area Served by a Healthcare Organization over 2011–2022. We analyzed primary incidence of Diseases of the Circulatory System class (ICD-10: I00–I99) per 4 items and 10 nosologies among population aged 18 years and older and calculated extensive rates and chain growth / decline rates of primary cardiovascular incidence.

To establish attributable disease cases, a level – response function was used given by the relative risk rate per 10 μg/m^3 as well as frequency of diseases per 1000 people. Additional numbers of adverse health outcomes associated with exposure to pollutants were calculated using average daily PM_{10} and $PM_{2.5}$ levels at the upper exposure limit (P_{90}) per 1000 adult people (‰). Primary incidence of diseases associated with elevated blood pressure, myocardial infarction, stroke, chronic CHD and cardiovascular mortality among the adult Arkhangelsk population were estimated as adverse health outcomes in this study. An additional number of cardiovascular events was calculated using relative risk levels under exposure to $PM_{2.5}$ [2–8] and PM_{10} [9–12] (Table 1). The obtained data were statistically analyzed with STATA software, version 18.

Results and discussion. Levels of most chemical pollutants in ambient air, both average and at $P_{90,}$ conformed to safety standards in Arkhangelsk in 2011–2022 (Table 2). Formaldehyde levels were 2.3 and 3.3 times higher than average annual MPL at the average and P_{90} level respectively. Average benzene levels were 1.5 times higher than average annual MPL and 1.6 times higher than P_{90} over the analyzed period. We also detected chromium and copper levels that exceeded their MPLs and were 1.8 times higher at the average level and 2.8–3.3 times higher than MPLs at the upper exposure limit. Average TSP, PM_{10} and $PM_{2.5}$ levels conformed to safety standards but their levels at the upper exposure limit were 1.2, 1.1 and 1.6 times higher than average annual MPL respectively. Average benzo(a)pyrene and ozone levels were within safety ranges in ambient air in Arkhangelsk; at the upper exposure limit, their levels exceeded average annual MPLS 1.3 and 1.2 times respectively.

Essential (primary) hypertension accounts for the biggest proportion in cardiovascular incidence (23.9 %); it is followed by CHD, (the second place with 22.1 %) and cerebrovascular diseases (21.1 %). Over the 12-year analyzed period, primary cardiovascular incidence was established to decline in Arkhangelsk with its chain decline rate being -3.0 % (Table 3). Primary incidence went down for most items: average chain decline rates starting from -5.0 % identified for diseases of veins, lymphatic vessels and lymph nodes to -2.3 % identified for CHD. Essential primary hypertension was the only exclusion for which an average chain growth of 1.7 % was identified.

Table 1

A growth in effect on adult population per one-unit change in pollutant level (10 μg/m^3)

A growth in effect per one-unit change in level	ICD-10 code	$PM_{2.5}$	PM_{10}	Source ($PM_{2.5}$ / PM_{10})
Diseases of the circulatory system	I00–I99	1.36	1.10	[3] / [10]
– Ischemic heart diseases (coronary heart disease)	I20–I25	1.27	-	[2] / -
– Acute myocardial infarction	I21	1.28	1.03	[2] / [9]
– Cerebral infarction	I63	1.21	-	[6] / -
– Essential (primary) hypertension	I10	1.11	1.12	[7] / [12]
Cardiovascular mortality	I00–I99	1.12	1.10	[5] / [10]
Death due to cerebral infarction	I63	1.11	-	[4] / -
Death due to coronary heart disease	I20–I25	1.18	-	[8] / -
Death due to myocardial infarction	I21	-	1.05	- / [11]

Table 2

Ambient air quality in Arkhangelsk over 2011–2022

Chemical	The number of samples, total	Level				Average annual MPL, mg/m^3
		Average annual		At P_{90}		
		mg/m^3	Proprotion of average annual MPL	mg/m^3	Proprotion of average annual MPL	
Total solid particles	28,753	0.049	0.6	0.087	1.2	0.075
Sulfur dioxide	20,378	0.002	< 0.1[b]	0.003	0.1[b]	0.05[b]
Carbon oxide	22,240	1.26	0.4	1.685	0.6	3.000
Nitrogen dioxide	31,428	0.025	0.6	0.033	0.8	0.040
Nitrogen oxide	10,452	0.024	0.4	0.054	0.9	0.060
Hydrogen sulfide	30,555	0.001	0.4	0.001	0.5	0.002
Formaldehyde	31,429	0.007	2.3	0.010	3.3	0.003
Methyl mercaptan	5253	0.0001	–	0.0001	–	–
Benzo(a)pyrene	5606	0.875[c]	0.9	1.29[c]	1.3	1.00[c]
Benzene	5793	0.007	1.5	0.008	1.6	0.005
Toluene	5791	0.006	< 0.1	0.010	< 0.1	0.4
Ethyl benzene	5792	0.001	< 0.1	0.002	0.1	0.04
Xylenes	5792	0.010	0.1	0.012	0.1	0.1
Chromium	283	0.000014	1.7	0.000023	2.8	0.000008
Manganese	283	0.000014	0.3	0.000021	0.4	0.00005
Iron	283	0.000549	< 0.1	0.000718	< 0.1	0.04
Nickel	283	0.000010	0.2	0.000015	0.3	0.00005
Copper	283	0.000036	1.8	0.000066	3.3	0.00002
Zinc	283	0.000031	< 0.1	0.000045	< 0.1	0.035
Lead	283	0.000005	< 0.1	0.000010	0.1	0.00015
$PM_{2.5}$[a]	594	0.025	0.8	0.039	1.6	0.025
PM_{10}[a]	594	0.028	0.7	0.044	1.1	0.04
Ozone[a]	401	0.021	0.8	0.037	1.2	0.03

Note: [a] means data collected in 2021–2022; [b] means average daily MPL; [c] means ($\times 10^{-6}$).

Table 3

Primary cardiovascular incidence among adult population in Arkhangelsk in 2011–2022 (average frequency per 1000 people aged 18 years and older)

Diseases of the circulatory system	ICD-10 code	Proportion, %	Frequency per 1000 people	Chain growth / decline rate, %
Diseases of the circulatory system	I00–I99	100.00	29.70	-3.00
Essential (primary) hypertension	I10–I13	23.91	7.11	1.67
Ischemic heart diseases (coronary heart disease)	I20–I25	22.13	6.58	-2.58
– Angina pectoris	I20	5.44	1.61	-4.13
– Myocardial infraction	I21	4.20	1.25	-2.12
– Chronic ischaemic heart disease	I25	10.66	3.17	5.67
Cerebrovascular diseases	I60–I69	21.09	6.27	-2.32
– Subarachnoid haemorrhage	I60	0.29	0.09	2.18
– Intracerebral haemorrhage and other nontraumatic intracranial haemorrhage	I61, I62	1.46	0.43	-6.75
– Cerebral infarction	I63	9.53	2.83	-2.58
Atherosclerosis of arteries of extremities, thromboangiitis obliterans	I70.2,I73.1	1.86	0.55	-8.67
Diseases of veins, lymphatic vessels and lymph nodes	I80–I89	14.58	4.33	-5.00
– Phlebitis and thrombophlebitis	I80	2.59	0.77	-8.51
– Varicose veins of lower extremities	I83	8.17	2.43	-0.57

Average hazard quotients taken over 2011–2022 were higher than their permissible levels for formaldehyde (HQ = 2.3), copper (HQ = 1.8) and $PM_{2.5}$ (HQ = 1.7) and conformed to them for the remaining analyzed chemicals (Figure 1). HQ at the upper exposure limits equaled 3.3 for copper and formaldehyde, which corresponds to high risk of general toxic effects (Figure 2). HQ at the P_{90} level corresponded to alerting risk levels for $PM_{2.5}$, benzo(a)pyrene, ozone, and total solid particles.

HI for the cardiovascular system amounted to 6.6 over the analyzed period in Arkhangelsk, which corresponds to high risk of general toxic effects caused by exposure to ambient air pollutants. HI for the cardiovascular system was 10.8 at the upper exposure limit. Major contributions to the identified risk levels were made by copper (27 %), $PM_{2.5}$ (26 %), ozone (11 %), total solid particles, and PM_{10} (9 % both).

Adverse health outcomes associated with primary cardiovascular incidence among adult population in Arkhangelsk caused by $PM_{2.5}$ and PM_{10} exposure amounted to 10.69 and 2.96 ‰ respectively. Attributable cardiovascular incidence rates caused by $PM_{2.5}$ exposure

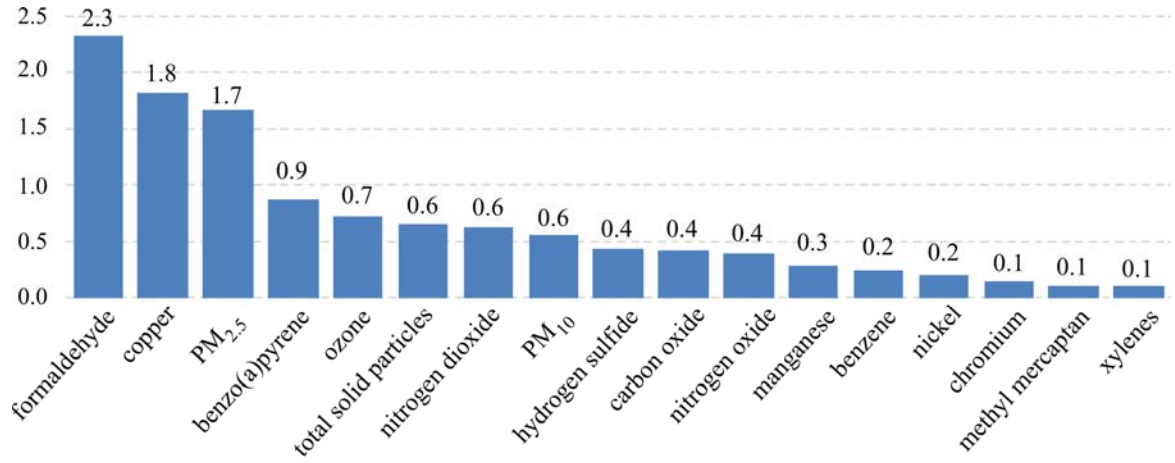

Figure 1. Hazard quotients of pollutants identified in ambient air in Arkhangelsk in 2011–2022 (taken as per average levels)

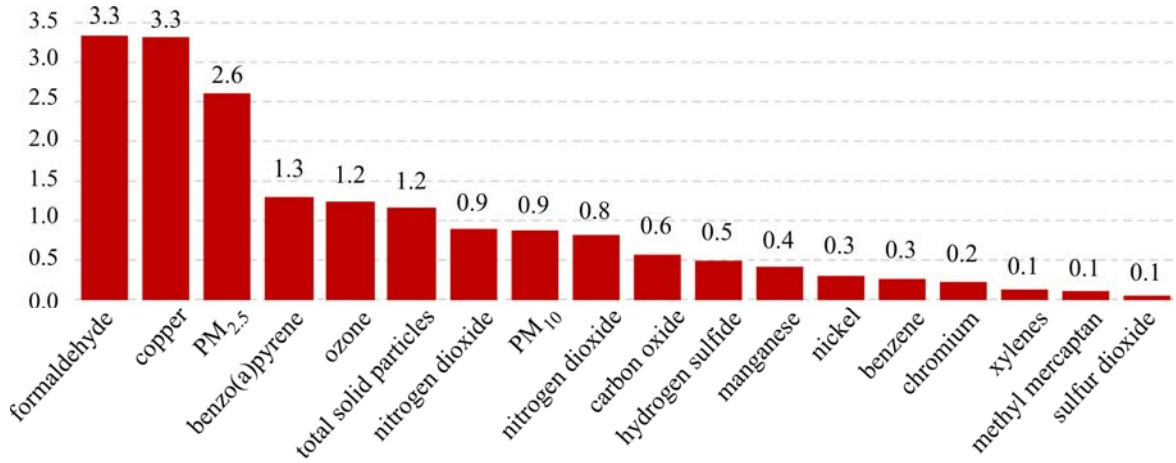

Figure 2. Hazard quotients of pollutants identified in ambient air in Arkhangelsk in 2011–2022 (taken as per average levels) (taken as per P_{90})

were established to be equal to 1.85 ‰; myocardial infarction. 0.48 ‰; cerebral infarction, 0.59 ‰. Likely additional numbers of first diagnosed essential hypertension upon $PM_{2.5}$ and PM_{10} exposure amounted to 0.78 and 0.85 ‰ respectively. Cardiovascular mortality caused by $PM_{2.5}$ exposure equaled 2.28 ‰ cases; PM_{10} exposure, 1.89 ‰ cases. Attributable deaths due to coronary heart disease and myocardial infarction upon $PM_{2.5}$ exposure amounted to 2.04 and 0.27 ‰ respectively.

Analysis of ambient air quality in Arkhangelsk established that levels of most pollutants were within their safe ranges; however, long-term average annual concentrations of formaldehyde, benzene, chromium and copper did not conform to safety standards. HQ for formaldehyde, copper and $PM_{2.5}$ was established to be higher than its permissible level. Hazard index for the cardiovascular system corresponded to high risk of general toxic effects. Major contributions to toxic effects were made by copper, $PM_{2.5}$, ozone, total soli particles, and PM_{10}. Primary cardiovascular incidence attributable to $PM_{2.5}$ exposure amounted 10.69 ‰; PM_{10} exposure, 2.96 ‰ cases.

Diversity of chemicals in ambient air, their structure and pollution intensity depend on pollution sources present on a given territory. Motor transport is a major source of ambient air pollution (more than 70 %) in many cities in Russia, for example, Kazan, Samara, Saint Petersburg, Tyumen, and Vladikavkaz. Our study findings are consistent with data obtained for Tyumen [13] and Vladikavkaz [14] where HI values were higher than 6 for the cardiovascular system, which corresponds to high risk of general toxic effects (23.2 and 6.7 respectively). Major contributions to pollution in these cities are made by carbon oxide and nitrogen dioxide.

In Kazan [15], Samara [16] and Saint Petersburg [17], where motor transport is a major source of ambient air pollution, HI established for the cardiovascular system is not higher than its permissible level (1.9; 0.74; 1.4 respectively). In Kazan, the greatest contributions to HI value are made by PM_{10} (79 %) and carbon oxide (21 %); in Samara, carbon oxide (55 %) and phenol (45 %); in Saint Petersburg, carbon oxide and $PM_{2.5}$.

Differences in HI values established for the cardiovascular system in different cities with similar major sources of ambient air pollution might be due to different chemicals included into monitoring programs, different methods for identifying chemicals in ambient air, or different approaches to selecting priority chemicals for health risk assessment.

Stationary sources make the main contribution to ambient air pollution in the Perm region [18], Novokuznetsk [19], Tula [20], Donetsk region [21], Orenburg region [22], and Irkutsk region [23]. High risk of general toxic effects for the cardiovascular system has been established in Makeevka (Donetsk region), a city with developed metallurgic production (HI = 9.33), where the greatest contributions are made by total solid particles (45 %) and ammonia (30 %) [21]. In Novokuznetsk, HI for the cardiovascular system was 4.6, which corresponded to alerting risk [19]. According to research data, areas with permissible risks of general toxic effects include Perm [18], Berezniki (Perm region) [18], Tula [20], Orenburg region [22], and Irkutsk region [23], where HI values established for the cardiovascular system equaled 0.76, 1.9, 0.48, 2.1 and 2.23 respectively. Major contributions to HI in these areas are made by benzene, phenol, carbon oxide, ammonia, and total solid particles.

Additional number of deaths due to CVDs per year caused by $PM_{2.5}$ exposure is higher in Arkhangelsk (11.9 %) than in Ust-Kamenogorsk (3.9 %), a city with developed metallurgic industry [24]. According to some foreign research, additional number of deaths due to CVDs under $PM_{2.5}$ exposure amounts to 0.072 ‰ in one of the most polluted Iranian cities with developed metallurgic and petrochemical industry [25]. This is considerably lower against Arkhangelsk (2.28 ‰). A study accomplished in an industrially developed area in Northern Italy with intense motor traffic established that cardiovascular mortality caused

by $PM_{2.5}$ exposure amounted to 4.33 ‰ [26], which is higher than in Arkhangelsk. In Tallinn, health losses due to cardiovascular incidence caused by $PM_{2.5}$ exposure amounted to 3.38 ‰ [27] additional diseases per year, which was lower than In Arkhangelsk (10.69 ‰).

Conclusion. Ambient air quality in Arkhangelsk conforms to safety standards as per levels of most pollutants. Long-term average annual concentrations of formaldehyde, benzene, chromium and copper did not conform to safety standards; additionally, non-conformity was also established at the upper exposure limit for total solid particles, PM_{10} and $PM_{2.5}$, ozone and benzo(a)pyrene. Risk of general toxic effects was determined as high for the cardiovascular system under exposure to ambient air pollution. Attributable levels of primary cardiovascular incidence caused by PM_{10} and $PM_{2.5}$ exposure amounted to 10.7 and 2.9 ‰ cases per year respectively among adult population in Arkhangelsk. It is necessary to expand lists of chemicals that are subject to monitoring in Arkhangelsk at stationary posts of the Northern Office for Hydrometeorology and Environmental Monitoring so that monitoring programs cover fine-dispersed fractions of solid particles.

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Research article

ASSESSING RISKS OF SECONDARY IMMUNODEFICIENCY IN CHILDREN WITH ALUMINUM CONTAMINATION IN BIOLOGICAL MEDIA AND POLYMORPHISM OF THE CELL DEATH GENE *FAS RS1159120* AND THE ANTIGEN-RECOGNIZING GENE OF THE TOLL-LIKE RECEPTOR *TLR4* RS1927911

O.V. Dolgikh, N.V. Zaitseva, O.A. Kazakova, A.V. Yaroma, T.S. Ganich

Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation

Secondary immunodeficiency remains an incompletely understood medical problem, and despite a significant number of international and scientific studies, there is no complete picture of the causes and consequences of this pathology. Metal cations have been proven to participate in the formation of acquired immunodeficiency. In particular, aluminum properties as an immune suppressor have been established and its targets in the body have been identified in case aluminum was present in biological media. However, no evaluations have been accomplished so far as regards the role of specific point genetic changes, that is, polymorphisms in the genes of immune system compartments that determine the risk of negative effects caused by contamination with metal cations, including aluminum. It is quite relevant to search and substantiate immunogenetic markers to create an indicator system for diagnostics and prevention of secondary immunodeficiency states in children associated with aluminum contamination in biological media.

We examined 97 preschool children exposed to elevated levels of airborne aluminum (in an area influenced by a metallurgic production). The study groups were divided depending on either presence or absence of secondary immunodeficiency as immune system pathology (common variable immunodeficiency D83). Several markers of the immune system were evaluated: aluminum-specific IgG, $CD3^+$, $CD4^+$, $CD8^+$, $CD127^-$, $CD95^+$, $CD284^+$, and phagocytic activity; we also evaluated polymorphism of TLR4 A8595G (rs1927911) and FAS C14405T (rs1159120) genes of innate and acquired immunity.

According to the results obtained by examining biological media composition, children with secondary immunodeficiency had 1.8 times higher aluminum levels in urine (0.0095 ± 0.0014 vs. 0.0054 ± 0.0009 mg/dm^3, reference range < 0.0075 mg/dm^3) as opposed to their conditionally healthy peers. We established an authentic inverse dependence between the expression level of the main CD clusters ($CD3^+$: r = -0.38; $CD4^+$: r = -0.39; $CD8^+$: r = -0.26) as well as indicators of phagocytic activity (r = -0.22–0.23) and the level of aluminum contamination in biological media (urine). Expression of T-mature lymphocyte clusters was found to be inhibited by 1.3–3.1 times (including T-helpers, effector T-lymphocytes, NK-killers, regulatory lymphocytes) and we also detected some changes in expression of specific immunoglobulin of IgG class to aluminum. All this results in an unacceptable level of relative risk (RR = 1.23–1.63) of developing secondary immunodeficiency against increased frequency of allele C and genotype CC of FAS gene (rs1159120) by 1.2 and 1.5 times respectively, as well as minor allele G of TLR4 gene (rs1927911) by 1.8 times relative to the comparison group (OR = 4.05; CI: 1.41–11.59; p = 0.006; RR = 1.23; CI: 1.02–1.48) and (OR = 2.01; CI: 1.04–3.91; p = 0.037; RR = 1.64; CI: 1.46–1.94). Toll-dependent and FAS-dependent mechanism of this risk is associated with aluminum contamination. It is recommended to use a combination of immune and genetic markers as indicator ones when evaluating the immune system state, in order to prevent the risk (RR = 1.23–1.63) of secondary immunodeficiency associated with aluminum contamination in biological media.

Keywords: *aluminum, children, relative risk, secondary immunodeficiency, cell differentiation clusters, FAS gene, TLR4 gene.*

Oleg V. Dolgikh – Doctor of Medical Sciences, Professor, Head of the Department of Immunobiological Diagnostic Methods (e-mail: oleg@fcrisk.ru; tel.: +7 (342) 236-39-30; ORCID: http://orcid.org/0000-0003-4860-3145).

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

Olga A. Kazakova – Candidate of Biological Sciences, Senior Researcher-Head of the Laboratory of Immunogenetics (e-mail: chakina2011@yandex.ru; tel.: +7 (922) 646-56-87; ORCID: https://orcid.org/0000-0002-0114-3930).

Alesya V. Yaroma – Junior Researcher at the Laboratory of Cellular Diagnostic Methods (e-mail: lady.yaroma@mail.ru; tel.: +7 (919) 499-66-41; ORCID: https://orcid.org/0000-0002-0932-3063).

Tatyana S. Ganich – Junior Researcher at the Laboratory of Immunogenetics (e-mail: tatka.kuleshova@yandex.ru; tel.: +7 (952) 318-57-45; ORCID: https://orcid.org/0009-0004-2582-162X).

Identification of imbalance in microelements, like aluminum, is an important task that has to be tackled to prevent development of chronic intoxications. Aluminum does not have any biological function in the human body; however, when this element or its compounds persist in biological media for a long time, they may affect the hematopoietic system, bones, nervous system and immunological indicators[1] [1, 2]. This metal has adverse effects not only on cell structures but also on their functions, in particular, ability to proliferate [3]. Aluminum promotes the expression of the apoptosis gene *FAS* [4]. D. Cheng with colleagues established and proved that aluminum was able to damage macrophages thus promoting secondary immunodeficiency development [1, 5]. Experimental data reported by B. Wang with colleagues allowed establishing that aluminum could also affect gut microbiota decreasing its diversity and disrupt its overall structure, which, in its turn, had a negative effect on the immune system [6].

Secondary (acquired) immunodeficiency is a specific dysfunction of the immune system that can occur in different age groups. Children aged 4–6 years are exposed to elevated viral and bacterial loads and any disruption of cell differentiation, proliferation and adaptation, together with decline in their number and activity, results in higher respiratory incidence with possible bacterial infections [7].

Although secondary immunodeficiency is not innate, nevertheless, polymorphism of genes that regulate the immune response has a significant role in making the body resistant to chemical and biological environmental exposures.

Toll-like receptors *TLR* belong to the innate immunity. They are the first to recognize ligands of viruses, bacteria, protozoa and fungi and to launch an immune reaction [8]. There is evidence that polymorphism of *TLR* genes, in particular, *TLR4* that is a signaling molecule responsible for cytokine expression, is associated with allergic, infectious and autoimmune diseases [9, 10].

The *FAS*-mediated apoptosis system ($CD95^+$) contributes to physiological and pathological cellular processes, such as differentiation and survival. Studies on mutations in the *FAS* gene can promote better understanding of the pathogenesis of autoimmune diseases and immunodeficiency formation [11]. *FAS* antigen plays the key role in regulating programmed cell death and is expressed on B- and T-lymphocytes [12, 13].

Search for new immunological and genetic markers associated with the development of aluminum-modified secondary immunodeficiency in children is relevant given new challenges and threats that arise due to changes in the sanitary-hygienic situation caused by exposure to new airborne chemicals able to affect human health.

The aim of this study was to assess risks of secondary immunodeficiency associated with specific polymorphism of the cell death gene *FAS* rs1159120 and the antigen-recognizing gene of the toll-like receptor *TLR4* rs1927911 in children with aluminum contamination in biological media.

Materials and methods. We examined 97 children aged between 3 and 6 years who lived in an area influenced by emissions from a non-ferrous metallurgy plant in Western Siberia. The observation group consisted of 50 children aged 5.0 ± 0.3 years with secondary immunodeficiency (common variable

[1] Zaitseva N.V., Dolgikh O.V., Krivtsov A.V., Otavina E.A., Bubnova O.A., Dianova D.G., Bezruchenko N.V., Perminova I.V. Sposob otsenki vliyaniya alyuminiya na immunnyi status [The method for assessing effects of aluminum of the immunity]: patent No. 2629597 Russian Federation, MPK G01N33/53], no. 2016126799; submitted on July 04, 2016; published on August 30, 2017, Bulletin No. 25; Federal Scientific Center for Medical and Preventive Health Risk Management Technologies is the applicant and patent holder (in Russian).

immunodeficiency, D83); the reference group was made of 47 presumably healthy children aged 4.3 ± 0.3 years. Both groups were comparable in terms of age, socioeconomic status and ethnicity.

All examinations were conducted at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (the study was approved by the Ethics Committee, the meeting report No. 8 dated July 17, 2023) in conformity with the ethical standards stipulated in the WMA Declaration of Helsinki.

Aluminum was quantified in urine using a quadrupole mass spectrometer with inductively coupled plasma Agilent 7500cx (Agilent Technologies, USA) with the octopole reaction system (ORS) in accordance with the methodical guidelines MUK 4.1.3589-19 "Measurement of aluminum mass concentration in biological media (blood, urine) by inductively coupled plasma mass spectrometry".

Immunological tests of blood serum indicators were performed using the unified research technique, namely, ELISA tests, on a Biotek ELx808 (USA).

We examined CD-clusters of cell differentiation and intracellular apoptosis markers on a flow cytometer BD FACSCalibur (USA) using relevant monoclonal antibodies and the universal software CellQuestPrO. The examinations included determining $CD127^-$, $CD16^+56^+$, $CD19^+$, $CD3^+$, $CD3^+$ $CD8^+$ counts with flow cytometry.

Phagocytic activity levels were identified using formalized sheep erythrocytes as phagocytosis objects following the V.N. Kaplin technique.

We examined polymorphism of the cell death *FAS* rs1159120 gene and antigen-recognizing toll-like receptor *TLR4* gene by real-time polymerase chain reaction performed on a CFX96 Real Time System.

Research data were statistically analyzed using Statistica 12.0 (StatSoft, Inc., USA) and Microsoft Excel 2013. We determined the simple mean (X) and its standard deviation (SD). Distribution was checked for normalcy using the Shapiro – Wilk test. Mean values were compared using the Student's t-test. The relationships between the analyzed immune markers and levels of aluminum contamination in biological media were assessed using Pearson Correlation. Risk assessment involved calculating relative risks (RR) of negative processes associated with candidate gene polymorphism. Differences were considered authentic at the significance level being above 95 % ($p < 0.05$).

Results and discussion. Chemical tests established that children from the observation group had authentically 1.8 times higher aluminum levels in urine against the reference group (0.0095 ± 0.0014 against 0.0054 ± ± 0.0009 mg/dm^3, the reference level is below 0.0075 mg/dm^3).

Specific immune response was established to be authentically higher in the observation group as per the level of IgG to aluminum, which was 1.8 times higher than in the reference group ($p < 0.05$) [14].

Comparative analysis of cell differentiation clusters established an authentic imbalance of regulatory mediators in the observation group associated with immunodeficiency. Thus, the absolute count of T-mature CD3+-lymphocytes responsible for cell-mediated immunity was 1.4 times lower. We established an authentic inverse correlation between CD3+ levels and levels of aluminum contamination in biological media (urine) ($r = -0.38$; $p < 0.05$). Alpatova N.A. with colleagues reported a decrease in the proportion of $CD3^+$-lymphocytes in animals upon aluminum administration[2] [15]. We found defi-

[2] Zaitseva N.S., Sizyakina L.P., Bagmet A.D., Kharitonova M.V. Sposob diagnostiki vtorichnogo immunodefitsita [The method for diagnosing secondary immunodeficiency]: patent No. 2749781 C1 Russian Federation, MPK G01N 33/53, no. 2020137481, submitted on November 16, 2020, published on June 16, 2021; Rostov State Medical University of the RF Ministry of Health is the applicant (in Russian).

ciency of T-helpers $CD3^+CD4^+$ (1.3 times lower in the observation group), which are responsible for antigen recognition and immune response regulation, and also established an authentic inverse correlation with aluminum contamination in biological media (urine) (r = -0.39). In another study, Y. She with colleagues reported less active Th1 immunocytes that secreted pro-inflammatory cytokines under exposure to aluminum [16]. In our study, deficiency of effector T-cytotoxic lymphocytes $CD3^+$ $CD8^+$ (1.4 times lower) was shown to result in cancelled apoptosis launch, which was associated with negative effects produced by aluminum on cellular immunity (r = -0.26; $p < 0.05$) [16, 17]. The present study verifies inhibited production (by 3.1 times lower) of T-regulatory lymphocytes $CD127^-$ as well as an authentic decline in expression of the membrane receptor of *FAS*-receptor cell death $CD95^+$ (by 1.6–1.8 times lower), which is an apoptosis trigger, which control the intensity of an immune response given by T-effector cells [18]. We also established an authentically inhibited expression of the toll-like receptor 4 ($CD284^+$ rel./abs. 1.6–1.8 times lower), which eliminates adequate pro-inflammatory mediator effects of antiviral and antibacterial immunity against the same indicators in the children from the reference group ($p < 0.05$).

Phagocytic activity assessment established that the relative count of phagocytic cells and their absorbing capacity was 10 % lower than in the observation group against the reference one (Table 1). A rise in the aluminum level in biological media (urine) has an inverse correlation with the level of phagocytic activity; this verifies that the chemical is able to suppress immunity[3] [19, 20].

We examined peculiarities of polymorphism of the cell death *FAS* (rs1159120) gene and antigen-recognizing toll-like receptor *TLR4* (rs1927911) gene in children with immunodeficiency exposed to aluminum. As a result, we found significantly ($p < 0.05$) higher frequency of C allele of the *FAS* (rs1159120) gene and its CC genotype, 1.2 and 1.5 times higher accordingly, as well as significantly ($p < 0.05$) 1.8 times higher frequency

Table 1

Peculiarities of the immune profile of the children from the analyzed groups

Indicator	Reference range	Observation	Reference	*p(t)*
IgG to aluminum, atb. units	0–0.1	0.16 ± 0.05	0.09 ± 0.02	***0.0220***
$CD3^+$-lymphocytes, abs., 10^9/l	0.69–2.54	1.54 ± 0.13	2.08 ± 0.24	***0.0000***
$CD3^+CD4^+$-lymphocytes, abs., 10^9/l	0.41–1.59	0.84 ± 0.08	1.10 ± 0.13	***0.0010***
$CD3^+CD8^+$-lymphocytes, abs., 10^9/l	0.19–1.14	0.60 ± 0.07	0.83 ± 0.09	***0.0000***
$CD127^-$ lymphocytes, abs., $10^9/dm^3$	0.015–0.04	0.07 ± 0.03	0.22 ± 0.05	***0.0000***
$CD95^+$ lymphocytes, abs., $10^9/dm^3$	0.43–0.87	0.290 ± 0.04	0.511 ± 0.05	***0.0008***
$CD95^+$ lymphocytes, rel., %	20–40	15 ± 1.05	25 ± 2.15	***0.0000***
$CD284^+$ lymphocytes, abs., $10^9/dm^3$	0.2–0.4	0.208 ± 0.03	0.380 ± 0.04	***0.0008***
$CD284^+$ lymphocytes, rel., %	10–20	11 ± 0.95	18 ± 0.97	***0.0000***
Phagocyte proportion, %	35–60	48.44 ± 1.98	53.76 ± 3.16	***0.0060***
Phagocytic number, atb. units	0.8–1.2	0.88 ± 0.05	1.02 ± 0.08	***0.0070***
Absolute phagocytosis, $10^9/dm^3$	1–2	1.24 ± 0.13	1.93 ± 0.31	***0.0000***

[3] Immunoterapiya: rukovodstvo dlya vrachei [Immunotherapy: guide for physicians]. In: R.M. Khaitov, R.I. Ataullkhanov eds. Moscow, GEOTAR-Media Publ., 2012, 672 p. (in Russian).

Table 2

The results obtained by genotyping of candidate genes *FAS* (rs1159120) and *TLR4* (rs1927911)

Gene	Allele	Observation % (N)	Reference % (N)	$x^2(p)$	OR (CI)	Genotype	Observation % (N)	Reference % (N)	$x^2(p)$	OR (CI)
FAS C14405T (rs1159120)	C	92.4 (85)	75.0 (30)	7.52 (0.006)	4.05 (1.41–11.59)	CC	84.8 (39)	55.0 (11)	7.70 (0.02)	4.56 (1.38–15.03)
						CT	15.2 (7)	40.0 (8)		0.27 (0.08–0.90)
	T	7.6 (7)	25.0 (10)		0.25 (0.09–0.71)	TT	0 (0)	5.0 (1)		0.14 (0.01–3.58)
TLR4 A8595G (rs1927911)	A	72.9 (70)	84.4 (103)	4.34 (0.037)	0.50 (0.26–0.97)	AA	58.3 (28)	73.8 (45)	3.50 (0.17)	0.50 (0.22–1.12)
						AG	29.2 (14)	21.3 (13)		1.52 (0.63–3.64)
	G	27.1 (26)	15.6 (19)		2.01 (1.04–3.91)	GG	12.5 (6)	4.9 (3)		2.76 (0.65–11.68)

of G allele of the *TLR4* (rs1927911) gene against the reference group. The wild C allele of the *FAS* (rs1159120) gene and the minor G allele of the *TLR4* (rs1927911) gene are both factors that determine greater likelihood of unfavorable scenarios (likely manifestations of an immunodeficiency) associated with aluminum contamination in biological media (urine) (OR = 4.05; CI: 1.41–11.59; p = 0.006; RR = 1.23; CI: 1.02–1.48) and (OR = 2.01; CI: 1.04–3.91; p = 0.037; RR = 1.64; CI: 1.46–1.94) (Table 2). The absolute expression of CD3+CD95+ activated T-lymphocytes and CD284+ as markers of apoptosis, cytokine expression and effectiveness of anti-infection immunity was authentically 1.6-1.8 times lower in the observation group in carriers of these polymorphisms of the *FAS* gene (rs1159120) and the toll-like receptor gene *TLR4*. This reflected expected inhibition of cell death and protein elimination controlling as regards both infectious agents and own nonfunctional proteins (Table 2).

Therefore, the present study verifies the hypothesis, which confirms the results reported in previous studies as regards effects produced by aluminum in vitro as immunosuppressant. As an immunosuppressant, the chemical affects certain priority regulatory clusters in cell differentiation, namely, some phenotypes of T-mature lymphocytes, including T-helpers, effector cells, T-regulatory lymphocytes. Among the latter, we should mention those having receptors of apoptosis and cytokine regulation controlling ($CD284^+$; $CD95^+$), imbalance of which is authentically combined with changed frequency of risk-associated alleles of the genes responsible for innate and adaptive immunity, *TLR4* A8595G (rs1927911) and *FAS* C14405T (rs1159120) in children with diagnosed secondary immunodeficiency and aluminum contamination in biological media (RR = 1.23–1.64).

Conclusions. Our study findings indicate that polymorphism of the apoptosis *FAS* rs1159120 gene and antigen-recognizing toll-like receptor *TLR4* rs1927911 gene creates risk (RR = 1.23–1.63) of secondary immunodeficiency in children with elevated aluminum levels in biological media (1.3 times higher than the reference level).

The study reports immune-suppressing effects produced by aluminum on the immune system cells (T-regulatory lymphocytes

$CD127^-$, T-lymphocytes with the $CD95^+$ phenotype, their production is 1.6–3.1 times lower; the $CD284^+$ phenotype of toll-like receptors, production 1.6–1.8 times lower). The effects are verified by authentic models of the relationship between inhibited expression of T-lymphocytic clusters and aluminum levels in urine ($CD3^+$: r = -0.38; $CD4^+$: r = -0.39; $CD8^+$: r = -0.26), which manifests itself as cancelled apoptosis controlling, inhibited activation of its receptor-mediated pathway and anti-infection immunity ($CD284^+$; $CD95^+$).

Authentically higher frequency of candidate alleles and genotypes of innate and adaptive immunity was established; C allele and CC genotype of the *FAS* gene (rs1159120) were 1.2 and 1.5 times more frequent accordingly and minor G allele of the *TLR4* gene (rs1927911) was 1.8 times more frequent than in the observation group. This higher frequency was associated with aluminum contamination in biological media (urine) (OR = 4.05; CI: 1.41–11.59; p = 0.006; RR = 1.23; CI: 1.02–1.48) and (OR = 2.01; CI: 1.04–3.91; p = 0.037; RR = 1.64; CI: 1.46–1.94). This reduces the body resistance to viral and bacterial pathogens in the environment ($CD284^+$) and creates the elevated risk of secondary immunodeficiency (D83).

It is recommended to use immunologic ($CD284^+$; $CD95^+$) and genetic (C allele and CC genotype of the *FAS* (rs1159120) gene and G allele of the *TLR4* (rs1927911) gene) indicators as markers of effect and sensitivity associated with the risk (RR = 1.23–1.64) of aluminum-modified secondary immunodeficiency in children.

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Research article

PERSONAL RISK FACTORS OF INTERNET ADDICTION IN ADOLESCENTS AGED 15–17 YEARS

O.Yu. Kocherova, O.M. Filkina, A.V. Boboshko, E.A. Vorobyova, N.V. Dolotova, A.I. Malyshkina

Ivanovo Research Institute of Motherhood and Childhood named after V.N. Gorodkov, 20 Pobedy St., Ivanovo, 153045, Russian Federation

Internet addiction formation and manifestations are a relevant trend in contemporary research. It is important to identify adolescents who have risk of developing Internet addiction so that early interventions can be made and certain impacts can be exerted on personal characteristics that contribute to formation of persistent addictive behavior able to affect health.

The aim of this study was to establish personal risk factors of developing Internet addiction in adolescents aged 15–17 years.

A comprehensive study was carried out on 407 adolescents aged 15–17 years who attended comprehensive secondary schools in Ivanovo. To assess the Internet addiction in adolescents, S. Chen's method was used; personal characteristics of adolescents were determined using the Eysenck test. Mental performance was assessed based on the results of proofreading tests (M.V. Antropova); academic performance was estimated per the average score. Data were statistically analyzed using conventional methods of variation statistics.

High neuroticism, introversion in girls, and centroversion in boys are risk factors able to cause formation of Internet addiction in adolescents; an increase in the severity of neuroticism in these groups combined with a decrease in accuracy, mental performance and academic performance is also a risk factor of Internet addiction in adolescents aged 15–17 years. A direct correlation was found between the Internet addiction score and the level of neuroticism. At risk of Internet addiction, introversion was detected in girls more often than in boys, and extraversion and high neuroticism were detected in the group with Internet addiction. Boys with Internet addiction were more likely to have centroversion than girls. Adolescents with severe Internet addiction had the lowest levels of accuracy and mental performance quotient and lower academic performance.

High neuroticism, introversion in girls, centroversion in boys, an increase in the severity of neuroticism, a decrease in accuracy, mental performance and academic performance are risk factors of Internet addiction formation in adolescents. This justifies the need to carry out sanitary and hygienic measures that should involve development of standards for safe Internet use and inclusion of psycho-prophylaxis.

Keywords: *adolescents 15–17 years old, Internet addiction, risk factor, sex-dependent characteristics, neuroticism, personality type, mental performance, academic performance.*

Rapidly expanding access to the Internet has serious influence on adolescents' physical and mental health. Modern educational programs involve more active implementing computer technologies into the educational process. However, these new developments

Olga Yu. Kocherova – Doctor of Medical Sciences, Leading Researcher at the Department of Children's Health (e-mail: ivniideti@mail.ru; tel.: +7 (961) 246-24-41; ORCID: https://orcid.org/0000-0002-2473-8339).

Olga M. Filkina – Honored Doctor of the Russian Federation, Honored Scientist of the Russian Federation, Doctor of Medical Sciences, Professor, Head of the Department of Children's Health (e-mail: omfilkina@mail.ru; tel.: +7 (903) 888-91-94; ORCID: https://orcid.org/0000-0003-2228-748X).

Aleksey V. Boboshko – post-graduate student of the Department of Children's Health (e-mail: lesha.boboshko.96@mail.ru; tel.: +7 (910) 685-26-36).

Elena A. Vorobyova – Doctor of Medical Sciences, Leading Researcher of the Department of Children's Health (e-mail: ivniideti@mail.ru; tel.: +7 (909) 249-88-44; ORCID: https://orcid.org/0000-0003-2820-9714).

Natalya V. Dolotova – Doctor of Medical Sciences, Senior Researcher at the Department of Children's Health (e-mail: dolotovan@inbox.ru; tel.: +7 (903) 879-17-05; ORCID: https://orcid.org/0000-0002-2449-0580).

Anna I. Malyshkina – Doctor of Medical Sciences, Professor, director (e-mail: ivniimid@inbox.ru; tel.: +7 (493) 233-62-63; ORCID: https://orcid.org/0000-0002-1145-0563).

are not properly regulated and this can result in excessive fatigue among students, Adolescent psyche is in the making and its traits are unsteady; therefore, it is especially prone to formation of Internet addiction [1–3].

Internet addiction is an obsessive desire to use the Internet and it always means its problematic use; it typically involves spending too much time in the Internet [4]. Several diagnostic methods are employed to detect formation of Internet addiction. They all rely on specific indicators. The Chen Internet Addiction Scale (CIAS), which we used in this study, relies on five scales for addiction identification: compulsive use symptoms; withdrawal symptoms; tolerance; interpersonal and health problems; time management.

Internet addiction is a relevant trend in contemporary research. A lot of attention is being paid to Internet addiction prevalence as well as sex-specific and psychological peculiarities of Internet users [2, 4, 5].

Many studies report that prevalence of Internet addiction varies between 4.3 % and 12.0 % among adolescents in Russia and up to 48 % of them are in a risk group. This phenomenon has become the focus of attention in modern studies that investigate its prevalence as well as psychological traits and sex differences of those suffering from Internet addiction [4, 5].

Internet addiction affects mental and somatic health, personality development, and psychosocial adaptation of adolescents.

Previous researches identified personality traits able to create elevated risks of Internet addiction for adolescents, in particular, impulsiveness [4, 8–10], poor self-control [4] and impaired inhibitory control [11]. Some researchers [5, 7, 11] also found higher aggressiveness in adolescents to be associated with their pathological behavior in computer games [3, 6, 7, 10]. Other studies also identified risk factors of Internet addiction including harm avoidance [12], emotional reactivity [11], and anxiety [13, 14].

Internet addiction affects adolescents' attention and memory. D.D. Fedorova, D.T. Pirog and others established in their studies that most adolescents were prone to become addicted to the Internet and to anxiety; the authors reported weaker short-term and long-term memory, attention and mental function [15, 16].

Long-term PC use leads to the carpal tunnel syndrome, facial and hand tick, dry eye symptoms and burning in the eyes, headaches, spine aches, irregular meals, neglected hygiene, impaired sleep, and disrupted sleep-wake patterns [1, 7, 9].

Use of digital devices for more than 6 hours a day was established to create elevated risks of myopia [17]. Hypodynamia, irrational eating habits, and elevated stress levels in Internet-addicted adolescents promote cardiovascular pathology and disrupt physical development.

It is important to identify risk factors able to cause Internet addiction in order to make early intervention and diagnostics and to develop relevant strategies aimed at preventing adverse effects of Internet use.

The aim of this study was to establish personal risk factors of developing Internet addiction in adolescents aged 15–17 years.

Materials and methods. A comprehensive study was carried out on 407 adolescents aged 15–17 years (209 boys and 198 girls) who attended comprehensive secondary schools in Ivanovo. All schools included in the study provided the identical comprehensive educational programs for their students.

Therefore, the study has age-related (adolescents aged 15–17 years), regional (Ivanovo) and educational (students attending comprehensive secondary schools) limitations.

Adolescents gave their voluntary informed written consent to take part in the study; after that, they took part in a comprehensive examination. The Chen Internet Addiction Scale (CIAS, S. Cnen, 2003, adapted by V.L. Malygin and K.A. Feklisov) was applied to estimate Internet addiction in adolescents [18]. Personal characteristics of each adolescent were estimated using a score system and the results obtained by questioning and structured interviews. We estimated com-

pulsive use symptoms, withdrawal symptoms, tolerance, interpersonal and health problems, as well as time management. The result obtained for each participant was estimated using the following scale:

– from 27 to 42 scores, no Internet addiction;

– from 43 to 64 scores, a risk of developing Internet addiction / pre-addiction;

– 65 scores and higher, well-grounded presence of Internet addiction (a person's behavior includes problematic Internet use).

We identified 3 groups: Group 1 included 50 adolescents without Internet addiction, Group 2 was made of 50 adolescents who had a risk of developing Internet addiction, and Group 3 included 50 adolescents with already present Internet addiction.

Personal characteristics of adolescents were determined using the Eysenck test (adapted by A.G. Shmelev)[1]. We determined extroversion – introversion and neuroticism (emotional stability – instability).

Mental performance was assessed using the results obtained by accomplishing proofreading tests (as per the methodology suggested by M.V. Antropova)[2]. A task given to students was to be accomplished in 2-minute time and its aim was to find a specific combination of letters (for example, AIE) that stood before a certain letter. The following indicators were then estimated for each participant: a as the number of correctly found combinations; b as the number of missed combinations; c as the number of errors; d as the number of all revised letters.

Accuracy k was calculated as follows: $k = a - (b + c) / a + b$; mental performance quotient i, as follows: $i = k \cdot d$.

When adolescents with different intensity of Internet activity perform the test at the same time, it allows identifying peculiarities of their mental performance and functional peculiarities of the central nervous system.

Adolescents' academic performance was analyzed relying on their average grades in basic school subjects.

The obtained data were analyzed using variation statistic techniques in Microsoft Office 2010, Statistica for Windows 6.0, and OpenEpi 303 licensed software packages. Differences in relative indicators were estimated using Pearson's χ^2-test with Yates correction. The statistical significance was taken at $p < 0.05$. Correlation analysis involved calculating the pair correlation coefficient (r).

Results and discussion. The study identified the risk of Internet addiction for the half of the examined adolescents (51 %) aged 15–17 years; one third (33 %) did not have any signs of Internet addiction; 16 % were found to have persistent Internet addiction.

Likelihood of Internet addiction turned out to be a bit higher among girls than among boys, 56.6 against 45.9 % ($p > 0.05$). In addition, girls tended to have Internet addiction more frequently than boys, 17.2 against 12.9 % respectively ($p > 0.05$). Higher scores were also identified for girls per the scales of key Internet addiction symptoms ($p = 0.00$) and negative outcomes of problematic Internet use ($p = 0.02$).

Using the Eysenck test, we established that most adolescents with Internet addiction had high neuroticism levels (68 %). High levels of neuroticism were much frequently found in the adolescents with Internet addiction than among their peers without any addiction or only with the risk of its development ($p < 0.02$, $p < 0.005$) (Table 1).

High levels of neuroticism were much more prevalent among the adolescents with Internet addiction, 42 % against 16 % among those without it ($p < 0.02$). Low levels of

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Table 1

Personal characteristics of adolescents aged 15–17 years with different levels of Internet addiction (%)

Group / conclusion		Without Internet addiction ($n = 50$)		At risk of Internet addiction ($n = 50$)		With Internet addiction ($n = 50$)		Validity of differences		
		1		2		3		4		
		n	%	*n*	%	*n*	%	*p*1-2	*p*2-3	*p*1-3
Type	extrovert	9	18.0	13	26.0	18	36.0	> 0.05	> 0.05	< 0.05
	centrovert	32	64.0	25	50.0	21	42.0	> 0.05	> 0.05	< 0.05
	introvert	9	18.0	12	24.0	11	22.0	> 0.05	> 0.05	> 0.05
Neuroticism	high	8	16.0	21	42.0	34	68.0	< 0.02	< 0.05	< 0.1
	medium	14	28.0	16	32.0	7	14.0	> 0.05	< 0.05	< 0.05
	low	28	56.0	13	26.0	9	18.0	< 0.02	< 0.001	> 0.05

neuroticism were rarer among the adolescents with the risk of addiction, 26 % against 56 % among their peers without Internet addiction. Medium levels of neuroticism were detected with comparable frequency in both groups.

Half of the adolescents with the risk of Internet addiction were centroverts whereas extraversion or introversion was not so frequent ($p < 0.05$) in one quarter of the examined adolescents. Centroversion also prevailed among the adolescents without Internet addiction (64 %), extroversion and introversion were not so frequent as they were detected in 18 % of the adolescents in this group ($p < 0.05$).

Extroversion was found more frequently among the adolescents with Internet addiction than among their peers without it (36 and 18 % respectively, $p < 0.05$); introversion was found more rarely (42 and 64 % respectively, $p < 0.05$).

Therefore, high levels of neuroticism were found more frequently in the examined adolescents with the risk of Internet addiction and already existing addiction than among their peers without any risks or addiction. This means apparent difficulty in emotional self-regulation and weaker behavior control [5, 14, 15]. Centroversion was a prevailing psych type among the adolescents with the risk of Internet addiction and without it; extraversion prevailed in the group with Internet addiction.

Analysis of personality characteristics of boys and girls in groups with different intensity of Internet addiction allows more precise identification of target groups for preventive and correction programs.

The study emphasizes the importance of the sex aspect in examining Internet addiction among adolescents. Centroversion was much more frequent among the examined girls without Internet addiction than among their addicted peers (63.16 and 31.25 % respectively, $p < 0.05$). Introversion was more frequently found among the girls with the risk of Internet addiction than among those without it (36 and 10.53 % respectively, $p < 0.05$). High levels of neuroticism were more frequent among the examined girls with Internet addiction and the risk of its development (52 and 31.58 %, $p < 0.05$ and 78.13 and 31.58 % respectively, $p < 0.05$) (Table 2).

In contrast to girls, centroversion prevailed among the boys from the analyzed groups with different intensity of Internet addiction; similar to girls, high levels of neuroticism were much more frequent in the groups with the risk of Internet addiction and Internet addiction.

Our comparison of sex-specific personal characteristics established that the girls without Internet addiction had high levels of neuroticism 5.2 times as frequently (31.58 and 6.5 % respectively, $p < 0.05$) and extraversion twice as frequently (26.32 and 12.9 % respectively) than the boys without addiction.

Table 2

Personality characteristics of boys and girls with different intensity of Internet addiction (%)

Group / conclusion		Without Internet addiction (n = 50)		Risk of Internet addiction (n = 50)		Internet addiction (n = 50)		Validity of sex-specific differences		
		1		2		3		Without IA	IA risk	IA
		g	b	g	b	g	b	$P_{g\text{-}b}$	$P_{g\text{-}b}$	$P_{g\text{-}b}$
Type	extra	26.32	12.9	24.0	28.0	46.88	16.67	> 0.05	> 0.05	< 0.02
	centro	63.16	64.52	40.0	60.0	31.25	61.11	> 0.05	> 0.05	< 0.05
	intro	10.53	22.58	36.0	12.0	21.87	22.22	> 0.05	< 0.05	> 0.05
Neuroticism	high	31.58	6.5	52.0	32.0	78.13	50.0	< 0.05	> 0.05	< 0.05
	medium	26.31	29.0	28.0	36.0	9.37	22.22	> 0.05	> 0.05	> 0.05
	low	42.11	64.5	20.0	32.0	12.5	27.78	> 0.05	> 0.05	> 0.05

Note: g means girls; b means boys; IA = Internet addiction.

Table 3

Mental performance of adolescents aged 15–17 years with different intensity of Internet addiction ($M \pm m$)

Mental performance indicators	Without Internet addiction (n = 40)	Risk of Internet addiction (n = 36)	Internet addiction (n = 32)	Validity of differences p
	1	2	3	4
Accuracy k	0.78 ± 0.04	0.76 ± 0.05	0.50 ± 0.06	$p_{1\text{-}3} < 0.001$ $p_{2\text{-}3} < 0.001$
Mental performance quotient i	238.85 ± 12.39	231.48 ± 14.67	155.42 ± 20.0	$p_{1\text{-}3} < 0.001$ $p_{2\text{-}3} < 0.01$

The girls with the risk of Internet addiction were introverts more frequently than the boys with such risk (36.0 and 12.9 % respectively, $p < 0.05$). Extroversion (46.88 and 16.67 % respectively, $p < 0.02$) and high levels of neuroticism (78.13 and 50.0 % respectively, $p < 0.05$) were more frequent among the girls with Internet addiction in comparison with the addicted boys. Centroversion was more frequent among the boys with Internet addiction than among their female peers (61.1 and 31.25 % respectively, $p < 0.05$).

We established a direct correlation between the score of Internet addiction per the Chen scale and the level of neuroticism in all adolescents, boys and girls, $r = 0.4$, $p < 0.05$.

Our examination of mental performance found that accuracy and the mental performance quotient were the highest in the adolescents without Internet addiction and the lowest in those with Internet addiction (Table 3).

Accuracy (k) was authentically lower in the adolescents with Internet addiction than in their peers with the risk of it (0.50 ± 0.06 and 0.76 ± 0.05, $p < 0.001$) and without Internet addiction (0.50 ± 0.06 and 0.78 ± 0.04, $p < 0.001$). The mental performance quotient (i) was also authentically lower in the addicted adolescents than in their peers with the risk of Internet addiction (155.42 ± 20.0 and 231.48 ± 14.67, $p < 0.01$) and without Internet addiction (155.42 ± 20.0 and 238.85 ± 12.39, $p < 0.001$). There were no authentic differences in accuracy and the mental performance quotient identified for the adolescents with the risk of Internet addiction and those without Internet addiction ($p > 0.05$).

We established authentic inverse correlations between intensity of Internet addiction and mental performance indicators, namely, accuracy (R = -0.315, p = 0.0009) and the mental performance quotient (R = -0.311, p = 0.001). The lowest values of accuracy and

the mental performance quotient were established for the adolescents with apparent Internet addiction (the highest scores per the Chen scale).

We examined sex-specific peculiarities of mental performance indicators in adolescents aged 15–17 years with different intensity of Internet addiction. As a result, we did not establish any authentic differences in accuracy and the mental performance indicators between boys and girls in the compared groups ($p > 0.05$).

Mental performance indicators were authentically lower both in boys and girls with Internet addiction than in their peers with the risk of it or without Internet addiction ($p < 0.05$).

Given that attention function has significant influence on academic performance, we analyzed the average grade of academic performance for the examined adolescents with different intensity of Internet addiction. We did not find any authentic differences between the adolescents without Internet addiction and the risk of it since the average grade amounted to 3.905 and 3.914 respectively.

The adolescents with already existing Internet addiction had considerably lower average grades than their peers with the risk of Internet addiction (3.736 and 3.914 respectively, $p = 0.0031$). These findings confirm that Internet addiction can affect mental performance and academic performance, which emphasizes the importance of developing effective strategies aimed at preventing and correcting Internet addiction among adolescents.

The average grade of academic performance did not have any authentic differences in the groups of girls with different intensity of Internet addiction (3.997; 4.018; 3.824 respectively); still, there was a descending trend in it identified for the examined girls with Internet addiction.

The average grade of academic performance was a bit lower among the boys with Internet addiction and with the risk of it than among their peers without Internet addiction.

The average grade was lower in all groups of boys than in all groups of girls; however, authentic differences were established only between the boys and girls with the risk of Internet addiction (3.790 and 4.018 respectively, $p = 0.002$).

Our study findings emphasize how important it is to understand personal traits and psychological factors for effective prevention of Internet addiction in adolescents. Prevention of Internet addiction and adverse outcomes of problematic Internet use is a complex interdisciplinary task that should be solved if we want to ensure adolescents' mental and physical health as well as their social adaptation. Internet-addicted behavior involves compulsive symptoms (irresistible obsession), loss of any control when using a PC, and apparent withdrawal syndrome when Internet use is limited. All these outcomes are caused by personal and inter-personal problems. Therefore, simple direct limitations imposed on PC use only lead to hypodynamia and health issues and are therefore ineffective. It is important to spot out a risk group for early and effective prevention. High levels of neuroticism in adolescents have been found to be a significant risk factor.

The established direct correlation between the score of Internet addiction per the Chen scale and the level of neuroticism in adolescents is explained by similar neurobiological pathways of these two states, namely, impaired impulse control [4, 8, 19, 20].

The established lower accuracy and mental performance quotients in the adolescents with the risk of Internet addiction and already addicted ones against their peers without Internet addiction give evidence of difficulty in attention concentration, which has negative influence on academic performance.

Our study findings indicate that there are several sex-specific personal differences in formation of Internet addiction in older adolescents. The risk of Internet addiction and persistent Internet addiction was a bit more frequently identified for girls than boys; key symptoms and negative outcomes of Internet addiction were also authentically higher in girls in comparison with boys [2, 4, 8, 20–22].

These differences are associated with sex-specific personal peculiarities.

High levels of neuroticism and extroversion were more rarely detected in the boys without Internet addiction than in the girls without it. Frequency of high neuroticism levels was twice as frequent in the girls with Internet addiction as opposed to boys; extroversion was also more frequent. Therefore, higher prevalence of Internet addiction among girls occurs due to more frequent high levels of neuroticism and extroversion in them. Formation of Internet addiction is usually accompanied with their growing prevalence.

Introversion was detected more frequently in the examined girls with the risk of Internet addiction than among their male peers, which means certain difficulty in real life communication. Many authors point out that underdeveloped communication skills are the reason why many adolescents are so eager to socialize in the Internet [23–25]. Internet-socializing compensates for difficulties in real life communication for people with such personality traits [6, 8, 16].

Boys with Internet addiction, similar to addicted girls, tend to have high levels of neuroticism but centroversion prevails among them in contrast to girls.

Identification of personality-related risk factors has great significance for prediction and early detection of Internet addiction. These factors include high levels of neuroticism, introversion in girls, centroversion in boys, and growing intensity of these traits together with declining accuracy, mental and academic performance. Early detection of high neuroticism, decrease in mental performance, identification of risk groups and prevention activities implemented at early stages in the development of Internet addiction can prevent its actual formation in adolescents. This creates the necessity to take relevant sanitary-hygienic measures together with developing standards of safe Internet use that include psychological prevention.

Conclusions:

1. High levels of neuroticism and extroversion were more frequently identified in adolescents with persistent Internet addiction than in their peers without it. A direct correlation was established between the score of Internet addiction and the level of neuroticism.

2. Introversion was more frequent in the girls than in boys with the risk of Internet addiction; extroversion and high levels of neuroticism were more typical for the girls with Internet addiction than for their male peers. The boys with Internet addiction tended to be centroverts more frequently than their female peers.

3. The adolescents with Internet addiction had the lowest values of accuracy and mental performance quotients as well as poorer academic performance.

4. High neuroticism, introversion in girls, centroversion in boys, an increase in the severity of neuroticism together with a decrease in accuracy, mental performance and academic performance are risk factors of Internet addiction formation in adolescents aged 15–17 years.

5. To prevent risks of Internet addiction, it is necessary to conduct sanitary-hygienic activities together with developing standards for safe Internet use that includes psychological prevention.

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Research article

STUDY OF CHILI PEPPERS AND PAPRIKA CONTAMINATION WITH *ALTERNARIA* TOXINS AS HEALTH RISK FACTORS

Z.A. Chalyy[1], I.B. Sedova[1], A.L. Spiridonova[1], M.I. Glavica[1], D.M. Kompaintseva[1], U.V. Ivanova[1], V.A. Tutelyan[1,2]

[1]Federal Research Centre of Nutrition and Biotechnology, 2/14 Ust'inskii pr., Moscow, 109240, Russian Federation
[2]Sechenov University, 8/2 Trubetskaya St., Moscow, 119991, Russian Federation

At present, researchers show considerable interest in unregulated toxins of widespread fungi in nature, in particular, metabolites of microfungi of Alternaria genus. These toxins are potentially hazardous for human health and, under unfavorable conditions, capable of producing toxic metabolites, Alternaria toxins (AT), which have shown genotoxicity, mutagenicity and acute toxic effects. They are frequently detected not only in cereals and oilseeds, fruits, nuts, vegetables, but also in spices, especially dried red peppers.

The aim of the study was to investigate the occurrence of Alternaria toxins in paprika and chili peppers sold on the domestic market as well as to assess their intakes by humans through their consumption.

Concentration of 5 Alternaria toxins (alternatiol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tentoxin (TEN), tenuazonic acid (TeA)) was detected in 37 samples of dried red peppers marketed in Moscow and Moscow region in 2024 year including paprika (20 samples) and chili pepper (17 samples). The analysis of Alternaria toxins contamination was carried out by high-performance liquid chromatography coupled to tandem mass-spectrometric detection (HPLC-MS/MS).

TeA was detected in 84 % of red pepper samples in concentration from 43 to 3295 μg/kg and TEN – in 40 % cases, in low levels ranging from 1.0 to 11 μg/kg. The occurrence of AT in paprika was higher than in chili pepper. Combined (2 or more toxins) contamination with Alternaria toxins was found only in paprika samples (65 %; the predominant combination is TeA and TEN). Intakes of Alternaria toxins associated with consumption of chili and paprika did not exceed the reference values and ranged from 0.0003 % (for TEN) to 0.24 % (for TeA) of the threshold of toxicological concern (TTC).

Keywords: *Alternaria toxins, tenuazonic acid, tentoxin, paprika, chili pepper, contamination, HPLC-MS/MS, risk assessment.*

Among the most common contaminants leading to crop spoilage, mycotoxins (MTs), including *Alternaria* toxins (ATs) produced by such mold fungi of the genus *Alternaria* as *A. alternata, A. tenuissima, A. solani*, etc., are of interest [1−4]. Their accumulation can begin both at the pre-harvest stage and in the post-harvest period, during transportation and storage, and the possibility of their simultaneous contamination of products makes this

Zakhar A. Chalyy – Junior Researcher at the Laboratory of Nutrition Enzymology (e-mail: brew@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-9371-8163).

Irina B. Sedova – Candidate of Biological Sciences, Senior Researcher at the Laboratory of Nutrition Enzymology (e-mail: isedova@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-6011-4515).

Anastasia L. Spiridonova – Laboratory Researcher of the Laboratory of Nutrition Enzymology (e-mail: a_l_spiridonova@mail.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0009-0008-6075-9963).

Monika I. Glavica – Laboratory Researcher of the Laboratory of Nutrition Enzymology (e-mail: glavicamonika1@mail.ru; tel.: +7 (495) 698-53-65; ORCID: http://orcid.org/0009-0003-0673-7097).

Darya M. Kompaintseva – Laboratory Researcher of the Laboratory of Nutrition Enzymology (e-mail: pestrolistay@mail.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0009-0006-5232-8659).

Ulyana V. Ivanova – Laboratory Researcher of the Laboratory of Nutrition Enzymology (e-mail: ds557@mail.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-8962-9133).

Victor A. Tutelyan – Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor, Head of the Laboratory of Nutrition Enzymology, Scientific Supervisor (e-mail: tutelyan@ion.ru; tel.: +7 (495) 698-53-46; ORCID: https://orcid.org/0000-0002-4164-8992).

problem extremely urgent [5, 6]. According to the literature data, not only food raw materials but also processed foods, in particular, red pepper, are exposed to AT contamination [2, 7, 8]. Among ATs, a real threat to public health is formed by alternariol (AOH), its methyl ester (AME), tentoxine (TEN), tenuazonic acid (TEA) and altenuane (ALT) [9−13]. According to the European Food Safety Agency (EFSA), AOH and TeA are the most widespread of them [8].

Consumption of foods contaminated with ATs can result in various diseases, for example, esophageal cancer in Henan, China, and Onyalai disease in African countries [3].

At present, there are no national or international regulations on the content of AT in food products. At the same time, European countries have introduced indicative levels for TEA, AOH and AME for a number of foodstuffs, exceeding which are grounds for additional research[1].

Chili pepper and paprika are the most susceptible to molds able to produce MTs, especially during prolong storage in inappropriate storage conditions. Spice processing methods can also influence fungal growth and subsequent accumulation of toxins [14]. According to the literature, MTs produced by mold fungi *Aspergillus* spp. and *Penicillium* spp. are priority contaminants in species [14−17]. Previous studies have shown that spices were most frequently contaminated with aflatoxins and ochratoxin A. As regards contaminated chili pepper and paprika, TEN was found in 41–57 % cases; AOH, 19 % cases; AME, 6–14 %; and ALT, 6–10 % [16].

The aim of this study was to investigate the occurrence of *Alternaria* toxins in paprika and chili peppers sold on the domestic market as well as to assess health risks associated with their consumption for the population of the Russian Federation.

Materials and methods. Dried red pepper samples were bought in retail outlets in Moscow and the Moscow region in 2024. Overall, 37 samples were examined including 20 paprika samples and 17 chili pepper samples produced in Uzbekistan (15), India (6), Spain (6), China (3), Armenia (2) and 5 samples of unknown origin.

Preparation of samples for screening of AT in spices was carried out in accordance with the developed methodology. A ground sample weighing 1 gram was put in a 50 cm^3 Falcon tube, added with 10 cm^3 of distilled water, shaken until wetting was complete and then left to swell in ultrasonic cleaner Elmasonic S15H (Elma, Germany) for 10 minutes. Next, the sample was added with 10 cm^3 of acetonitrile acidified with 1 %-solution of acetic acid, shaken for 10 minutes and then again treated with ultrasound for 10 minutes. The sample was added with 1 gram of NaCl and 4 grams of anhydrous $MgSO_4$, and mixed intensively after each addition, either by hand or using a vortex. The extract was centrifuged for 10 minutes at 10,000 rpm (Hettich, Rotina 38). Five cm^3 of supernatant were put into a 15 cm^3 falcon tube, added with 3 cm^3 of hexane saturated with acetonitrile and mixed in a shaker for 20 minutes. Next, the sample was centrifuged for 1 minute at not less than 4000 rpm (Hettich, Rotina 38); 3 cm^3 of defatted acetonitrile layer were taken and vapored until dry in a rotor evaporator (BioChromato, Japan). The rest was again dissolved in 0.1 cm^3 of methanol and added with 0.4 cm^3 of water. The resulting solution was put into a 1.5 cm^3 Eppendorf tube and centrifuged for 10 minutes at 15,000 rpm in a SL 16R centrifuge (Thermo Scientific, USA). 0.4 cm^3 of supernatant were put into a chromatographic vial.

ATs were identified using a HPLC system Agilent Technologies 1100 consisting of

[1] Commission recommendation (EU) 2022/553 of 5 April 2022 on monitoring the presence of *Alternaria* toxins in food: recommendations. *Official Journal of the European Union*, 2022. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022H0553&from=EN (May 14, 2024).

the gradient pump, column thermostat and autosampler, which was connected with a triple quadrupole mass spectrometric detector (Triple Quad 6400). Reversed phase high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) in the positive electrospray mode at atmospheric pressure and multiple reaction monitoring (MRM) was used to identify ATs.

Analytes were divided on a column filled with silica gel with added octadecylsilane groups (Zorbax SB-C18, 150 × 4.6 mm, 3.5 µm, pore size is 80Å, Agilent). The mixture of water : acetonitrile : formic acid (95 : 5 : 0.1 % vol.) was used as the mobile phase A; acetonitrile : formic acid (100 : 0.1), the mobile phase B. The gradient scheme was as follows: the start at 0 % B; the 7th minute, linear growth to 75 % B; the 10th minute, linear growth to 100 % B; the column equilibration from 17th to 19th minute at 100 % B; linear decline to 0 % during 0.5 minute; the 25th minute, the column equilibration at 0 % B. The eluent flow rate was 0.4 cm^3/min; the column temperature, 30 °C; the injection volume, 20 mm^3. Each sample was analyzed twice. The column temperature was 30 °C. Calibrations with a relevant 'clean' matrix were used for quantification. The MT recovery varied between 70 and 91 %; limits of quantification (LOQ) for the method amounted to 1, 4, 6, 8 and 20 µg/kg for TEN, AME, AOH, ALT and TeA respectively.

The following standards were used: AOH (99.3 %), AME (99.77 %), ALT (98 %), and TEN (99.84 %) (Fermentek, Israel). Stock solutions of the toxins were prepared in methanol in the concentration of 200 µg/cm^3. The TeA standard, concentration of 100.6 µg/cm^3 was bought from Romer Company, Biopure, Austria. All stock solutions were stored at -18 °C.

Results and discussion. The study of frequency and levels of contamination in 37 dried red chili samples established TeA (84 %) and TEN (40 %) to be the priority contaminants in these products (Table 1).

Table 1

Contamination of dried red pepper samples with *Alternaria* toxins

Toxins	The number of contaminated samples		Contamination range, µg/kg	Average MT contents in contaminated samples, µg/kg	MT contents in total samples, µg/kg	
	abs.	%			average	90 %
Paprika (n = 20)						
TeA	20	100	42.5–3295.4	1746.9	1746.9	2849.40
TEN	12	60	1.0–10.5	4.4	2.7	8.1
AOH	1	5	41.4	41.4	2.1	0
AME	1	5	8.2	8.2	0.4	0
ALT	0	0	< 8.0			
Chili pepper (n = 17)						
TeA	11	65	143.6–2481.5	640.4	943.3	1492.2
AOH	0	0	< 6.0			
AME	0	0	< 4.0			
TEN	0	0	< 1.0			
ALT	0	0	< 8.0			
Dried red pepper (n = 37)						
TeA	31	84	42.5–3295	1225	1462	2604
TEN	12	40	1.0–10.5	2.7	1.4	5.6
AOH	1	3	41.4	41.4	1.12	< LOQ
AME	1	3	8.20	8.20	0.22	< LOQ
ALT	0	0	< 8.0			

Chili pepper samples were less contaminated with ATs; only TeA was detected in them with frequency of 65 %. The levels of contamination varied between 143.6 and 2481.5 μg/kg, 640.4 μg/kg on average. Four out of 5 analyzed ATs were found in paprika samples: TeA was found in 100 % cases in concentrations between 143.6 and 2481.5 μg/kg, 1746.9 μg/kg on average; TEN was found in 60 % in low concentrations up to 10.5 μg/kg. AOH and AME were detected in single cases in concentrations of 41.4 and 8.2 μg/kg.

In contrast to our study findings, C. Mujahid et al. found four ATs (TeA, AOH, AME and TEN) in chili pepper samples with much higher TeA concentrations varying between 4510 and 20,478 μg/kg [18]. As regards paprika samples, all analyzed samples were contaminated with TeA, both in our study and in [18, 19]; however, according to their data, contamination levels were significantly higher and reached 18,856 and 37,300 μg/kg respectively.

More detailed analysis of TeA occurrence in dried red pepper samples established that the highest frequency of contamination was in samples obtained from Spain and Uzbekistan. TeA was founded less frequently and in lower concentrations in spice samples delivered from China (33 %, the average concentration was 740 μg/kg) and Armenia (50 %, 674.2 μg/kg) (Table 2). The highest average and maximum contamination levels were found in samples produced in Spain, India and Uzbekistan. Four ATs were simultaneously found in only one sample from Uzbekistan.

Sixty percent of the analyzed paprika samples were contaminated with two and more ATs. TeA and TEN were found in 11 samples; simultaneously 4 MTs were found in one sample: TeA, 1234.8 μg/kg; AOH, 41.4 μg/kg; AME, 8.2 μg/kg; and TEN, 7.9 μg/kg. According to [18], simultaneous contamination with three and more ATs was more frequent and amounted to 88 % and the levels of contamination were also higher. Simultaneous exposure to several ATs can have a significant adverse effect on the general toxicity in comparison with individual exposures [13, 20].

The contributions made by the analyzed spices into ATs intake were assessed considering the data on dried red pepper consumption provided by the Federal Customs Service of the Russian Federation and the levels of ATs contamination found in its samples in this study (Table 3). In Russia, average consumption of chili pepper and paprika is 0.206 gram per person[2]. An average body weight of a person is 70 kg. ATs burden was

Table 2

Occurrence of tenuazonic acid in dried red pepper samples depending on a country of origin

Country of origin	The number of analyzed samples	The number of contaminated samples		Contamination range, μg/kg	Average TeA contents in total samples, μg/kg
		abs.	%		
Uzbekistan	15	14	93	160.1−2284.3	1549.8
Spain	6	6	100	1588.4−2910.9	2103.0
India	6	4	67	42.5−3295.0	1026.4
China	3	1	33	2221.3	740.4
Armenia	2	1	50	1348.4	674.2
Unknown	5	5	100	389.0−2842.6	1508.6

[2] Federal'naya Tamozhennaya sluzhba. Tamozhennaya statistika vneshnei torgovli RF [Federal Customs Service. Customs Statistics of Foreign Trade of the Russian Federation]. Available at: http://stat.customs.gov.ru (May 27, 2024) (in Russian).

Table 3

Calculated average daily intake of *Alternaria* toxins for the population due to consumed chili pepper and paprika

Toxin	Calculated average daily intake of *Alternaria* toxins		Threshold of toxicological concern (TTC) for a toxin, ng/kg of body weight per day
	ng/kg of body weight	% of TTC	
TeA	3.6	0.24	1500
AOH	0.003	0.13	2.5
AME	0.0005	0.03	2.5
TEN	0.004	0.0003	1500

assessed by comparing the calculated average daily consumption with a relative value of threshold of toxicological concern (TTC) (1500 ng/kg of body weight for TeA and TEN, 2.5 ng/kg of body weight for AOH and AME) [2, 8].

According to the study results, ATs intake for the population calculated for daily consumption of dried red pepper is considerably below their TTC and does not create any significant health risks for the RF population.

Conclusions:

1. *Alternaria* toxins are established to contaminate dried red pepper; tenuazonic acid is the priority contaminant with its occurrence reaching 100 % in paprika (42.5–3295.4 μg/kg), and 60 % in chili pepper (143.6–2481.5 μg/kg). Combined contamination of paprika with tenuazonic acid and tentoxin was found in 65 % cases. Frequency of AOH and AME contamination in paprika was low.

2. Estimated intake of *Alternaria* toxins with paprika and chili pepper give evidence of their low exposure and the absence of any serious hazards for population health.

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Research article

HEALTH RISK ASSESSMENT TAKING INTO ACCOUNT N-NITROSAMINES' CONCENTRATIONS IN FOOD PRODUCTS

D.V. Suvorov, P.Z. Shur, V.A. Fokin, D.N. Lir, T.V. Nurislamova, D.Yu. Subbotina

Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation

N-nitrosamines formed in meat products, especially during heat treatment, are potentially hazardous to human health. Smoked sausages are a product with a high content of N-nitrosamines, including those not regulated in Russia, which can cause health risks for population.

The aim of this study was to perform health risk assessment considering ratios of N-nitrosamine concentrations in food products.

The data on N-nitrosamines' concentrations were analyzed using MS Excel and Statistica 10.0 programs. The relationship between these concentrations was evaluated using Spearman correlation coefficient. Parameterization of dependencies was carried out by regression analysis with evaluation of model significance by Fisher's criterion. Differences at the level of $p \leq 0.05$ were considered statistically significant. Risk assessment was performed in accordance with the Guideline R.2.1.10.3968-23.

Correlation analysis between N-nitrosamines in smoked sausages revealed a significant correlation between concentrations of NDMA and NEMA ($r_s = 0.77$) and NDMA and NPyRA ($r_s = 0.81$) at $p < 0.05$. The level of total carcinogenic risk ($\sum CR$) derived from data based on calculated concentrations of NEMA and NPyRA was determined to be unacceptable for the adult population. It should be noted that the contribution to $\sum CR$ value (11.0 %) is related to NEMA and NPyRA that are not regulated in Russia.

Our study results support the findings of earlier EFSA publications indicating that not only NDMA and NDEA, but also other N-nitrosamines may form a health risk upon simultaneous exposure.

Correlation and regression analyses allowed us to assess the concentrations of NEMA and NPyRA in sausages. Use of both these concentrations and actual NDMA and NDEA concentrations established unacceptable carcinogenic risk for certain consumer groups in case N-nitrosamines concentrations were above regulated level. The contribution of NEMA and NPyRA in $\sum CR$ value was 11.0 %.

Keywords: *correlation analysis, regression analysis, meat products, smoked sausages, N-nitrosamines, risk assessment, carcinogenic risk, consumers' health, NDMA.*

N-nitrosamines are food contaminants able to produce multiple negative effects on the human body. However, carcinogenic effects are considered the most hazardous among them upon oral introduction of N-nitrosamines. This is due to the fact that most N-nitrosamines upon oral introduction are transformed into carcinogens under in-

Dmitrii V. Suvorov – Researcher at the Health Risk Analysis Department (e-mail: suvorov@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-3594-2650).

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).

Vladimir A. Fokin – Candidate of Medical Sciences, 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).

Daria N. Lir – Candidate of Medical Sciences, Leading Researcher-Head of the Health Risk Analysis Department (e-mail: lir@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-7738-6832).

Tatyana V. Nurislamova – Doctor of Biological Sciences, Leading Researcher-Head of the Department of Analytical Chemistry Analysis (e-mail: nurtat@fcrisk.ru; tel.: +7 (342) 233-10-37; ORCID: https://orcid.org/0000-0002-2344-3037).

Daria Yu. Subbotina – Junior Researcher at the Department of Analytical Chemistry Analysis (e-mail: subbotina@fcrisk.ru; tel.: +7 (342) 233-10-37; ORCID: https://orcid.org/0000-0003-3030-3275).

fluence of enzyme systems of cytochrome p450[1] [1–6]. N-nitrosamines enter the human body predominantly with food products [1, 2]. Meat products, including boiled, smoked and summer sausages, are major sources of these chemicals since technologies of their production involve adding nitrites to them to achieve better properties (the mass concentration should not exceed 0.005 % per 100 grams of a product)[2]. Nitrites support nitrosation and, consequently, formation of N-nitrosamines[3] [7–9]. In addition, meat products are sources of biogenic amines, which are precursors of N-nitrosamines [10]. Use of up-to-date methods for chemical identification in food products allows identifying a wide range of N-nitrosamines [7]. Thus, at present, it is possible to quantify nine nitroso compounds in food products; all of them are classified by the IARC as strong carcinogens for humans[4] [1, 2, 11–13].

Nevertheless, the federal state statistic forms contain data on contamination in food products only for two compounds, N-nitrosodimethylamine (NDMA) and nitrosodiethylamine (NDEA)[5] [14]. International organizations that deal with food safety highlight the necessity to estimate safety of all identifiable N-nitrosamines that can occur in food products [1] including estimations based on assessing health risks for consumers.

Given that, assessment of health risks for consumers associated with N-nitrosamines intake with smoked sausages seems quite relevant. It should rely on using levels of exposure to N-nitrosamines, which are calculated considering their actual contents in analyzed food products.

The aim of this study was to assess additional health risk considering ratios of N-nitrosamine concentrations in food products on the example of smoked sausages.

Materials and methods. The study relied on using data on estimated levels of N-nitrosamines in smoked meat products taken from previous publications [14].

Results obtained by quantification of N-nitrosamines in the examined food products were analyzed with standard MS Excel 2014 and Statistica 10.0 applied statistical software package. Data that deviated from normal distribution (established by using the Shapiro – Wilk test) were described with median (Me), upper and lower quartiles ($Q_{25} - Q_{75}$).

Associations between regulated N-nitrosamines (NDMA and NDEA) and other nitrosamines identified in smoked sausages were estimated by using the Spearmen nonparametric correlation coefficient (r_s). The obtained relationships were parameterized by regression analysis together with assessing significance of models per the Fischer's test (F) stating the model parameters (b_0), regression coefficient (b_1) and determination coefficient (R^2). Differences were considered significant at $p \leq 0.05$.

The linear regression model in general is as follows:

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[1] Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – N-Nitrosodimethylamine. Ottawa, Ontario, Health Canada, 2011, 45 p.; Toxicological profile for N-nitrosodimethylamine. Athlanta, Agency for Toxic Substances and Disease Registry (ATSDR), 2023, 231 p.

[2] GOST R 55455-2013. Boiled-smoked meat sausages. Specifications: State Standard. *Baza GOSTov*. Available at: https://allgosts.ru/67/120/gost_r_55455-2013 (August 15, 2024) (in Russian); GOST 16351-86. Semi-smoked sausages. Specifications: Inter-State Standard. *The library for regulatory documents*. Available at: https://files.stroyinf.ru/Data2/1/4294836/4294836073.pdf (August 15, 2024) (in Russian).

[3] Nitraty, nitrity i N-nitrozosoedineniya [Nitrates, nitrites and N-nitrosamines]. Geneva, World Health Organization, 1981, 120 p. (in Russian).

[4] Agents Classified by the IARC Monographs, Volumes 1–136. *IARC*. Available at: https://monographs.iarc.who.int/agents-classified-by-the-iarc/ (June 05, 2024).

[5] Formy federal'nogo gosudarstvennogo statisticheskogo nablyudeniya [The federal state statistic forms]. Available at: https://47.rospotrebnadzor.ru/content/формы-федерального-государственного-статистического-наблюдения (August 13, 2024) (in Russian).

$$Y_i = b_0 + b_1 \cdot X_i , \quad (1)$$

where Y_i is the dependent variable; X_i is the independent variable; b_0 and b_1 are the parameters of the mathematical model.

Validity of differences between actual and calculated concentrations of associated chemicals was estimated using the Mann – Whitney test (*U*-test).

Levels obtained by using regression models considering the 90th percentile were employed to assess exposure for subsequent assessment of health risk caused by associated chemicals. Health risk was assessed in conformity with the Guide R.2.1.10.3968-23 Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment[6]. According to the Guide, the value typical for malignant neoplasms of the digestive organs was taken as a severity measure ($g = 0.495$).

Results and discussion. Earlier chemical and analytical identification of quantitative levels of N-nitrosamines in the analyzed sausages established their actual levels to be within a range from 0.0002 to 0.35 mg/kg of a product (Table 1).

Analysis of pair correlations between actual NDMA and NDEA levels and other N-nitrosamines in sausages established authentic correlations between levels of two pairs of N-nitrosamines: NDMA → NEMA ($r_s = 0.77$) and NDMA → NPyRA ($r_s = 0.81$) at $p < 0.05$.

Table 1

Levels of N-nitrosamines in smoked sausages identified by chromato-mass-spectrometry (mg/kg of a product)

Sample No.	Levels in smoked sausages			
	NDMA	NEMA	NDEA	NPyRA
1	0.0147	0.0014	0.0018	0.0073
2	0.23	0.062	0.0003	0.042
3	0.09	0.024	-	0.012
4	0.35	0.105	0.0009	0.086
5	0.189	0.043	0.0003	0.0278
6	0.043	0.0021	0.0007	0.0047
7	0.0006	0.0116	-	0.0031
8	0.0359	0.0037	0.0002	0.0012
9	0.0006	0.0005	-	0.0029
10	0.0003	0.0003	-	0.0018
11	0.0003	0.0014	-	0.0007
12	0.0008	0.0003	0.0003	0.0032
13	0.0732	0.0323	-	0.0073
14	0.0165	0.0014	-	0.0016
15	0.075	0.0004	0.0003	0.0044
16	0.0916	0.022	0.0003	0.0044
Me	0.0395	0.0029	0.0003	0.0044
[Q_{25}]	0.0008	0.0012	0.0003	0.0026
[Q_{75}]	0.0904	0.0261	0.0007	0.0085

Note: NPyRA is for N-nitrosopyrrolidine amine; NEMA is for N-nitrosoethylmethylamine.

[6] Guide R.2.1.10.3968-23. Rukovodstvo po otsenke riska zdorov'yu naseleniya pri vozdeistvii khimicheskikh veshchestv, zagryaznyayushchikh sredu obitaniya [Health Risk Assessment upon Exposure to Chemical Pollutants in the Environment]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, 2023, 221 p. (in Russian).

Table 2

Parameters of significant regression models

Independent variable (X)	Dependent variable (Y)	b_0	Error	b_1	Fischer's test (F)	Model authenticity (p)	Determination coefficient (R^2)
NDMA	NEMA	-0.0019	0.002	0.282	172.4	$p < 0.001$	0.93
NDMA	NPyRA	-0.0029	0.007	0.211	119.6	$p < 0.001$	0.89

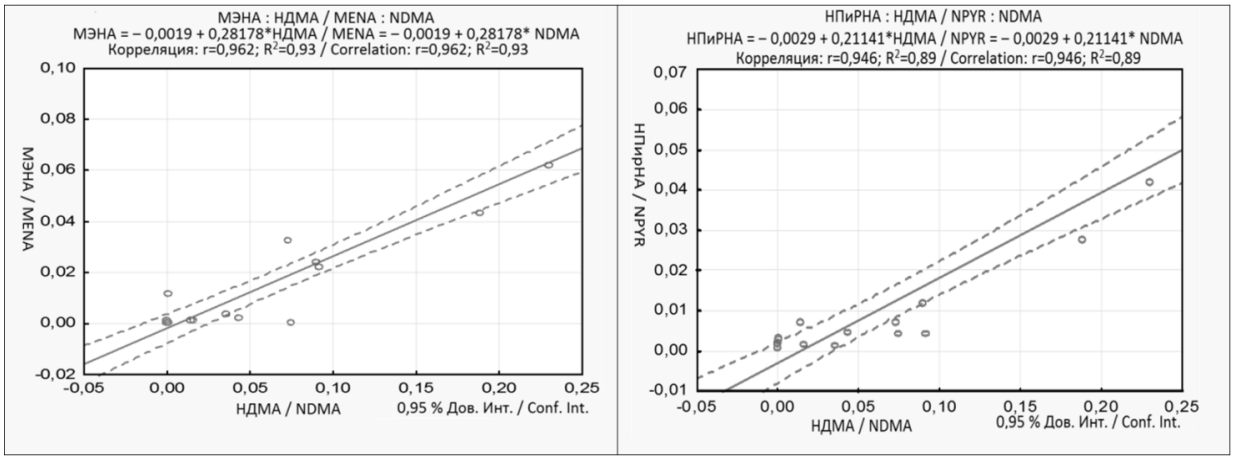

Figure. The results obtained by using the regression model of th eraltisnhios between levels of N-nitrosamines

A strong positive correlation ($r_s \geq 0.7$) between levels of these chemicals may indicate their probable co-occurrence in the analyzed food products. The obtained relationships were parameterized by using regression analysis and this showed obvious associations between these two n-nitrosamines and NDMA levels. Table 2 provides the parameters of regression models.

The models that describe the relationships between two of the analyzed N-nitrosamines and NDMA levels make it possible to calculate their levels in smoked sausages (Figure).

Calculated levels, 90th percentile taken into account, were as follows: NEMA, the actual level 0.053 mg/kg of a product (the calculated one, 0.057 mg/kg of a product); NPyRA, the actual level, 0.035 mg/kg of a product (the calculated one, 0.043 mg/kg of a product). The calculated levels, which were used in subsequent exposure assessment, did not have any authentic differences from the actual ones as confirmed by the Mann – Whitney test (the value of the U-Mann – Whitney test was 120 and 115 for NEMA and NPyRA respectively at the critical U-test value being equal to 83).

Exposure was assessed within carcinogenic risk calculation by using an average volume of consumed sausages equal to 0.002 kg per day for the whole population. The figure was established in previous publications [15]. However, according to a study by E.E. Keshabyants with colleagues [16], smoked meat products are consumed regularly by 18.8 % of the adult population and average intake of all types of sausages amounts to 56.4 grams per day. Bearing in mind that smoked sausages account for 7.9 % of the total sausage consumption [17], we can estimate average daily consumption of smoked sausages as equal to 0.005 kg per day. Given that, volumes of smoked sausage consumption were estimated per two scenarios to assess exposure: for the total popula-

tion (scenario 1) and basing on data obtained for adults who consume smoked sausages regularly [16] (scenario 2).

Carcinogenic risk assessment (*CR*) that considered contributions made by specific N-nitrosamines established that the permissible risk level was surpassed under the exposure scenario 2 ($CR \geq 1.0 \cdot 10^{-4}$) (Table 3).

Carcinogenic risk assessment under the scenario 1 established that the total carcinogenic risk (∑CR) was equal to $7.39 \cdot 10^{-5}$, which is considered permissible (acceptable). At the same time, the total carcinogenic risk for adults who consume smoked sausages regularly was established to be alerting ($\sum CR = 1.85 \cdot 10^{-4}$) and this is unacceptable. NDMA contribution to carcinogenic risk amounted to 87.6 %. NEMA and NPyRA each contributed 10.3 % into carcinogenic risk levels.

In Russia, levels of two N-nitrosamines, namely the sum of NDMA and NDEA, are monitored in sausages[7]. In its turn, no strict regulations are introduced for such chemicals in food products in the European Union [18]. Nevertheless, we should not rule out probable unacceptable health risks caused by intake of all detected potentially hazardous N-nitroso compounds. This is confirmed by a recent study conducted by the European Food Safety Authority (EFSA), which highlights the necessity to assess risks for human health caused by all detected N-nitrosamines in food products and to develop safe standards of their contents in foods [1, 2].

In some countries, normative documents have been already adopted that stipulate maximum permissible levels (MPLs) of N-nitrosamines in meat products. Thus, the Canadian Food Inspection Agency (CFIA) established the following MPLs for N-nitrosamines in dried meat: 0.01 mg/kg for NDEA, N-nitrosodipropylamine (NDPA), NDMA, N-nitrosodibutylamine (NDBA) and 0.015 mg/kg for NPyRA. In Chile, the MPL for N-nitrosodimethylamine (NDMA) in meat is fixed at 0.03 mg/kg[8] [18].

Table 3

Carcinogenic risk levels caused by NDMA, NDEA, NEMA and NPyRA intake with smoked sausages

Calculated values	NDMA	NEMA	NPyRA	NDEA	∑CR
Cancer slope factor, Sfo	51	22	2,1	150	
Scenario 1					
Average daily dose (carcinogen), mg/kg of body weight	$2.57 \cdot 10^{-6}$	$7.00 \cdot 10^{-7}$	$5.23 \cdot 10^{-7}$	$1.35 \cdot 10^{-8}$	$7.39 \cdot 10^{-5}$
Carcinogenic risk, *CR* considering disease severity	$6.48 \cdot 10^{-5}$	$7.62 \cdot 10^{-6}$	$5.43 \cdot 10^{-7}$	$1.00 \cdot 10^{-6}$	
Scenario 2					
Average daily dose (carcinogen), mg/kg of body weight	$6.41 \cdot 10^{-6}$	$1.75 \cdot 10^{-6}$	$1.31 \cdot 10^{-6}$	$3.37 \cdot 10^{-8}$	$1.85 \cdot 10^{-4}$
Carcinogenic risk, *CR* considering disease severity	$1.62 \cdot 10^{-4}$	$1.90 \cdot 10^{-5}$	$1.11 \cdot 10^{-6}$	$2.50 \cdot 10^{-6}$	
Contribution to carcinogenic risk level, %	87.6	10.3	0.7	1.4	100

[7] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: Tekhnicheskii reglament Tamozhennogo soyuza (s izmeneniyami na 23 iyunya 2023 goda) [CU TR 021/2011. On Food Safety: Technical Regulations of the Customs Union (as of June 23, 2023)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902320560 (June 24, 2024) (in Russian).

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The findings reported in this study highlight the importance to take a complex approach to assessing health risks caused by N-nitrosamines in food products. It is necessary to consider not only specific chemicals but their cumulative effect as well. The established correlations between levels of various N-nitrosamines prove the necessity to consider them in combination in health risk assessment as well as the possibility and even necessity to regulate their levels in food [19–21]. In its turn, assessment of only a limited number of N-nitrosamines in foods may result in underestimating adverse effects of chemicals on the human body. Moreover, when assessing health risks, it is necessary to consider not only levels of analyzed chemicals in foods but also a share of population who consumes an analyzed product regularly ant not the population as a whole, which is confirmed by our findings.

Conclusion. Correlation-regression analysis of data on NDMA levels made it possible to calculate NEMA and NPyRA levels in smoked sausages. Considering these results, the total carcinogenic risk was established to be unacceptable ($\sum CR = 1.85 \cdot 10^{-4}$) for adults who consume smoked sausages regularly. It should be noted that two N-nitrosamines (NEMA and NPyRA) that are not regulated in Russia account for 11.2 % of the total carcinogenic risk. At the same time, it was established that using data on volumes of consumption calculated for the whole population in exposure assessment reduced the total carcinogenic risk down to $7.39 \cdot 10^{-5}$. This highlights how important it is to consider actual consumption patterns in health risk assessment.

Therefore, the results of this study emphasize that it is relevant to consider all N-nitrosamines detected in food products in health risk assessment relying both on actual data and calculations to identify exposure levels more precisely. It is also very important to consider actual consumption patterns in health risk assessment.

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Research article

CRITERIA THAT DETERMINE CONSUMER CHOICE OF FOOD PRODUCTS WITHIN HEALTH RISK ANALYSIS

N.A. Lebedeva-Nesevria[1,2], M.D. Kornilitsyna[3], A.O. Barg[1]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation
[2]Novosibirsk State Technical University, 20 K. Marks Av., Novosibirsk, 630073, Russian Federation
[3]Perm State University, 15 Bukireva St., Perm, 614068, Russian Federation

Provision of food quality and safety is among national priorities. The strategy aimed at improving quality of food products in the Russian Federation for the period up to 2030 highlights a relevant issue as regards Russians consuming low-quality food products. The aim of this study is to identify criteria that determine consumer choice of food products and its influence on health risks.

The empirical data used in the study are represented by the results of national social surveys (Russian Public Opinion Research Center, NAFI Research Center, Public Opinion Fund, 2020–2023) and materials obtained by focus interviews with Russian megacity residents (n = 26, spring 2024).

Price is the top criterion that determines consumer choices in Russia. We identified three behavioral strategies based on subjective perception of food quality and price: 1) quality is priority regardless of a price (the strategy is typical for middle-aged consumers with higher incomes); 2) a balance between quality and price (including orientation at discounts and special promotion campaigns at points-of-sail); 3) refusal from subjectively more qualitative food products in favor of less qualitative but cheaper ones (the strategy is typical for consumers from senior age groups). Consumer orientation at product price leads to changes in diets in case population incomes are dropping or market prices are growing; in particular, it means a decline in fruit, meat and fish consumption. Significance a consumer places on food taste as a selection criterion results in choosing food products with low 'objective' consumer value but a higher 'subjective' one, for example, chips, sweetened carbonated beverages, and products with high saturated fat contents. Risky consumer choice is also determined by low interest in healthy diets and absence of any faith in possibility to get objective information.

A conclusion is made that it is necessary to make food products, which are subjectively perceived by consumers as more qualitative, more affordable in money terms; to intensify educational activities and to create suitable conditions for making consumers refuse from buying products with low nutrition value.

Keywords: *food products, food quality, food safety, consumer behavior, subjective criteria of quality and safety, risk behavior.*

The WHO (World Health Organization) Global Strategy for Food Safety considers food safety a priority issue of public healthcare and socioeconomic development[1]. The Strategy for Food Quality Improvement up to 2030 approved by the RF Government Order in June 2016 defines provision of food quality as a 'key component in health protection, achieving longer life expectancy and raising quality of life of the country population'[2]. Great attention is

Natalia A. Lebedeva-Nesevria – Doctor of Sociological Sciences, Head of the Laboratory for Social Risks Analysis (e-mail: natnes@fcrisk.ru; tel.: +7 (342) 237-25-47; ORCID: https://orcid.org/0000-0003-3036-3542).

Mariya D. Kornilitsyna – post-graduate student of the Department of Sociology (e-mail: maruromanova@mail.ru; tel.: +7 (342) 237-25-47; ORCID: https://orcid.org/0000-0003-2291-4316).

Anastasiya O. Barg – Candidate of Sociological Sciences, Senior Researcher at the Laboratory for Social Risks Analysis (e-mail: an-bg@yandex.ru; tel.: +7 (342) 237-25-34; ORCID: https://orcid.org/0000-0003-2901-3932).

[1] EB150/25, WHO Global Strategy for Food Safety: Report by the Director-General. *WHO*, December 10, 2021. Available at: https://apps.who.int/gb/ebwha/pdf_files/EB150/B150_25-en.pdf (August 14, 2024).

[2] Strategiya povysheniya kachestva pishchevoi produktsii v Rossiiskoi Federatsii do 2030 goda, utv. rasporyazheniem Pravitel'stva RF ot 29 iyunya 2016 g. № 1364-r [The Strategy for Food Quality Improvement in the Russian Federation up to 2030, approved by the RF Government Order on June 29, 2016 No. 1364-r]. *The Russian Federation Government*. Available at: http://static.government.ru/media/files/9JUDtBOpqmoAatAhvT2wJ8UPT5Wq8qIo.pdf (August 14, 2024) (in Russian).

paid to food safety and quality due to products with *inadequate* consumer properties (falsified food products or foods that do not conform to sanitary-epidemiological requirements) and foods with poor consumer properties (products with low nutrient and biological value, with high contents of trans fats) being distributed on the market. In Russia, turnover of low quality and falsified goods is supervised by Rospotrebnadzor, Rosselkhoznadzor and some other relevant public authorities. A consumer is not usually able to recognize a falsified product and, as a rule, does not choose it consciously[3]. But consumers buy food products with objectively low consumer properties, which, however, are safe according to a formal approach, following their own choice. It is usually based on 'consumer quality' [1] or 'subjective quality as perceived by consumers' [2], 'subjective consumer utility' [3].

Subjectively perceived quality of foods is determined by two groups of factors, 1) internal (they describe how a consumer perceives internal properties of a product (appearance, smell, composition, number of calories, etc.)) and 2) external (they describe how a consumer perceives modifiable properties of a product, namely, its price, brand, manufacturer, etc.). Within the Search / Experience / Credence model, three types of quality are viewed as components in the structure of perceived quality. They are 'search quality' (it can be determined prior to purchase, for example, a product appearance or composition declared by a manufacturer), 'experience quality' (it can be determined only upon consumption, for example, a product taste), and 'credence quality' (consumers cannot determine it on their own and have credence in others, as it is the case with utility of organic products declared by mass media) [4]. Criteria used by consumers to determine food quality have been studied in great detail; as a result, more than 50 such criteria, or cues, have been identified including price, smell, taste, vitamins and minerals, easiness to prepare, country of origin, being a product for diabetics / children / pregnant women etc. All cues have different significance for various groups of consumers [5]. For example, a study accomplished by NAFI Research Center in October 2022 revealed that a) a product utility was more important for people aged 55 years and older; b) a product taste was more significant for men, middle-aged people and people without higher education; c) a product price was rather a key cue for Russians close to retirement age and already retired as well as low-income consumers[4].

Consumer demands are dynamic and tend to change when influenced by a socioeconomic situation, sociocultural contexts, advertising, fashion, etc. New social norms and values that become fixed in a society also transform consumer demands to food products [6]. Thus, a trend for environmental-friendly consumption has spread globally over the last decade [7] and this has resulted in including 'environmental friendliness' into priority consumer criteria. According to the data provided by the Russian Public Opinion Research Center (VCIOM), in 2021, 55 % of respondents in Russia considered whether a product was 'environmental-friendly' when making a choice and 64 % of respondents would rather buy a more expensive product if it was 'environmental-friendly'[5].

Consumer choice is only partially rational [8] and very often impulsive and rather shortsighted when a consumer is oriented only at getting momentary benefits and not long-term

[3] Ignatova O. Glava Rospotrebnadzora rasskazala o sposobakh vyyavleniya fal'sifikata [The Head of Rospotrebnadzor has told us how to detect falsified products]. *Rossiiskaya gazeta: Internet portal*. Available at: https://rg.ru/2018/10/10/glava-rospotrebnadzora-rasskazala-o-sposobah-vyiavleniia-falsifikata.html (August 14, 2024) (in Russian).

[4] Aimaletdinov T.A., Bychkova E.A. Trendy potrebleniya rossiyanami produktov pitaniya. Rezul'taty kompleksnogo issledovaniya [Trends in food consumption by Russians. The results of complex survey]. *NAFI Research Center*, 2023. Available at: https://nafi.ru/projects/potrebitelskoe_povedenie/trendy-potrebleniya-rossiyanami-produktov-pitaniya/ (August 17, 2024) (in Russian).

[5] Ekologichnoe potreblenie [Environmental-friendly consumption]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/ehkologichnoe-potreblenie (August 15, 2024) (in Russian).

effects [9]. When buying food products, consumers, as a rule, act 'out of habit' or use their own intuitive judgment [10]. Declared values may fail to determine actual consumer behavior (for example, accepting health as a key life value may be combined with 'unhealthy diets' or choosing food products with low nutritional value).

The aim of this study was to identify subjective consumer criteria that describe safety and quality of foods distributed on the Russian market and to determine their relationship with health risk behavior.

Materials and methods. The study is based on a secondary analysis of national social surveys (Domestic and Imported Foods by the Public Opinion Fund, August 2020, n = 1000; Russians' Health and Diet by the Russian Public Opinion Research Center, May 2021, n = 1600; Trends in Food Consumption by Russians by NAFI Research Center, October 2022, n = 1600; Foods: Domestic or Imported? by the Russian Public Opinion Research Center, April 2023, n = 1600) as well as materials obtained by focus interviews with Russian megacity residents (a criterion sample, n = 26), which were conducted by experts from the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies in March – April 2024.

Results and discussion. Consumer criteria of food safety and quality are not identical with consumer choice criteria but they overlap to a certain extent. According to the results obtained by formalized surveys, price is the top criterion that determines consumer choices in Russia. According to the data provided by the NAFI Research Center, in 2022, 82 % of Russians primarily relied on a product price as the most significant criterion when buying foods (87 % among consumers aged 35–44 years and 86 % among those aged 55 years and older). Significance of price as a selection criterion was also confirmed by 88 % of the respondents agreeing with the statement: 'I pay great attention to discounts, campaigns, or special offers when buying foods'[6]. According to VCIOM data, price was one of top three criteria employed to choose food products by 52 % of the respondents in 2020 and by 59 % in 2021. It is noteworthy that in 2021, the share of consumers who were primarily guided by product prices was higher among people aged 18–24 years and consumers with a very poor / poor financial position than on average in the sample and reached 67 and 68 % respectively[7]. Growing significance of a product price in consumer groups with lower incomes was also evidenced for foreign samples [11, 12].

Analysis of the interviews revealed that consumers, regardless of their age or sex, tended to believe that more expensive products had the highest quality when discussing the 'price' category: *'Price means quality' (female, 19 years old, Novosibirsk), 'More expensive buckwheat has better quality and is cleaner. When you wash cheaper one, you get only husk' (female, 77 year old, Nizhnii Novgorod), 'The more expensive a product is the better quality it has. I'm sure of it' (female, 83 years old, Perm), '.. depends on a price, of course. If a product is more expensive, it is going to have higher quality. Cheap products do not have high quality.' (male, 22 years old, Perm)*[8]. However, it is price that makes qualitative food products less affordable for many consumers: '*Quality very often means high prices […] So, we have to buy cheaper products and they are not so high-quality' (female, 21 years old, Novosibirsk).*

In general, when consumers who gave an interview talked about food prices in connec-

[6] Aimaletdinov T.A., Bychkova E.A. Trendy potrebleniya rossiyanami produktov pitaniya. Rezul'taty kompleksnogo issledovaniya [Trends in food consumption by Russians. The results of complex survey]. *NAFI Research Center*, 2023. Available at: https://nafi.ru/projects/potrebitelskoe_povedenie/trendy-potrebleniya-rossiyanami-produktov-pitaniya/ (August 17, 2024) (in Russian).

[7] Zdorov'e i pitanie rossiyan: monitoring [Russians' Health and Diet: monitoring]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/zdorove-i-pitanie-rossijan-monitoring (August 15, 2024) (in Russian).

[8] Here and later on the text contains informants' original statements.

tion to quality, they could be divided into three groups: those who chose products, which, in their opinion, were of higher quality, regardless of their price (*'We certainly do not buy very expensive products but we try not to be economical when it comes down to quality' (female, 46 years old, Perm*)), 2) those who looked for a balance between quality and price (*'I try to buy discounted fish and meat […] always inspect a product appearance so that it would be satisfying. Meat is expensive and fish is as well, so we buy such products in… large retail networks. …as a rule, when they are sold with discounts' (female, 51 years old, Novosibirsk*)), 3) those who refused from buying products, which, in their opinion, were of higher quality, and chose less qualitative but cheaper products (*'If pension is enough, we buy qualitative products, and when it is not … we look for something cheaper' (female, 78 years old, Nizhnii Novgorod), 'If a product is sold at a cut-price, yes, our pensioners buy it and low-income families do it. I do it as well even if I know that this product is not healthy. But I, just as people like me, who get very small salary, cannot afford to buy … such food products that are qualitative and healthy' (female, 49 years old, Perm)*). Therefore, limited purchasing power in Russia is the primary factor that influences consumer choices making them buy products with lower subjective quality.

When consumers are guided by prices, it results in changes in their diets in a situation when incomes are declining or market prices are growing. A study by the Public Opinion Fund revealed that growing prices for food products made 27 % of Russians to buy less qualitative products and 22 % of Russians to refuse from buying certain expensive products. One quarter of consumers economize on meat and poultry; 18 %, on cheese, sausages, fish and other seafood; 14 %, on fruit[9]. Foreign studies established that healthy food on average tended to cost more than unhealthy one [13] and prices for the former tended to grow faster during a socioeconomic crisis [14].

In consumers' minds, quality of a product is closely connected with its freshness: *A high-quality product is a fresh one. For me, quality is first of all freshness.' (female, 64 years old, Nizhnii Novgorod).* Freshness is usually estimated through an expiration date and appearance. Some respondents considered these quality characteristics to be quite comprehensive without any clarifying questions by an interviewer: *"First of all, you have a look at how fresh a product is and when it was manufactured. As for more … I guess there is nothing else to look at' (male, 63 years old, Nizhnii Novgorod), '[I judge freshness] by appearance…. Only appearance' (female, 71 years old, Perm).* This may indicate that consumers either have rather low demands or are poorly aware of methods used to estimate food quality. If we pay attention to contexts of interviews, we can see that the financial position of this respondents' group does not allow them to buy expensive products.

Consumers have several ways to estimate food quality connecting it with determining how fresh it is: 1) by seeing the declared expiration date on a package in case it is provided there (according to the NAFI Research Center data, among all consumers who pay attention to a product package to a certain extent (89 % of the sample), 92 % of the respondents do it to see a date when a product was manufactured and its expiration date[10]); 2) estimating a product by using the sense organs judging its color, smell, and consistency; 3) asking sellers: *'[When determining whether a product is fresh, rely on] only on sellers' words who tell us that everything is fresh' (male, 67 years old, Nizhnii Novgorod).* It is interesting that such two criteria as 'quality' and 'freshness' were

[9] Produkty i tovary: stat'i ekonomii [Products and goods: items to economize on]. *FOM (Public Opinion Fund): official web-site*. Available at: https://fom.ru/Ekonomika/14189 (August 14, 2024) (in Russian).

[10] Aimaletdinov T.A., Bychkova E.A. Trendy potrebleniya rossiyanami produktov pitaniya. Rezul'taty kompleksnogo issledovaniya [Trends in food consumption by Russians. The results of complex survey]. *NAFI Research Center*, 2023. Available at: https://nafi.ru/projects/potrebitelskoe_povedenie/trendy-potrebleniya-rossiyanami-produktov-pitaniya/ (August 17, 2024) (in Russian).

offered to respondents separately in the quantitative survey by the Russian Public Opinion Research Center (2021). As a result, 53 % of the respondents told that they primarily paid attention to quality; 37 %, to freshness[11].

It is noteworthy that quality, freshness included, is estimated in a different way for various product groups. For example, some specific estimates are used for a) fish and meat: specific appearance (*'Well, some yellow film on fish, fat on its surface,…so to speak. You can always tell a fresh fish from spoilt' (male, 67 years, Nizhnii Novgorod)*, *'I try to notice whether a fish has a good color and its scale is shining; if yes, it means it is more or less edible' (female, 71 years old, Perm*)), consistency (*'… if it is not a frozen product, I estimate consistency' (female, 50 years old, Perm*)); b) fruit and vegetables: freshness (*'Probably, how ripe they are, if there are no rotten spots … they do not look spoilt' (female, 46 years old, Perm*)), size (*'Well, appearance mostly, color and shape. Some people also judge by size nowadays because sometimes products are of an enormous size and you can't help doubting their quality' (female, 50 years old, Perm*)), tactile perception (*'Whether an apple is soft or not', (female, 21 years old, Novosibirsk*)). It is the most difficult for consumers to estimate in a shop whether fruit and vegetables are 'qualitative' due to, among other things, absence of any label with product description: *'They do not provide the composition and I cannot read anywhere what this fruit or vegetable contains. I can only have a look at it and see whether I want to buy it or not or whether I like it or not, something like that' (female, 51 years old, Novosibirsk).*

Consumers with different level of income tend to have different attitudes towards products with a close expiration date. According to the NAFI Research Center survey, when answering the question 'If you see products that are sold at cut-off prices but with their expiration date being in the next few days, are you going to buy them or not?', 25 % of the respondents answered that they would consider buying such products above all. The share of those who would not buy such products was 8 % higher among wealthy respondents than on average in the sample (36 against 28 % respectively). Moreover, a product with a close expiration date attracted primary attention of low-income consumers (due to its more attractive price) but wealthy consumers saw it as a reason not to buy it even if this product was still safe for consuming. Therefore, choosing a product of lower quality estimated basing on its freshness was again connected with its greater affordability.

In consumers' opinion, food quality is closely connected with a place of origin, be it a country, a region, or even a specific manufacturer. For example, according to the Public Opinion Fund survey, in 2016, 14 % of Russian respondents believed the quality of Russian food products to be lower than that of imported ones[12]. The NAFI 2022 survey revealed that the share of consumers who thought imported goods to be more qualitative almost did not change and amounted to 15 %. The younger a respondent was the more often he or she stated that Russian products were less qualitative than imported ones: 24 % among people aged 18–24 years and 27 % – among those aged 25–34 years and only 8 % among people aged 60 years and older. This preference for domestic foods as more qualitative, which was typical for elder age groups, could also be traced in the interviews: *'It's better to buy our Russian fruits' (male, 67 years old, Nizhnii Novgorod*), *'We never buy imported foods. We always choose our home manufacturers' (female, 77 years old, Nizhnii Novgorod*).

Consumers believe more qualitative products to be a) those grown in their vegetable gardens (*'Our own vegetables, which we have*

[11] Zdorov'e i pitanie rossiyan: monitoring [Russians' Health and Diet: monitoring]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/zdorove-i-pitanie-rossijan-monitoring (August 15, 2024) (in Russian).

[12] Otechestvennye vs. importnye produkty pitaniya [Domestic vs. Imported foods]. *FOM (Public Opinion Fund): official web-site.* Available at: https://fom.ru/Ekonomika/12587 (August 14, 2024) (in Russian).

grown in our garden, are high-quality of course' (female, 64 years old, Nizhnii Novgorod)), b) farmer products without any industrial processing (*'We buy milk from private farmers, fresh cow milk' (male, 63 years old, Nizhnii Novgorod*)), c) products manufactured in a region where an informant lives (*'We know what manufactures are located not far from us in Perm region. Well I hope they stick to all necessary production processes at their milk processing plant' (female, 46 years old, Perm*)). 'Being natural' is the key argument here, which is commonly interpreted as absence of any artificial components in a product, refusal from using antibiotics or hormones in production, and a natural environment: *'… some E-numbers, additives, I guess, I'd be cautious with them, just as with palm oil. I'm not going to buy foods with them' (female, 51 years, Novosibirsk), 'Meat should be grown without any antibiotics, without any... Well, everything should be natural about it' (female, 49 years, Perm).* However, consumers not always choose local products because they believe such products are more 'natural' and sometimes their reasons can seem irrational: *'Interviewer: And why do they seem the most qualitative to you? Informant: I don't know. I guess … home is home' (female, 63 years old, Nizhnii Novgorod*). Preference to local producers and consumption of farmer products can be called a trend that has been developing across the globe starting from the mid 2010ties [15]; it became only 'stronger' during the pandemic [16].

A product 'naturalness' can be seen not only as a sign of its quality but of its safety as well. For some consumers, safe products are those that do not contain genetically modified organisms or cultures or artificial chemical additives: *'safe food products are those without any chemical coloring agents, primarily, GMOs and so on and so forth' (female, 49 years old, Perm), 'For me, safe foods are those with low levels of chemicals or sugar as well as those with low GMO contents' (female, 19 years, Novosibirsk).* In general, Russian consumers tend to be highly suspicious of genetically modified organisms. According to VCIOM data, in 2020, 66 % of Russians agreed with the statement 'Genetically modified food products (GMO-containing foods) are extremely hazardous for human health. However, the population is not allowed to have this information'; 17 % had difficulty in expressing their unambiguous attitude towards the statement; and only 20 % of respondents believed genetically modified food products to be safe for people[13]. In 2022, 44 % of Russians agreed with the statement that 'GMO-containing products induce cancer'[14]. The smaller settlement respondents lived in the sooner they would agree with this statement ($p < 0.001$, Fi = 0.194).

Food safety is a basic criterion of consumer choice. It is closely connected with product quality and is interpreted as a) commonly as food being edible (*'Unsafe [foods] means they are not edible' (female, 71 years old, Perm)*) and b) in a more narrow sense, as absence of relatively prompt adverse health outcomes (food poisoning or allergic reactions) (*'[safe food product] means I don't get, for example, stomach ache after I've had it' (female, 83 years old, Perm); '[Unsafe foods] means you can eat them and get stomach ache in the best case, in the worst case, you get poisoned with …bacteria' (female, 42 years old, Novosibirsk)*). Product freshness, identified based on its expiration date or visually, is the basic operational safety criterion. Still, a) safe foods can be 'unhealthy' (*'Suppose, a hamburger is unhealthy food. Well, I still think it to be safe, yeah? That is, it is fresh, made of meat*

[13] Teorii zagovora – i chto lyudi o nikh dumayut? [Conspiracy theories – and what do people think about them?]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/teorii-zagovora-i-chto-lyudi-o-nikh-dumayut (September 16, 2024) (in Russian).

[14] Pochemu neobkhodimo prosveshchenie, ili snova o rasprostranennykh zabluzhdeniyakh [Why do we need education or again about commonly spread delusions]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/pochemu-neobkhodimo-prosveshchenie-ili-snova-o-rasprostranennykh-zabluzhdenijakh (September 14, 2024) (in Russian).

and vegetables. And I think it is safe basically but still unhealthy' (female, 24 years old, Perm)), b) safe food products can be low-quality (*'well, for example, vegetables have been on the shelf for too long, carrots are flabby but they are still safe. You can buy them and cook something' (female, 71 years old, Perm)*).

A 2019 survey by the Russian Public Opinion Research Center revealed that only 17 % of Russians were guided by food safety when buying agricultural products such as vegetables or greenery. On the other hand, 'being fresh' turned out to be a significant criterion for 48 % of the respondents (the most common choice among all options)[15], which in general supports the thesis that customers are primarily guided by food safety even if there is no verbal confirmation of the fact.

A product taste is another important criterion that determines consumer choice [17]. According to a survey that was accomplished among Europeans by Euromonitor International, in 2022, 51 % of European consumers were guided by taste when choosing foods[16]. A survey that was conducted in Portugal in October 2022 revealed that 58 % of the respondents thought a product taste to be of critical importance[17]. Quantitative surveys aimed at determining how significant a product taste is for Russians have yielded somewhat ambiguous results. Thus, according to a survey by the Russian Public Opinion Research Center, in 2021, only 17 % of the respondents named a product taste among three basic cues in choosing food products. An all-Russia survey conducted in 2021 by the Research Scientific Center for Social and Political Monitoring of the Institute for Social Sciences, on the contrary, established that 51 % of consumers thought a product taste to be a priority criterion [18]. Data collected by the NAFI Research Center show that in 2022 64 % of the respondents aged 35–44 years were guided by a product taste when choosing food products.

Significance of a product taste as a consumer choice cue results in preferring foods with low 'objective' consumer properties but high subjective value such as chips, sweetened carbonated drinks, products with high saturated fat contents, etc.: *'Coca-Cola, energy drinks, yes, I have sinned. They are very tasty.' (male, 23 years old, Novosibirsk).* Still, in the foregoing survey by the Russian Public Opinion Research Center, 43 % of the respondents told they 'would rather agree' with the statement that 'tasty food is never healthy'. It is important that consumers understand low nutritional value of foods they consume since they label such products as 'unhealthy' or 'useless' (*'Unhealthy foods have a lot of salt or fats, like chips, also Coca-Cola, crackers, croutons and many fast foods, all this is unhealthy'* (*female, 71 years old, Perm)*) and their consumption as non-conforming to a healthy diet (for example, by using such words as 'sin' or 'we have sinned': *'Sometimes, you just want sausage or herring. Sometimes, we sin and have some chocolates as well' (female, 78 years, Nizhnii Novgorod)*). Still, an attractive taste of a product turns out to be the predominant criterion in making a decision to buy (*'I like all these unhealthy stuff very much, so delicious, even if they have E-numbers … It's very difficult to refuse from eating them' (female, 27 years, Novosibirsk)*).

A product taste is an important cue when choosing fast food as a basic diet. Thus, according to the Russian Public Opinion Research Center data, in 2022, 25 % of young consumers in Russia aged between 18 and 24 years visited a fast food café at least once a week. More than a half of them (65 %) did it

[15] Pitanie: pravil'noe i bezopasnoe [Diet: healthy and safe]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/pitanie-pravilnoe-i-bezopasnoe (August 14, 2024) (in Russian).

[16] Food and Nutrition. *Euromonitor International*. Available at: https://www.euromonitor.com/insights/food-and-nutrition (August 15, 2024).

[17] Food choice criteria in Portugal in 2022, by level of importance. *Statista*. Available at: https://www.statista.com/statistics/1394568/portugal-food-choice-criteria-by-importance/ (August 14, 2024).

to save time and 23 % stated they did it because 'the food was delicious'[18]. The second place as per popularity belonged to the answer 'children ask / children like' in the age group 35–44 years as regards going to fast food cafes. This again confirms that fast food has an attractive taste not so much for adults as for children.

When consumers are guided by product tastes (without considering their nutritional value, vitamin and mineral contents), this leads to prevalence of consumer behavior that creates health risks. Persistent consumption of sweetened carbonated drinks and fast food (in particular chicken and potato fried in deep fat, hamburgers, etc.) authentically increases obesity risk [19], among children and adolescents as well [20]. Excessive consumption of sugar in foods is associated with caries both in adults [21] and children [22].

Customers' opinions about product tastes are to a great extent based on their own previous experience *('I mostly buy the same brand of macaroni that I like, that I've had eaten and know that they are tasty' (female, 24 years old, Perm*). Overall, experience is an important component of the mechanism for consumer choice stabilization ('making a habit'). Thus, most Russians (90 %) try to buy products of those brands and manufactured by those companies they 'know well'. 'Conservative' decisions are more popular among middle-age people, women and consumers with higher middle incomes than on average in the sample[19]. Well-established habits of informants' consumer behavior [23] are usually manifested by using such words as 'habit' or 'life experience' ('*Well, we have been buying this butter for a long time, like, got used to it. So we keep buying it' (female, 64 years old, Nizhnii Novgorod)).* On the one hand, habitual consumption makes choice easier; on the other hand, it is viewed as a way to mitigate risks: *'Actually, you buy the same stuff,…you know this product is alright, you eat the same foods and they seem to not affect your body or health so much. So to say, no poisonings, nothing bad. Therefore, I try to buy the same products, so to say, not to face any risk of poisoning' (male, 64 years old, Nizhnii Novgorod*).

Several reasons, apart from attractive tastes and lower prices, determine risky consumer choices:

– Low interest in the subject and no motivation to learn any useful data on product features *('[studying product contents in detail] No, I've never done that, I'm not so good at it, you have to learn how to do it properly <…> I know that many people are able to count calories and something like that but that's not my cup of tea...' (female, 64 years old, Nizhnii Novgorod)*);

– Consumers do not believe they can get objective information about products; they think they have no choice as available ranges of products are limited ('*We go shopping and buy what we see on the shelf. In most cases, we just buy a cat in a bag and we do not know what we eat' (female, 49 year old, Perm)*);

– Health risks associated with consuming foods that possibly contain some adverse chemicals are estimated as low ('*Well, cucumbers and tomatoes that are sold in winter… people say they contain a lot of poisons. But nobody has died of it, right? Everybody eats them' (female, 64 years old, Nizhnii Novgorod*));

– Lack of time and peculiar lifestyles ('*You often just don't have enough time to, say, cook anything; it just takes a lot of time. And a lot of homework, all this makes me have just a snack on foot, and these snacks can be rather unhealthy, I don't know, maybe, too stuffed with nutrients or whatever' (female, 21 years old, Novosibirsk)*).

[18] Fastfud: ot epokhi Makonal'dsa – vo vremena «Vkusno i tochka» [Fast food: from the McDonald's era to Vkusno I Tochka times]. *VCIOM (Russian Public Opinion Research Center): official web-site*. Available at: https://wciom.ru/analytical-reviews/analiticheskii-obzor/fastfud-ot-ehpokhi-makdonaldsa-vo-vremena-vkusno-i-tochka (August 17, 2024) (in Russian).

[19] Aimaletdinov T.A., Bychkova E.A. Trendy potrebleniya rossiyanami produktov pitaniya. Rezul'taty kompleksnogo issledovaniya [Trends in food consumption by Russians. The results of complex survey]. *NAFI Research Center*, 2023. Available at: https://nafi.ru/projects/potrebitelskoe_povedenie/trendy-potrebleniya-rossiyanami-produktov-pitaniya/ (August 17, 2024) (in Russian).

Conclusion. Our findings allow us to make the following conclusions. First, such cues as food quality and food safety overlap in consumers' minds in many respects. In particular, 'freshness' of a product or its expiration date being remote indicate that a product is high-quality and safe. 'Naturalness' of a product is estimated by presence / absence of chemical additives or genetically modified organisms and cultures in it and this makes such a product both safe and high-quality for consumers. Given that, it seems unadvisable to offer consumers such an estimation criterion as 'food quality' without its additional operationalization within formalized empirical research.

Second, criteria that describe food quality and those determining consumer choices do not coincide completely. Price is the key priority for Russians when they choose what food products to buy. Consumers giving preference to a cheaper product often means that they do not keep strict demands to quality. An attractive taste is a significant motive to choose a product with low nutritional value but consumers do not think it to be a sign of good quality.

Third, consumers' credence to a manufacturer or supplier is a mediator allowing to estimate a product as high-quality one. Thus, local food manufacturers are seen as 'close', 'domestic' and, accordingly, they deserve more credence as compared with manufacturers from other regions or countries.

Fourth, routine practices of buying foods have been made a habit and are determined by a habit to choose the same product. It can be based on various motives including absence of any interest in keeping a 'healthy diet', wish to mitigate health risks by keeping this habit, lifestyle peculiarities, etc. A considerable part of consumer practices being old habits moves the issue of assessing food quality and safety out from the space of everyday reflexion.

Reduction in prevalence of risky consumer behaviors requires the following:

1) making 'natural' products more affordable, offering wider ranges of farmer products affordable for consumers with middle and low incomes;

2) intensifying educational activities aimed at forming stable public opinions about safety of genetically modified products and about healthy food that can also be tasty;

3) creating suitable conditions for refusal from consuming food products with low nutritional value by young students including wider ranges of dishes offered to them by food providers in vocational educational establishments and higher education institutions.

Limitation of the study. Primary empirical data the study relied on are qualitative. The sample corresponds to the requirement of being representative fixed for qualitative research.

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Research article

POSTERIOR ASSESSMENT OF OCCUPATIONAL RISKS ASSOCIATED WITH WORK HARDNESS BASED ON WORKERS' SUBJECTIVE PERCEPTION OF THEIR HEALTH

N.V. Zaitseva[1], P.Z. Shur[1], D.N. Lir[1,2], V.B. Alekseev[1], V.A. Fokin[1], A.O. Barg[1,4], T.A. Novikova[3], E.V. Khrushcheva[1]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation
[2]E.A. Vagner's Perm State Medical University, 26 Petropavlovskaya St., Perm, 614990, Russian Federation
[3]Saratov Hygiene Medical Research Center, 1A Zarechnaya St., Saratov, 410022, Russian Federation
[4]Perm State University, 15 Bukireva St., Perm, 614068, Russian Federation

Work hardness causes health impairments in workers of some occupations. Posterior assessment should be considered priority one in health risk analysis instead of relying solely on results obtained by prior assessment based on descriptions of working conditions,

This paper presents the results obtained by posterior occupational risk (OR) assessment associated with work hardness; the assessment is based on analyzing workers' subjective perception of their health (workers employed at a bearing production were used as an example).

A survey was accomplished within this study followed by analyzing subjective perception of one's health. It gave an opportunity to accomplish quantitative posterior OR assessment (that considered both likelihood of diseases and their severity) at the group and individual levels. Work hardness creates unacceptable group health risks associated with diseases of the musculoskeletal system and connective tissue ($1.93 \cdot 10^{-2}$–$2.56 \cdot 10^{-2}$), nervous system ($4.03 \cdot 10^{-2}$–$6.77 \cdot 10^{-2}$), genitourinary system ($2.04 \cdot 10^{-2}$–$2.7 \cdot 10^{-2}$) and cardio-vascular system ($1.47 \cdot 10^{-2}$–$1.69 \cdot 10^{-2}$). Such indicators as 'weight of constantly lifted cargo and cargo moved by hand' (35–58 %) and 'uncomfortable working posture / working upright' (29–54 %) make major contributions to the integral risk.

Risk categories were adjusted at the individual level (considering parameters of the relationship that describe how likelihood of disease is influenced by work hardness, age and working records). This allowed establishing that OR was predominantly caused by diseases of the musculoskeletal system and connective tissue ('medium risk' for 19–83 % of the workers and 'high risk' for 75–81 %), nervous system ('high risk' for 84–85 % and 'extremely high risk' for 15–16 % of the workers) and genitourinary system ('moderate risk' for 1 %, 'medium risk' for 8 %, and 'high risk' for 87 %).

Occupational health risk assessment allowed identifying priority indicators of work hardness ('weight of constantly lifted cargo and cargo moved by hand' and 'uncomfortable working posture / working upright') and establishing proper scope of relevant prevention activities at the group and individual level.

Keywords: *occupational risk, health risk, work hardness, posterior assessment, health disorder, subjective perception, methodical approaches, work-related diseases.*

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).

Darya N. Lir – Candidate of Medical Sciences, Leading Researcher – Head of the Health Risk Analysis Department; Associate Professor at the Department of Hygiene of Medical-Preventive Faculty (e-mail: lir@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-7738-6832).

Vadim B. Alekseev – Doctor of Medical Sciences, director (e-mail: alekseev@fcrisk.ru; tel.: +7 (342) 236-32-70; ORCID: https://orcid.org/0000-0001-5850-7232).

Vladimir A. Fokin – Candidate of Medical Sciences, 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).

Anastasiya O. Barg – Candidate of Sociological Sciences, Senior Researcher at the Laboratory for Social Risks Analysis (e-mail: an-bg@yandex.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0003-2901-3932).

Tamara A. Novikova – Candidate of Biological Sciences, Leading Researcher – Head of the Laboratory for Occupational Hygiene and Common Pathology (e-mail: novikovata-saratov@yandex.ru; tel.: +7 (845) 234-71-84; ORCID: https://orcid.org/0000-0003-1463-0559).

Ekaterina V. Khrushcheva – Senior Researcher acting as a Head of the Laboratory for Risk Management Methods and Technologies (e-mail: khrusheva@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0003-2107-8993).

Over the last 10 years, work hardness has caused a growth in the share of diseases associated with physical overloads (the growth rate is 5.12 %)[1]. Assessment of occupational risk (OR) associated with work hardness is among relevant tasks of occupational hygiene. In practice, it mostly relies on prior health risk assessment based on SAWC (Special Assessment of Working Conditions) data [1–3]. Several publications report some results obtained by posterior assessment that involved establishing cause-effect relations between health impairments and work hardness as per results of epidemiological studies [4–8] as well as posterior assessments with the use of one-figure indexes [2]. However, this does not always allow quantifying OR levels and describing them in detail at the individual level.

To achieve optimal functioning of a system for preventive activities, it seems advisable to consider both working conditions for prior assessment and health for posterior assessment as comprehensively as only possible within OR assessment. Another important thing is a possibility to predict individual health risks over the whole period of working [9–11]. Given that a registered occupational incidence (OI) rate can fail to fully describe an actual situation [12, 13], it seems interesting to analyze aggregated health outcomes to describe workers' health. These outcomes should cover both occupational diseases (ODs) and prevalence of general somatic diseases that are likely to be associated with working conditions (work-related diseases or WRDs), which tend to grow as work records become longer[2]. International experience in assessing prevalence of work-related diseases of the musculoskeletal system [14–16] allows employing questioning with subsequent analysis of subjective health perception to solve these tasks. Such an approach, on one hand, can supplement available data obtained by medical check-ups; on the other hand, it ensures an independent information source about aggregated health responses in the body prior to disease development and putting a clinical diagnosis. This is especially vital for preventive medicine since this helps ensure substantial effects of implemented preventive activities.

The present study has been accomplished considering the priority given to posterior quantitative OR assessment in relevant guidelines and it supplements the previously published results of prior assessment of OR associated with work hardness [17].

The aim of this study was to perform posterior assessment of occupational risk associated with work hardness based on analyzing data about workers' subjective perception of their health (exemplified by workers employed at a bearing production facility).

Materials and methods. Hygienic health risk assessment was accomplished considering the basic provisions stated in the Guide R 2.2.3969-23 Assessment of Occupational Health Risk for Workers / Organization and Methodical Essentials, Principles and Assessment Criteria[3] (hereinafter R 2.2.3969-23). They stipulate relevant assessment stages, use of adequate methods for measuring exposure, quantitative characteristics and priority of posterior assessment, that is, use of data on actual workers' health.

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[1] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2022 godu: Gosudarstvennyi doklad [On sanitary-epidemiological welfare of the population in the Russian Federation in 2022: the State Report]. *The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing.* Available at: https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=25076 (June 11, 2024) (in Russian).

[2] Professional'naya patologiya: natsional'noe rukovodstvo [Occupational pathology: national guide]. In: N.F. Izmerov ed. Moscow, GEOTAR-Media Publ., 2011, 784 p. (in Russian).

[3] Guide R 2.2.3969-23. Rukovodstvo po otsenke professional'nogo riska dlya zdorov'ya rabotnikov. Organizatsionno-metodicheskie osnovy, printsipy i kriterii otsenki; utv. Federal'noi sluzhboi po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka 7 sentyabrya 2023 g. [Assessment of Occupational Health Risk for Workers. Organization and Methodical Essentials, Principles and Assessment Criteria; approved by the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing on September 7, 2023]. *GARANT: information and legal support*. Available at: https://base.garant.ru/408890207/ (June 11, 2024) (in Russian).

Work hardness was assessed based on measures and criteria according to the Guide R 2.2.2006-05 Hygienic Assessment of Factors Related to Working Environment and Work Process / Criteria and Classification of Working Conditions[4]. This assessment involved analyzing workers' subjective perception of this factor including a survey with a specifically designed questionnaire and a template for automated risk calculation. The results obtained by implementing the foregoing approach were quite valid (sensitivity was 94 % and predictive value was 84 %) [17].

Data on workers' health were also obtained by questioning. A questionnaire as a survey tool was developed considering international experience in health assessment using the standardized Nordic questionnaire for the analysis of musculoskeletal symptoms[5]. The questionnaire consisted of 19 questions and was aimed at revealing symptoms of diseases associated with work hardness over the last 12 months; visits to a doctor to treat these symptoms; an established diagnosis (if any).

A list of relevant symptoms was created basing on data about likely negative changes associated with work hardness. They were established at the hazard identification stage. Symptoms typical for identified health outcomes were determined by expert estimates performed by occupational pathologists at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies under the guidance by O.Yu. Ustinova, Doctor of Medical Sciences, Professor. The symptoms were then used to create a matrix for identification of a likely disease.

Likelihood of health impairments (diseases) was calculated considering already existing symptoms associated with these diseases from a relevant disease class.

Health risk levels for workers were quantified according to R 2.2.3969-23 considering additional likelihood of negative outcomes (diseases) and their severity. Severity coefficients were determined as per assessments of disability-adjusted life years (DALY) recommended by the WHO[6]. When determining a risk category, quantitative values that corresponded to negligible and low OR were considered acceptable (permissible).

The study was accomplished on a sample made of workers employed at a bearing production facility (BPF) with various occupations (blacksmith, founder, driver, setter, furnace tender, roll-on handler, repairman, sorter, heat-treater, turner, polisher, electrician etc.) who worked in five divisions (workshops) (n = 97, average age was 45.1 ± 1.2 years) [17]. The test group was made of workers exposed to work hardness that was above its permissible level per some specific indicators. The reference group was made of workers from the same workshops provided they were not exposed to impermissible work hardness. A relationship between a factor and disease was considered significant ($p < 0.05$) at a relative risk

[4] Guide R 2.2.2006-05. Rukovodstvo po gigienicheskoi otsenke faktorov rabochei sredy i trudovogo protsessa. Kriterii i klassifikatsiya uslovii truda; utv. Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 29 iyulya 2005 g. [Hygienic Assessment of Factors Related to Working Environment and Work Process. Criteria and Classification of Working Conditions; approved by G.G. Onishchenko, the RF Chief Sanitary Inspector on July 29, 2005]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200040973 (June 11, 2024) (in Russian).

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[6] Global Burden of Disease Study 2019 (GBD 2019) Disability Weights. Seattle, USA, Institute for Health Metrics and Evaluation (IHME), 2020. Available at: https://ghdx.healthdata.org/record/ihme-data/gbd-2019-disability-weights (June 17, 2024); WHO methods and data sources for global burden of disease estimates 2000–2019. *WHO*, 2020. Available at: https://cdn.who.int/media/docs/default-source/gho-documents/global-health-estimates/ghe2019_daly-methods.pdf?sfvrsn=31b25009_7 (June 17, 2024).

(*RR*) level and the lower limit of the confidence interval of 95 % (CI) being above 1. Additional likelihood of negative health outcomes was determined based on epidemiological analysis and an established relationship between a factor and disease as difference between likelihood of disease in the test and reference groups.

The integral group risk (R_{int}) associated with some specific indicators of work hardness was defined per the following formula 1:

$$R_{int} = 1 - \prod_{i=1}^{n} \cdot \left(1 - p_{ji} \cdot g_i\right), \qquad (1)$$

where

p_{ij} is likelihood of the *j*-th disease associated with the *i*-th indicator of work hardness;

g_j is the severity coefficient for the *j*-th disease (response).

Severity coefficients corresponded to average weighted measures per disease classes[6]: diseases of the musculoskeletal system and connective tissue, 0.079; diseases of the nervous system, 0.166; diseases of the circulatory system, 0.07; diseases of the genitourinary system, 0.078.

Individual OR was calculated based on created logistic regression models that described relationships between diseases and work hardness (exposure level), age and work records (Formula 2):

$$p_1 = \frac{1}{1 + e^{-(b_0 + b_1 x_1 \cdot x_2 + b_2 x_3)}}, \qquad (2)$$

where

p_1 is likelihood of the *j*-th response;

x_1 is a level of exposure to work hardness as an adverse occupational factor per some specific indicators (kg·m; kg; km; % of time; kg·sec; abs. units);

x_2 is work records, years;

x_3 is age, years;

b_0, b_1, b_2 are parameters of a mathematical model.

Significance of parameters and model adequacy was estimated by using dispersion analysis per the Fischer's test ($p < 0.05$).

Results and discussion. A list of most likely unfavorable health outcomes was created at the hazard identification stage. They included both ODs and WRDs[7]. The list was systematized for groups of indicators that described work hardness (physical dynamic load for a local and total load; weight of constantly lifted cargo and cargo moved by hand; stereotype work movements; static loads; uncomfortable working posture; body bending at an angle more than 30 degrees; moving horizontally or vertically) and included diseases of the musculoskeletal system and connective tissue, nervous system, circulatory system and genitourinary system.

Prevalence of the analyzed symptoms was established based on workers' subjective perception of their health as well as on frequency of visits to a doctor in case of health complaints and already diagnosed diseases. Over the last 12 months, more than 50 % of the workers felt discomfort, strain and / or pain in the small of the back; less than 50 % but more than 40 %, discomfort, strain and / or pain in arms and / or legs; less than 40 % but more than 20 % of the workers, discomfort, strain and / or pain in the neck, shoulder blades, shoulders, restriction of movements in joints of upper and / or lower extremities; less than 20 % of the workers complained about vascular asterisks on lower extremities, shin and foot edemas, impaired sensitivity, and changes in skin color of upper and / or lower extremities (Table 1). In terms of occupations, malaise signs were the most frequently identified in turners (pains in different part of the spine, upper and / or lower extremities 6.3–9.1 %), setters (pain in the small of the back 9.5 %, pain in the legs 7.8 %), founders and polishers (pain in the small of the back 6.8 %).

[7] Ob utverzhdenii perechnya professional'nykh zabolevanii: Prikaz Ministerstva zdravookhraneniya i sotsial'nogo razvitiya RF ot 27 aprelya 2012 g № 417n [On Approval of the List of Occupational Diseases: the Order by the Ministry for Healthcare and Social Development of the Russian Federation issued on April 27, 2012 No. 417n]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902346847/titles/64U0IK (June 11, 2024) (in Russian).

Table 1

Subjective perception of ones' health by workers employed at a bearing production facility

№	List of symptoms	Total	Symptoms occurred over the last 12 months		Black-smith	Foun-der	Setter	Repair-man	Sorter	Heat-treaterт	Turner	Polisher
			abs.	%	%	%	%	%	%	%	%	%
1	Restricted motion, discomfort, strain and pain in the neck	79	22	27.8	2.5	2.5	3.8	0.0	3.8	1.3	7.6	2.5
2	Neck noises at head turns	77	19	24.7	2.6	2.6	3.9	0.0	1.3	1.3	5.2	5.2
3	Headache (back to temple), which is not stopped by analge-sics	76	20	26.3	1.3	2.6	5.3	1.3	3.9	1.3	3.9	3.9
4	Discomfort, strain and pain in the shoulder blades	77	18	23.4	0.0	1.3	3.9	0.0	3.9	3.9	3.9	1.3
5	Discomfort, strain and pain in the shoulders	77	22	28.6	1.3	3.9	5.2	2.6	2.6	2.6	3.9	3.9
6	Discomfort, strain, weakness and pain in the arms and hands	70	28	40.0	2.9	4.3	4.3	1.4	4.3	2.9	8.6	4.3
7	Impaired sensitivity, changes in skin color of the shoulder girdle	74	5	6.8	0.0	1.4	2.7	0.0	0.0	1.4	0.0	1.4
8	Muscle weakness, reduced mus-cle force of the arms	76	17	22.4	1.3	1.3	1.3	1.3	2.6	0.0	3.9	2.6
9	Discomfort, strain and pain in the small of the back	74	38	51.4	4.1	6.8	9.5	1.4	4.1	5.4	6.8	6.8
10	Stiffness, restricted motion of the spinal column	79	20	25.3	1.3	1.3	5.1	0.0	0.0	2.5	6.3	5.1
11	Discomfort, strain and pain in the legs	77	33	42.9	2.6	3.9	7.8	0.0	3.9	5.2	9.1	5.2
12	Changes in the gait, swaying, fatigue	74	23	31.1	2.7	2.7	5.4	1.4	4.1	1.4	5.4	4.1
13	Discomfort, strain and pain of lower extremities	74	4	5.4	1.4	1.4	1.4	0.0	0.0	0.0	1.4	0.0
14	Impaired sensitivity and func-tions of the pelvis organs (con-stipation, enuresis; in females, bloody discharge, bulging of genital organs)	74	4	5.4	0.0	0.0	0.0	0.0	2.7	1.4	1.4	0.0
15	Restricted motion, pain in joints of upper extremities upon movement and physical loads	74	18	24.3	1.4	4.1	5.4	1.4	2.7	1.4	4.1	2.7
16	Restricted motion, pain in joints of lower extremities upon movement and physical loads	73	17	23.3	1.4	2.7	5.5	0.0	2.7	2.7	4.1	4.1
17	Spontaneous bone fractures	74	3	4.1	0.0	0.0	0.0	1.4	0.0	0.0	1.4	0.0
18	Vascular asterisks, varicose saphenous veins of lower ex-tremities	75	14	18.7	4.0	1.3	4.0	1.3	2.7	0.0	1.3	2.7
19	Shin and foot edemas by the end of a work day	74	13	17.6	1.4	0.0	1.4	1.4	2.7	1.4	4.1	4.1

Not more than 30 % of the workers who had health complaints visited a doctor. Thus, 29 % of the workers went to a clinic to treat discomfort, strain and / or pain in the small of the back and a diagnosis was put in 45 % of the cases (osteochondrosis, a herniated disk); 12 %, to treat discomfort, strain and / or pain in the legs; of them, a diagnosis was put in 25 % of the cases (osteoarthrosis); 7 %, to treat discomfort, strain and / or pain in the arms without any diagnosis at the moment the present study was being accomplished.

Within this study, presence of likely adverse health outcomes was established using symptoms mentioned by a respondent and according to the above mentioned matrix for identification of health responses. These health outcomes were determined per relevant classes of diseases (ICD-10): diseases of the musculoskeletal system and connective tissue (M00–M99), diseases of the nervous system (G00–G99), diseases of the circulatory system (I00–I99), and diseases of the genitourinary system (N00–N99).

To perform posterior OR quantification, the Exposure – Response relationship was estimated and cause-effect relations were established between adverse health outcomes in workers employed at BPF and levels of exposure to the analyzed factor per specific indicators that described work hardness (Table 2). Out of 17 indicators that described work hardness, significant cause-effect relations were identified for three (no relation was established for one indicator, and there were not enough observations to estimate 9 indicators for the test group).

Epidemiological analysis established that diseases of the musculoskeletal system and connective tissue were probably caused by too heavy weight of cargo that was constantly lifted and moved around by hand (1.67 (1.2; 2.4), EF = 40 %, $p < 0.05$) as well as too long time spent working in an uncomfortable posture (1.71 (1.2; 2.4), EF = 41 %, $p < 0.05$) or working upright (1.65 (1.0; 2.7), EF = 40 %, $p < 0.05$). Diseases of the nervous system were associated with the same indicators of work hardness with the etiological fraction of the factor being 44–52 %. A major contribution was made to diseases of the circulatory system by long periods spent working upright (2.29 (1.1; 5.0), EF = 56 %, $p < 0.05$) and too heavy weight of cargo that was constantly lifted and moved around by hand (1.77 (1.0; 3.0), EF = 43 %, $p < 0.05$). Diseases of the genitourinary system in the analyzed sample had the strongest association with weight of cargo that was constantly lifted and moved around by hand (2.19 (1.4; 3.5), EF = 54 %, $p < 0.05$) and uncomfortable working posture (1.75 (1.1; 2.8), EF = 43 %, $p < 0.05$).

Table 2

Relative risk of negative health outcomes (per classes of diseases) associated with some specific indicators of work hardness for BPF workers as per epidemiological analysis results

Indicators	Diseases of the musculoskeletal system and connective tissue (M00–M99)	Diseases of the nervous system (G00–G99)	Diseases of the circulatory system (I00–I99)	Diseases of genitourinary system (N00–N99)
Weight of a cargo lifted and moved by hand: interchanging with other work tasks, kg	0.6 (0.3; 1.0)	0.43 (0.2; 0.9)	0.39 (0.2; 1.0)	0.72 (0.4; 1.4)
Weight of a cargo lifted and moved by hand: constantly, kg	**1.67 (1.2; 2.4)**	**1.96 (1.3; 2.9)**	**1.77 (1.0; 3.0)**	**2.19 (1.4; 3.5)**
Uncomfortable working posture, % of time	**1.71 (1.2; 2.4)**	**2.09 (1.4; 3.0)**	1.57 (0.9; 2.7)	**1.75 (1.1; 2.8)**
Working upright, % of time	**1.65 (1.0; 2.7)**	**1.78 (1.0; 3.1)**	**2.29 (1.1; 5.0)**	1.69 (0.9; 3.1)

Note: significant cause-effect relations are given in bold, $p < 0.05$.

The risk characterization stage involved identifying levels of group OR associated with some specific indicators of work hardness considering additional likelihood and severity of negative health outcomes per various classes of diseases (Table 3).

The highest group OR values per specific indicators of work hardness were established for diseases of the nervous system and corresponded to the 'high risk' category. OR levels corresponded to the 'medium risk' category for all other classes of diseases. Still, the integral group risk associated with various characteristics of work hardness and caused by diseases of the musculoskeletal system and connective tissue ($6.64 \cdot 10^{-2}$), circulatory system ($4.31 \cdot 10^{-2}$) and genitourinary system ($6.13 \cdot 10^{-2}$) was estimated as 'high'; the integral group risk caused by diseases of the nervous system ($1.56 \cdot 10^{-1}$) was estimated as 'very high'.

Major contributions were made to the integral risk by such indicators as 'Weight of constantly lifted cargo and cargo moved by hand' (from 35 to 58 %) as well as 'uncomfortable working posture' (from 37 to 44 %) and 'working upright' (from 29 to 54 %). Estimated structure of contributions per various classes of diseases showed that 'uncomfortable working posture' made the greatest contribution to the risk of diseases of the nervous system (43 %).

Individual ORs were established using the results of mathematical modeling. Its parameters are provided in Table 4.

The individual risk was established to be caused by diseases of the musculoskeletal system and connective tissue, nervous system and genitourinary system. Lifting and moving heavy cargos (their weight being above safe standards) as a constant activity during a work shift created an individual OR caused by diseases of the musculoskeletal system and connective tissue with its value varying between $1.69 \cdot 10^{-2}$ and $7.9 \cdot 10^{-2}$ and with workers being distributed into groups of medium (25 %) and high risk (75 %). The same indicator created

Table 3

Levels of group occupational risks of negative health outcomes (per classes of diseases) caused by specific indicators of work hardness among workers employed at BPF

Indicators	Likelihood of disease		Additional likelihood	Risk level
	Test group	Reference group		
Diseases of the musculoskeletal system and connective tissue (M00–M99) (g = 0.079)				
Weight of constantly lifted cargo and cargo moved by hand, kg	0.727	0.435	0.292	$2.31 \cdot 10^{-2}$
Uncomfortable working posture, % of time	0.783	0.458	0.324	$2.56 \cdot 10^{-2}$
Working upright, % of time	0.619	0.375	0.244	$1.93 \cdot 10^{-2}$
Diseases of the nervous system (G00–G99) (g = 0.166)				
Weight of constantly lifted cargo and cargo moved by hand, kg	0.697	0.355	0.342	$5.68 \cdot 10^{-2}$
Uncomfortable working posture, % of time	0.783	0.375	0.408	$6.77 \cdot 10^{-2}$
Working upright, % of time	0.556	0.313	0.243	$4.03 \cdot 10^{-2}$
Diseases of the circulatory system (I00–I99) (g = 0.07)				
Weight of constantly lifted cargo and cargo moved by hand, kg	0.485	0.274	0.211	$1.47 \cdot 10^{-2}$
Working upright, % of time	0.429	0.188	0.241	$1.69 \cdot 10^{-2}$
Diseases of the genitourinary system (N00–N99) (g = 0.078)				
Weight of constantly lifted cargo and cargo moved by hand, kg	0.636	0.290	0.346	$2.7 \cdot 10^{-2}$
Uncomfortable working posture, % of time	0.609	0.347	0.261	$2.04 \cdot 10^{-2}$

Table 4

Parameters of models that describe relationships between likelihood of diseases and effects produced by various indicators of work hardness, age and work records

Work hardness indicator	Class of disease	Model parameters			
		b_0	b_1	b_2	p
Weight of constantly lifted cargo and cargo moved by hand, kg	Diseases of the musculoskeletal system and connective tissue (M00–M99)	-2.26	0.00095	0.048	0.0009
	Diseases of the nervous system (G00–G99)	-2.03	0.0011	0.036	0.0013
	Diseases of the genitourinary system (N00–N99)	0.54	-0.0013	0.002	0.002
Uncomfortable working posture, % of time	Diseases of the musculoskeletal system and connective tissue (M00–M99)	-2.08	0.0007	0.045	0.003
	Diseases of the nervous system (G00–G99)	-1.8	0.0008	0.032	0.005
Working upright, % of time	Diseases of the musculoskeletal system and connective tissue (M00–M99)	-1.96	0.0002	0.042	0.015

Table 5

Distribution of workers employed at BPF per levels of individual health risks, abs. (%)

Likely health outcome	Risk level and category						
	0–0.001 Negligible	0.0001–0.001 Low	0.001–0.01 Moderate	0.01–0.03 Medium	0.03–0.1 High	0.1–0.3 Very high	0.3–1 Extremely high
Weight of constantly lifted cargo and cargo moved by hand, kg							
Diseases of the musculoskeletal system and connective tissue (M00–M99)	0	0	0	22 (24.7)	67 (75.3)	0	0
Diseases of the nervous system (G00–G99)	0	0	0	0	76 (85.4)	13 (14.6)	0
Diseases of the genitourinary system (N00–N99)	2 (2.2)	2 (2.2)	1 (1.1)	7 (7.9)	77 (86.5)	0	0
Uncomfortable working posture, % of time							
Diseases of the musculoskeletal system and connective tissue (M00–M99)	0	0	0	17 (19.1)	72 (80.9)	0	0
Diseases of the nervous system (G00–G99)	0	0	0	0	75 (84.3)	14 (15.7)	0
Working upright, % of time							
Diseases of the musculoskeletal system and connective tissue (M00–M99)	0	0	15 (16.9)	74 (83.1)	0	0	0

an individual OR caused by diseases of the nervous system with its value varying between $3.54 \cdot 10^{-2}$ and $16.6 \cdot 10^{-2}$ and with workers being distributed into groups of high (85 %) and very high risk (15 %). And finally, it created an individual OR caused by diseases of the genitourinary system with its value varying between $3.52 \cdot 10^{-9}$ and $5.2 \cdot 10^{-2}$ and with workers being distributed into groups of negligible (2 %), low (2 %), moderate (1 %), medium (8 %) and high (87 %) risk (Table 5).

Long periods of time spent working in an uncomfortable posture and / or upright created an OR associated with diseases of the musculoskeletal system and connective tissue with its value varying between $1.99 \cdot 10^{-2}$ and $7.81 \cdot 10^{-2}$

and with workers being distributed into groups of moderate (17 %), medium (20–83 %) and high (81 %) risk. They also created an OR caused by diseases of the nervous system with its value varying between $4.15 \cdot 10^{-2}$ and $1.64 \cdot 10^{-1}$ and with workers being distributed into groups of high (84 %) and very high (16 %) risk.

Therefore, the accomplished quantitative posterior OR assessment established a relationship between negative health outcomes per classes of diseases and specific indicators of work hardness and determined contributions made by these indicators to the integral risk level. In addition, it allowed identifying relevant groups of workers for subsequent implementation of targeted medical and preventive activities relying on such a criterion as an unacceptable risk level.

Questioning as a method for establishing prevalence of diseases, on one hand, brings about some limitations to a study since it is based on subjective perception of one's health, which can cause both overestimation and underestimation of an actual health state. On the other hand, the questionnaire used in this study is an adapted version of the Nordic Musculoskeletal Questionnaire, which is used worldwide as an optimal instrument not for clinical diagnostics but rather for measuring prevalence of diseases of the musculoskeletal system under various working conditions within epidemiological research [18, 19]. It can supplement available results obtained by periodical medical check-ups.

Effects produced by work hardness on development of diseases (both ODs and WRDs) have been described in literature in detail [20–22]. Leading indicators of work hardness are described in some cases for some occupations [23, 24]. Our results obtained for workers employed at a bearing production facility do not contradict any previously described regularities. Thus, epidemiological analysis established occupational causation of diseases of the musculoskeletal system and connective tissue ('medium correlation', *EF* = 33–50 %), nervous system, circulatory system and genitourinary system ('medium' and 'high' correlation, *EF* = 33–66 % per specific indicators), which is similar to findings reported in other studies [1, 4]. Similar relationships between diseases of the musculoskeletal system and such indicators as 'lifting and moving heavy cargoes' (weighing above safe standards) and 'uncomfortable working posture / working upright' have also been confirmed in foreign studies with presented results of epidemiological research (*RR* = from 1.4 (1.3–1.5) to 4.1 (2.2–7.6) in various occupational groups) [14–16].

The published recommendations on how to use mathematical models make it possible to predict ODs of the peripheral nervous system and musculoskeletal system depending on a class of working conditions (CWC) and work hardness per such indicators as 'stereotype movements under local loads' and 'stereotype movements under regional loads'[8]. However, results of such assessment are limited by using only two indicators and this does not allow considering variable characteristics of work in some specific occupational groups. Moreover, the model includes CWC categories and not actual levels of exposure to an affecting factor, which can vary within one class and to a great extent reflect results obtained by individual assessments. Some other studies have focused on creating models for predicting likelihood of diseases of the circulatory system and musculoskeletal system. They allow assessing individual health risks but do not make it possible to establish a relationship between these diseases and some

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specific indicators of work hardness [25]. The parameters of the Exposure – Work Records – Age – Response relationship that are presented in this study supplement the available mathematical models for individual health risk assessment with such indicators as 'weight of constantly lifted cargo and cargo moved by hand' and 'uncomfortable working posture / working upright'.

The results obtained by quantitative posterior OR assessment established that the group risk level for workers employed at BPF varied from 'medium' to 'high' whereas preliminary prior assessment [17] had earlier established workers' distribution into groups with risks varying from 'low' to 'high'. Additionally estimated individual risk levels vary from 'negligible' to 'very high'. These adjusted data allow creating risk groups of workers which should be considered priority ones as regards provision of targeted medical and preventive activities.

Conclusion. Questioning employed in this study together with subsequent analysis of subjective perception of one's health makes it possible to perform preliminary quantitative posterior OR assessment (considering both likelihood of disease and its severity) at the group and individual level.

In this study, workers employed at a bearing production facility were used as an example in occupational risk assessment. As a result, unacceptable group health risks were established for them that were caused by diseases of the musculoskeletal system and connective tissue, nervous system, genitourinary system and circulatory system and were likely to be associated with work hardness (the risk category varied from 'medium' to 'high'). Such indicators as 'weight of constantly lifted cargo and cargo moved by hand' (35–58 %) and 'uncomfortable working posture / working upright' (29–54 %) made the greatest contributions to the integral risk level. Adjustment of a risk category at the individual level established OR to be caused predominantly by diseases of the musculoskeletal system and connective tissue, nervous system and genitourinary system (its levels varied from 'medium' to 'very high' risk categories).

OR assessment results should be considered when planning group sanitary-technical, organizational, as well as targeted medical and prevention activities.

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Research article

FACTORS AND LEVEL OF THE TOTAL CARDIOVASCULAR RISK FOR PEOPLE IN THE NORTH-EASTERN RUSSIA

I.N. Bezmenova, I.V. Averyanova

"Arktika" Scientific Research Center of the Far East Branch of the Russian Academy of Sciences, 24 Karla Marksa Av., Magadan, 685000, Russian Federation

In northern regions, cardiovascular pathology tends to be more aggressive, is likely to occur at a young age already and often results in disability and even mortality among people who have not yet reached the retirement age.

The aim of this study was to assess the structure and age-specific dynamics of basic factors able to cause cardiovascular diseases in men living in northern regions and the total cardiovascular risk.

We conducted a one-center experimental cross-sectional comparative one-sample study of 116 men living in the north of the Magadan region. The complex study program included questioning, anthropometric examination, laboratory biochemical tests and molecular-genetic screening of candidate genes of essential hypertension (AGT (rs 4762), AGTR1 (rs5186), ADD1 (rs4961), NOS3 (rs2070744), and ACE (rs4340)). Genotyping was performed using real-time polymerase chain reaction. The total cardiovascular risk was calculated using the SCORE scale.

The structure of basic risk factors that can cause cardiovascular diseases in men in the Magadan region includes both modifiable (smoking, obesity, essential hypertension, and dyslipidemia) and non-modifiable ones (climatic-geographic and genetic risk factors) in various age groups. With age, risk factors exert stronger influence on each other thus aggravating the clinical course of cardiovascular diseases and increasing risks of fatal cardiac events. This is especially important for northern regions where people are exposed to such non-modifiable risk factors as extreme climate. Moderate risk of cardiovascular diseases (according to the SCORE scale) was established for all men living in the North already at a young age. Therefore, a key task is to develop a strategy for active prevention of cardiovascular pathology that covers public at large starting from younger age groups.

Keywords: *total cardiovascular risk, genetic risk factors, SNP, cardiovascular prevention, essential hypertension, dyslipidemia, obesity, North.*

Cardiovascular diseases (CVDs) have long been persistently occupying leading places as regards their prevalence not only in Russia but across the globe as well. Most variable measures have been actively implemented to reduce cardiovascular mortality but despite that cardiovascular incidence and mortality rates in young people (younger than 45 years) continue to grow. In 2005–2007, incidence of acute myocardial infarction was 4.3 % in this age group but it grew up to 5.7 % in 2012–2014 and it should be noted that 90 % of patients with this diagnosis were males [1].

CVDs belong to a large group of multifactorial diseases that are caused by combined effects of hereditary and non-hereditary factors. Cardiovascular risks can be altered by modifying burden of some of these factors. The National Russian recommendations on cardiovascular prophylaxis establish that 'factors able to create elevated cardiovascular risks include dyslipidemia, smoking, abdominal obesity, psychosocial factors, and diabetes mellitus; factors able to mitigate them include consuming sufficient amounts of fruit and vegetables and regular physical activity' [2]. Some risk factors for CVDs, such as age, gender, and genetics, cannot be modified, but many others, including hypertension, obesity, diabetes, dyslipidemia, smoking, and air pollution, can be prevented or corrected [3]. RF modification can considerably (up to 75 %)

Irina N. Bezmenova – Candidate of Biological Sciences, Associate Professor, Researcher at the Laboratory of Physiology of Extreme Conditions (e-mail: lependina_bel@mail.ru; tel.: +7 (914) 037-68-50; ORCID: https://orcid.org/0000-0003-3251-5159).

Inessa V. Averyanova – Doctor of Biological Sciences, Head of the Laboratory of Physiology of Extreme Conditions, Chief Researcher (e-mail: inessa1382@mail.ru; ORCID: https://orcid.org/0000-0002-4511-6782).

prevent premature incidence and mortality caused by diseases of the circulatory system [4, 5].

Many various scales have been developed and are actively used to quantify total cardiovascular risk, for example, Framingham Risk Scale, SCORE (Systematic Coronary Risk Evaluation), PROGRAM (Prospective Cardiovascular Munster Study) and others [6]. Use of these scales makes it possible to analyze the whole set of likely risk factors and predict cardiovascular events in future.

In 2023, the WHO Western Pacific Office adopted the Regional Action Framework for Noncommunicable Disease Prevention and Control in the Western Pacific region aimed at promoting and encouraging responsible health behaviors among people living in the region [7]. Promoting healthy lifestyles among population and encouraging people to give up bad habits is a powerful prevention tool able to considerably reduce risks of cardiovascular pathology, diabetes mellitus, and obesity.

In Russia, the Federal Project 'Fighting against Cardiovascular Diseases' has been implemented within the Healthcare National project for a long time. Its major goal is to implement active CVDs prevention at the state level. However, despite the whole wide range of implemented prevention activities, etiological RF, pathogenetic RF and RF causing premature cardiovascular deaths are still prevalent in Russia. And it is absence of any traditions of keeping healthy lifestyles and responsible health behaviors among Russians that can be considered the main reason for wide CVDs prevalence [8].

It is especially vital to resolve health issues of working age population and to preserve labor potential in the Arctic region and areas close to it since there are plans for their active development for the period up to 2035 fixed by the President Order No. 645 dated October 26, 2020. Cardiovascular pathology is known to be more aggressive in northern regions, its onset usually occurs at younger ages and it often results in disability or even premature death among people who have not yet reached the retirement age [9–12]. Men are the most vulnerable population group in this respect. According to B.A. Revich with colleagues (2023), on average, over 2000–2021, cardiovascular mortality among men was higher than the national average in all regions in the Arctic, excluding the Yamal Nenets Autonomous Area. Thus, it was more than 30 % higher in the Murmansk region and Chukotka, more than 20 % higher in the Arkhangelsk region, Magadan region and Karelia, and more than 15 % higher in the Komi Republic and the Nenets Autonomous Area [13].

Higher mortality rates in the North can be due to extremely uncomfortable natural and climatic, geomagnetic and social-demographic conditions. In addition to that, burden of CVDs caused by metabolic risk factors is considerably higher for men than women, which might be associated with protective effects of female hormones and higher prevalence of such risk factors among men [3]. Epidemiology of CVDs and their RF as well as cardiovascular mortality is known to have considerable regional peculiarities [4].

Given all aforementioned, active CVDs prevention for people in the North aimed at creating the most effective mechanisms of health protection and promotion is among primary tasks of contemporary healthcare. J.S. Berger with colleagues suggested selective use of various prevention measures according to the total cardiovascular risk. It is important to reveal a target group with high and extremely high cardiovascular risks among relatively healthy asymptomatic people since they are likely to need some drug correction as well [14].

The aim of this study was to assess the total cardiovascular risk, structure, levels and age-specific dynamics of basic factors able to cause cardiovascular diseases in men living in northern regions.

Materials and methods. To achieve the study goals, the continuous sampling method was employed to create a sample of 116 men living in the North (their average age was 39.4 ± 12.30 years). The study participants were mostly Caucasians; they were considered tentatively healthy and were not relatives; they all had been born in the Magadan region and

lived there ever since, that is, were representatives of the 1st to 3rd generation of the local population. All study participants gave their informed written consent to take part in it and to have their personal data analyzed. The research was conducted in conformity with the ethical principles stipulated by the WMA Declaration of Helsinki (2013) and approved by the Ethics Committee of the "Arktika" Scientific Research Center of the Far East Branch of the Russian Academy of Sciences (the report No. 002/021 dated November 26, 2021).

The examined sample was divided into two age-specific groups, Group 1 made of men aged 20–40 years (59 people) and Group 2 made of men aged 40–65 years (57 people). Data on basic risk factors identified for men living in the Omsk, Ryazan, and Krasnodar regions were used as comparison indicators to identify regional peculiarities in the structure of cardiovascular risk factors typical for men living in the North [15–21]. All examined cohorts were comparable in terms of sex (men), age (20–64 years), ethnicity (Caucasians) and the range of analyzed indicators. All participants took part in questioning aimed at identifying family medical history and presence of CVD RF; they also took part in anthropometric, biochemical and molecular-genetic examinations. Whole blood taken in the morning on empty stomach from the ulnar vein was used as the test material in biochemical and molecular-genetic screening.

Anthropometric examination was conducted prior to lunchtime. The following somatic data were obtained for each participant: body height (cm); body weight; waist circumference; chest circumference. Body mass index (BMI) was calculated according to the conventional procedure (BMI = body weight (kg) / squared height (m) (kg/m^2)). Conventional WHO criteria were employed to identify BMI-specific groups where BMI < 18.5 kg/m^2 is considered underweight; BMI = 18.5–24.9 kg/m^2, normal weight; BMI = 25.0–29.9 kg/m^2, overweight; and BMI > 30 kg/m^2 is considered obesity [22].

To identify functional peculiarities of the cardiovascular system, we measured the participants' blood pressure (BP), systolic (SYS mm Hg) and diastolic (DIA mm Hg), and heart rate (HR, bpm). BP ≥ 130 / 85 mm Hg was considered as elevated normal blood pressure (ENBP). Essential hypertension (EH) was diagnosed at BP values ≥ 140 / 90 mm Hg [23].

The following lipid metabolism indicators were identified in all participants: total cholesterol (TCS, mmol/l), triglycerides (TG, mmol/l), high density lipoproteins cholesterol (HDL, mmol/l) and low density lipoproteins cholesterol (LDL, mmol/l). Atherogenicity index (AI) was calculated per the following formula: AI = (TCS – HDL) / HDL[1]. The following dyslipidemia criteria were employed in accordance with the Russian Recommendations of the 7th Revision issued in 2020 [24] and National Cholesterol Education Program (NCEP) expert report [25]: hypertriglyceridemia at TG > 1.7 mmol/l; hypercholesterolemia at TCS > 5.2 mmol/l; hypoalphacholesterinaemia at HDL < 1.03 mmol/l and at LDL > 3 mmol/l meant the high level of this indicator.

Several SNP of EH candidate genes were examined as genetic cardiovascular risk factors including AGT (rs4762), AGTR1 (rs5186), ADD1 (rs4961), NOS3 (rs2070744), and ACE (rs4340). Genome DNA was extracted for molecular-genetic testing by the standard phenol-chloroform method. The following genotyping was accomplished by the real-time polymerase chain reaction (PCR) using the commercial kits SNP-Screen (Syntol, Russia).

The total cardiovascular risk was calculated using the SCORE scale, which covers sex, age, SYS, smoking status and total cholesterol. The scale is based on results obtained by cohort studies with more than 205 thousand participants that were conducted in 12 European countries, Russia included. The resulting score is likelihood of death due to cardiovascular disease in the next 10 years given in percent. De-

[1] Klimov A.N., Nikul'cheva N.G. Obmen lipidov i lipoproteidov i ego narushenie: rukovodstvo dlya vrachei [Lipid and lipoprotein metabolism and its disruption: guide for physicians]. Saint Petersburg, Piter Kom Publ., 1999, 512 p. (in Russian).

pending on the resulting risk level (in percent), a patient is assigned into one of the following categories: below 5 % means low risk and 5 % and above means high risk. The relative risk scale was used additionally to the SCORE scale for the age group of 20–39 years [26].

The data were statistically analyzed using Statistica 7.0 software package. Quantitative data were given as $M \pm m$ (M is simple mean and m is error of mean). Inter-group differences were estimated using Student's t-test (differences were considered significant at $p < 0.05$). Frequencies of genotypes and alleles were estimated based on the Hardy – Weinberg equilibrium and compared using Pearson's χ^2 test (the equilibrium holds at $p > 0.05$).

Results and discussion. The following risk factors were established for the examined cohort made of male residents of the Magadan region: smoking, elevated normal blood pressure and essential hypertension, obesity (BMI 30 or above), dyslipidemia, significant frequency of genes that increase cardiovascular risks in the gene pool of the examined northern residents.

Smoking men accounted for 16.4 % in the analyzed cohort; higher prevalence of smoking was detected in Group 1 (people aged 20–40 years), 16.9 % against 15.8 % in Group 2 (people aged 40–65 years). However, we believe these figures to be greatly underestimated due to personalized methods applied to perform questioning. Therefore, the results do not give the actual situation with prevalence of smoking in our region. As reported by Yu.V. Barbaruk [27], anonymous questioning established that 42 % of Magadan region residents older than 15 years smoked regularly. Some researchers believe that the Magadan region is among those with the highest number of smokers; however, we compared the same indicators in other regions (the data are given in Figure 1) and as a result, prevalence of smokers in the Magadan region (42 %) turned out to be similar to that established in the Ryazan region (49.2 %) [18] and Krasnodar region (51.6 %) [21] and considerably lower ($p < 0.001$) than prevalence of smokers in the Omsk region (76.8 %) [15].

EH prevalence was established to be 37.72 % in the analyzed sample and this was quite similar to EH prevalence established in other Russian region (Figure 1). Analysis of age-specific dynamics in this factor revealed that the proportion of men with ENBP was 20.34 % (per SYS) and 5.08 % (per DIA) among men younger than 40 years (Group1). In addition to that, despite quite young age of people in this group, prevalence of stage 1 EH amounted to 8.47 % (per SYS) and 11.84 % (per DIA). The proportion of people with ENBP per SYS was a bit lower in Group 2 (aged 40–65 years) and amounted to 14.55 % but it was significantly higher per DIA, 16.36 %. The proportions of men with EH per both SYS and DIA grew significantly in Group 2 and amounted to 29.09 and 27.28 % respectively.

Tension in the cardiovascular system functioning was estimated in two analyzed groups of male northern residents as the sum of frequency of people with ENBP and stage 1 EH per SAS and DIA. As a result, its prevalence amounted to 45.73 % in Group 1 (people aged 20–40 years); it was significantly higher in Group 2 (people aged 40–65 years) where it was 87.28 %. We believe that higher frequency of ENBP and EH and generally higher prevalence of elevated BP in Group 2 (men aged 40–65 years) reflects age-specific dynamics and can also be due to significantly higher body weight in this group.

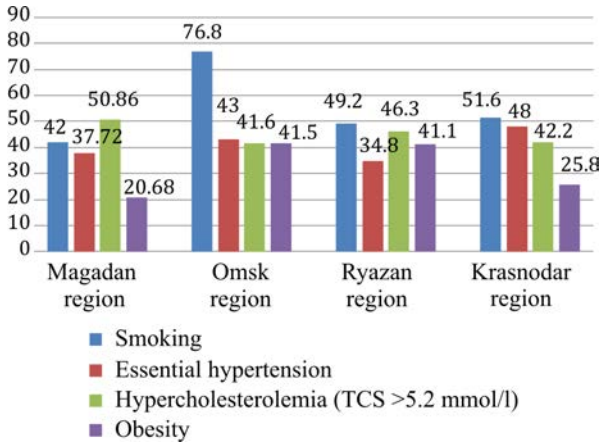

Figure 1. Prevalence of main cardiovascular risk factors in several Russian regions, %

The average BMI value is 26.9 ± 0.38 in the analyzed group of male northern residents, which is classified as overweight. Forty-one point seven percent of the examined men turned to be overweight. Obesity prevalence is 21.8 % in the analyzed population and the figure is comparable with its prevalence in the Krasnodar region and considerably lower than obesity prevalence in the Omsk (p = 0.001) and Ryazan regions (p = 0.002).

Analysis of sex-specific dynamics of unhealthy body weight in the analyzed group of male northern residents established overweight in 32.76 % among men from Group 1 (20–40 years old); Class 1 obesity was detected in 12.07 %; Class 2 obesity, in 3.45 %. Issues of unhealthy weight tend to aggravate with age in the analyzed cohort and there is a significant growth in BMI values deviating from its safe range. Thus, overweight was identified in 47.37 % of men from Group 2 (aged 40–65 years); Class 1 obesity, in 22.81 %; Class 2 obesity, in 5.26 %.

Hypercholesterolemia prevalence was the highest in the cohort of male Magadan region residents in comparison with their peers from the Omsk, Ryazan and Krasnodar region and amounted to 50.86 % (Figure 1). The reference group included populations from regions with various climatic and geographic conditions, including those located in the southern and central parts of Russia. The Magadan region is located in the north-eastern Russia and is considered an Arctic area. We believe that such a high proportion of people with elevated TCS levels in blood may indicate that metabolism of Caucasian immigrants had to adapt to this new environment and switch from its traditional protein-carbohydrate variant to a 'northern' or protein-lipid one. It is reported in literature sources that this switch usually leads to growing TCS levels in blood and energy support for adaptation is predominantly provided by lipids[2]. Table 1 characterizes lipid profile indicators in the examined groups of northern residents.

Analysis of age-specific dynamics of lipid profile established that total cholesterol levels, low density lipoproteins and AI values were already at the upper limit of the safe range already in young northern residents and the issue only aggravates with age. We established high prevalence of dyslipidemia in the analyzed groups of male northern residents (Figure 2).

The following RFs were identified in the structure of dyslipidemia prevalence in Group 1 (young men): hypercholesterolemia (30.51 %), elevated LDL levels (30.51 %), hypoalphaholesterolemia (8.48 %), and hypertriglyceridemia (10.71 %) Dyslipidemia prevalence was significantly higher in Group 2 (men aged 40–65 years) and more than a half of men in it had hypercholesterolemia (59.65 %), elevated LDL levels (61.40 %), hypoalphaholesterolemia (10.53 %), and hypertriglyceridemia (29.82 %). Atherogenicity index (AI) is widely used in calculating risks of deaths caused by atherosclerosis-associated

Table 1

Lipid profile indicators in the analyzed groups of northern residents ($M \pm m$)

	Total cholesterol, mmol/l	High density lipoproteins, mmol/l	Low density lipoproteins, mmol/l	Triglycerides, mmol/l	Atherogenicity index, arbitrary units
Group 1 (aged 20–40 years)	4.98 ± 0.14	1.31 ± 0.04	3.21 ± 0.12	0.99 ± 0.08	2.70 ± 0.11
Group 2 (aged 40–65 years)	5.75 ± 0.13	1.33 ± 0.05	3.79 ± 0.11	1.43 ± 0.10	3.60 ± 0.17
Significance of differences	p = 0.000	p = 0.691	p = 0.001	p = 0.001	p = 0.000

[2] Kaznacheev V.P., Kulikov V.Yu., Panin L.E., Sokolov V.P., Lyakhovich V.V., Shorin Yu.P., Mayanskii D.N. Mekhanizmy adaptatsii cheloveka v usloviyakh vysokikh shirot [Human adaptation mechanisms in high latitudes]. Leningrad, Meditsina Publ., 1980, 200 p. (in Russian).

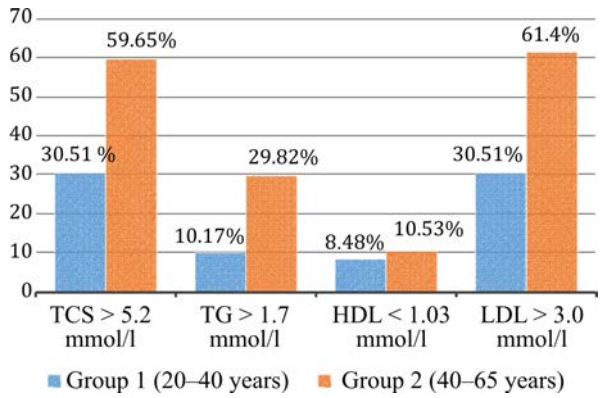

Figure 2. Dyslipidemia prevalence in the analyzed groups of male northern residents

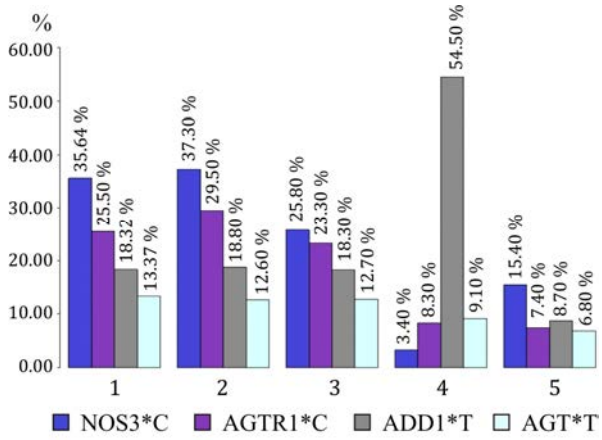

Figure 3. Prevalence of EH candidate genes in various populations: 1, northern male residents of the Magadan region; 2, Caucasian population; 3, American population; 4, Asian population; 5, African population

diseases as an effective prognostic marker. Comparative analysis of the Group 1 and 2 established this indicator to be higher than its reference level in Group 2 (men aged 40–65 years) and was also significantly higher than in Group 1 (men aged 20–40 years).

The present study is the first to analyze such a non-modifiable risk factor as prevalence of genes that increase cardiovascular risks. Frequency of alleles and genotypes in two analyzed groups corresponds to the Hardy – Weinberg equilibrium ($p > 0.05$). We established that frequency of alleles creating elevated risks of essential hypertension is *ADD1**T = 15.6 %, *AGT**T = 17.26 %, *AGTR1**C = 24.22 %, *NOS3**C = 33.63 % and *ACE**D = 49.54 % in the general male population in the Magadan region. Frequency of genes involved into blood pressure regulation has certain ethnic peculiarities; given that, we compared the analyzed population made of male northern residents with some basic world populations[3] (Figure 3). The analyzed population was found to demonstrate the Caucasian type of gene frequency as per the analyzed polymorphisms.

Table 2 provides data obtained by genetic-molecular testing performed in the analyzed groups.

Although genetic risk factors cannot be modified, they should be always considered when assessing the total cardiovascular risk. It is noteworthy that we did not establish any authentic differences in prevalence of the analyzed polymorphisms between the male northern population of the Magadan region and Caucasians living in much more comfortable environment. We believe this indicates that

Table 2

Prevalence of EH candidate genes in the analyzed groups of male northern residents

Gene	Frequency of alleles (in %) that increase cardiovascular risks	
	Group 1 (20–39 years old)	Group 2 (40–65 years old)
*AGTR1*C* (rs5186)	26.36	25.93
*AGT*T* (rs4762)	20.00	17.59
*NOS3*C* (rs2070744)	28.18	30.56
*ADD1*T* (rs4961)	14.29	16.98
*ACE*D* (rs4340)	43.64	55.56

[3] National Library of Medicine: The National Center for Biotechnology Information. Available at: https://www.ncbi.nlm.nih.gov/snp (February 01, 2024); ALFRED – the ALlele FREquency Database. Available at: https://alfred.med.yale.edu/ALFRED/index.jsp (February 01, 2024); SNPedia: Database catalogs of single nucleotide polymorphisms. Available at: https://www.snpedia.com/index.php/SNPedia (February 01, 2024).

early CVD onset and progressing in the North may be caused by a predominant contribution made by much more uncomfortable natural, climatic and geomagnetic environmental factors as well as peculiar lifestyles and not only by genetic RFs.

The 10-year cardiovascular risk was calculated per the SCORE scale using the parameters of the analyzed population obtained in the present study. The results were as follows:

– moderate risk (1–4 % per the SCORE scale) was detected in 100 % of the cases in Group 1 (people aged 20–39 years);

– 61.40 % of the respondents in Group 2 (people aged 40–65 years) had moderate risk (1–4 % per the SCORE scale) of fatal cardiovascular events over the next 10 years;

– frequency of high (5–9 % per the SCORE scale) and very high (> 10 % per the SCORE scale) risk of fatal cardiovascular events over the next 10 years amounted to 17.54 and 7.02 % respectively in Group 2 (people aged 40–65 years).

Smoking is a significant cardiovascular risk factor; this fact is evidenced by the total cardiovascular mortality risk growing fivefold in case this RF is present in the age group younger than 50 years [28]. The problem remains the most acute in our country since the population in Russia is among 'the most smoking' ones across the globe [29].

Elevated systolic blood pressure (SYS) or essential hypertension (EH) are the most significant risk factors of not only CVD-related disability but also cardiovascular mortality, both in Russia and all over the world [30]. The global trend of growing essential hypertension prevalence is obviously determined by not only population ageing but also growing influence of lifestyle-related risk factors including unhealthy diets and hypodynamia. According to the ESSE-RF-2 Study data, practically half of the Russian working age men (49.1 %) have EH [31]. Analysis of cardiovascular mortality rates gives evidence of their considerable variability per RF regions. For example, in 2021, the standardized cardiovascular mortality rate was 457.5 per 100 thousand people in Moscow whereas it was somewhat higher in the Magadan region equaling 583.8[4].

Obesity is not only a cardiovascular risk factor but also a factor that can influence or even aggravate other risk factors, including essential hypertension, dyslipidemia and diabetes mellitus, by various pathways [32–34]. Over the last 50 years, obesity prevalence has grown up to a global non-communicable pandemic since practically each fifth adult on the planet is overweight. In Russia, incidence of obesity-related EH has been growing among working age men (!) [2]. Thus, 14.3 % of Russian men aged 25–34 years are obese and the proportion grows steadily with age reaching 36.3 % by the age of 55 years [35].

Some experts report a strong positive correlation between TCS and LDL levels (within a wide range of concentrations) and cardiovascular risks[5]. The number of deaths associated with elevated LDL levels in patients with CVDs is predicted to reach 3.1 million by 2029 [2]. According to the ESSE-RF-2 Study data, the total cholesterol level is higher than 5 mmol/l in more than a half of the Russian population [36].

All the foregoing risk factors were present, though with various intensity of their manifestation, in the analyzed groups of male northern residents.

To verify the study findings, we analyzed the mortality structure due to diseases of the circulatory system in the analyzed groups of male northern residents living in the Magadan region over 2016–2019 (2020 and 2021 were excluded from the analysis due to the COVID-19 pandemic). The analysis was performed for the

[4] Demograficheskii ezhegodnik Rossii 2021: stat. sb. [The Annul Russia Demography Bulletin 2021: statistical data collection]. Moscow, Rosstat, 2021, 167 p. (in Russian).

[5] Neaton J.D., Blackburn H., Jacobs D., Kuller L., Lee D.J., Sherwin R., Shih J., Stamler J., Wentworth D. Serum cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. Multiple Risk Factor Intervention Trial Research Group. *Arch. Intern. Med.*, 1992, vol. 152, no. 7, pp. 1490–1500.

following blocks: deaths caused by hypertensive diseases (ICD-10 codes 110–114); deaths caused by ischaemic heart diseases (ICD-10 codes 120–125) with separate analysis of deaths caused by myocardial infarctiona (ICD-10 codes 121–122); as well as prevalence of deaths caused by cerebrovascular diseases (ICD-10 codes 160–169). The analyzed data were stratified by age with a 10-year step (Figure 4).

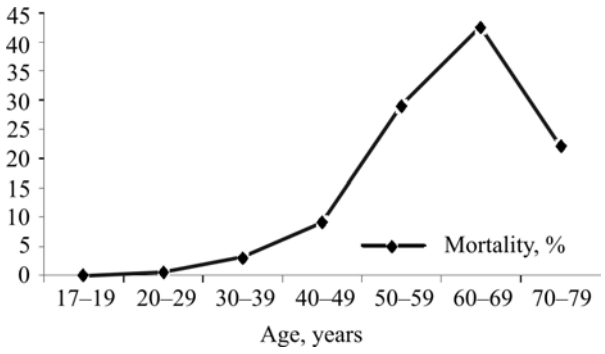

Figure 4. Age-specific cardiovascular mortality among men in the Magadan region in dynamics

The analysis of this mortality graph shows that cardiovascular mortality among males in the Magadan region starts to grow exactly in the age group of 20–29 years (the mortality rate is 0.6 %); an apparent growth in cardiovascular mortality occurs in the age group of 40–49 years (9 %) and the peak is reached in the age group of 60–69 years (the cardiovascular mortality is 42.4 %). The calculated data and the data on actual cardiovascular mortality rate seem to be quite consistent.

Conclusion. Modifiable cardiovascular risk factors identified for male northern residents of the Magadan region include smoking, obesity, EH, and dyslipidemia. All the examined male northern residents had moderate cardiovascular risk (1–4 % per the SCORE scale) already at a young age (20–40 years); with age, some men appeared to have high (5–9 % per the SCORE scale) and very high (> 10 % per the SCORE scale) cardiovascular mortality risk for the next 10 years with their prevalence being 17.54 and 7.02 % respectively.

Very high hypercholesterolemia prevalence (50.86 %) in male northern residents may be considered a regional peculiar cardiovascular risk factor. This is consistent with the concept described by V.P. Kaznacheev and others about occurrence of ‘northern’ metabolism as an adaptation response in immigrants to the specific climatic conditions in the North[6] . Another regional risk factor is a significant growth in BMI values deviating from their safe range with age.

The study findings on prevalence and age-specific dynamics of cardiovascular risk factors in male northern population emphasize the necessity to implement active prevention of cardiovascular diseases, which should start already at a young age and be aimed at reducing cardiovascular mortality. Prevention activities should be based on modifying all existing risk factors, active promotion of healthy lifestyles among population and creating suitable conditions for pursuing them.

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Competing interests. The authors declare no competing interests.

[6] Kaznacheev V.P., Kulikov V.Yu., Panin L.E., Sokolov V.P., Lyakhovich V.V., Shorin Yu.P., Mayanskii D.N. Mekhanizmy adaptatsii cheloveka v usloviyakh vysokikh shirot [Human adaptation mechanisms in high latitudes]. Leningrad, Meditsina Publ., 1980, 200 p. (in Russian).

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Research article

PRECONCEPTION MATERNAL EXPOSURE: RISK OF FETAL AND INFANT LOSSES

S.F. Sosnina, P.V. Okatenko, M.E. Sokolnikov

Southern Urals Biophysics Institute, 19 Ozyorskoe shosse, Ozyorsk, 456783, Russian Federation

Safety of female personnel exposed to occupational radiation is still a topical issue of radiation epidemiology. Mayak Production Association is the first Russian atomic enterprise and women made a quarter of its personnel. Fetal and infant losses (FILs) that include stillbirths and infant mortality could be used as an important criterion for assessing the effects of maternal preconception (prior to conception) exposure.

The aim of this study was to assess FILs risk among the offspring of female Mayak PA workers exposed to occupational preconception external gamma-radiation.

A retrospective analysis was performed among 15,307 children born in 1949–1973; mothers of 4880 of them were Mayak PA workers. FILs were analyzed taking into account sex of the offspring, period of their birth, nosologies, parental age, and dose categories of preconception exposure. Methods of non-parametrical statistics were used and calculation of relative risk was performed with 95 % confidence interval.

In general, fetal and infant losses demonstrated no statistical differences, 44.5 for 10^3 in the main group, 38.7 for 10^3 in the reference group, χ^2 =2.79, p = 0.95. A statistically significant increase of FILs, stillbirths and infantile mortality was detected among the offspring with only their mothers working at Mayak PA. Dynamics analysis established the period from 1949 to 1953, in which FILs, stillbirths and infantile mortality were higher among the offspring of exposed mothers. Statistically significant differences in the FILs structure were obtained for fetal death that was more often registered in the main group, 3.48 vs 1.34 for 10^3, χ^2 = 7.54, p = 0.006. FILs risk among mothers working at Mayak PA aged under 20 was statistically significantly higher for girls, 2.42 (1.25–4.67), and for both sexes, 2.16 (1.37–3.4). FILs were associated with the dose range of preconception external gamma-radiation of mothers from 0.16 mGy to 3006 mGy. The study also established certain categories of preconception exposure of the ovaries with significantly higher stillbirth risk in the main group as opposed to the reference one.

Keywords: *fetal and infant losses, stillbirths, infant mortality, offspring of exposed individuals, radiation, preconception exposure, dose to the gonads, external gamma-radiation, Mayak PA.*

Research of unfavorable health effects on the offspring of workers employed in the area of exposure to anthropogenic sources of ionizing radiation is of great scientific and practical interest for the purposes of regulating hygienic standards at radiation-hazardous facilities. A problem of radiation safety of female workers at nuclear energy enterprises, in particular for their reproductive health, is one of the topics for discussion.

The results of epidemiological studies of unfavorable pregnancy outcomes in women who had preconception (before conception) contact with the sources of ionizing radiation, are quite contradictory. Thus, researches among survivors of the atomic bombings in Hiroshima and Nagasaki pointed at increased frequency of unfavorable pregnancy outcomes in the form of severe congenital malformations and perinatal mortality of the offspring, although no statistically significant estimates of the direct effect of radiation was obtained [1]. A retrospective analysis in the cohort of the offspring born to mothers exposed to radiation therapy due to oncology in childhood, demonstrated a significant in-

Svetlana F. Sosnina – Candidate of Medical Sciences, Senior Researcher of Radiation Epidemiology Laboratory (e-mail: sosnina@subi.su; tel.: +7 (35130) 716-52; ORCID: https://orcid.org/0000-0003-1553-0963).

Pavel V. Okatenko – Head of the group on computational hardware and software at Radiation Epidemiology Laboratory (e-mail: okatenko@subi.su; tel.: +7 (35130) 769-03; ORCID: https://orcid.org/0000-0002-8260-1808).

Mikhail E. Sokolnikov – Doctor of Medical Sciences, Head of Epidemiology Department (e-mail: sokolnikov@subi.su; tel.: +7 (35130) 716-52; ORCID: https://orcid.org/0000-0001-9492-4316).

crease of stillbirth and neonatal death rates in case of exposure of the pelvic organs in accumulated doses over 10 Gy [2]. Considering maternal health as a factor for the formation of child health, Yu.E. Shmatova et al. [3] note that 'effect of harmful working conditions of future mothers (ionizing radiation, work on the conveyer, etc.) during a year before child's birth is a risk factor for retrogression of children's health group in future.

At the same time, M.P. Little et al. [4] do not find clear transgenerational effects following radiation exposure of parents in the cohorts of children born to liquidators of the Chernobyl accident consequences, of the offspring of workers who were in contact with radiation at workplaces and were exposed to medical (diagnostic and therapeutic) radiation. Up to date, no final conclusion related to the effects of mothers' exposure to the health of their offspring was obtained; so the issues of safety of radiation exposure for female organism require further research [5].

Mortality rates are among the most frequently analyzed in the course of epidemiological assessment of potential effects of radiation exposure [6]. However, the analysis of infant mortality has a special place due to exceptional demographic importance being one of the most significant indicators of the population health [7]. Fetal and infant losses (FILs) including stillbirth rate and mortality under the age of one year, belong to a subtle social and cultural indicator of the society [8].

Until recently, perinatal and infant mortality was assessed separately by medical statistics which made it impossible to carry out a comprehensive assessment of infant mortality [9]. An integrated indicator of fetal and infant losses expands the opportunities for the analysis and makes it possible to consider it as a valuable tool for assessing the effects of radiation exposure.

Mayak Production Association (PA) is the first production facility of Russian atomic energy industry that is in operation since 1948. The Mayak PA worker cohort is notable for high specific weight of female personnel (25 %) exposed to occupational radiation at reproductive age, which is not typical for other cohorts of nuclear industry workers in the world.

The aim of the work: risk assessment of fetal and infant losses among children of Mayak PA female workers exposed to occupational preconception radiation.

Material and methods. Information resources for the study included SUBI archived documents containing data on the Mayak PA regular personnel [10], on the population of the Closed Administrative Territorial Unit (CATU) Ozyorsk located near the facility [11], health and social data from medical records [12], data on death cases [13].

The 'Mayak Worker Dosimetry System – 2013' was the main source of data on individual doses of occupational exposure of Mayak PA personnel [14]. Accumulated absorbed doses of external gamma-radiation to gonads were estimated.

Criteria for the formation of the studied group of offspring (the main group) were the following: a child was born in the CATU in 1949–1973; the mother was a worker at Mayak PA main production facilities. Criteria for the formation of the comparison group were: a child was born in the CATU in 1949–1973; the parents were not resettled from radioactively contaminated areas, were not exposed to occupational radiation before conception (were not among regular personnel, were not military men or builders who were involved in working at a nuclear facility, were not liquidators of radiation accidents' consequences). So the main group included 4880 individuals: 2552 boys (52.3 %) and 2328 girls (47.7 %). Among them, 2768 children had both parents who were Mayak PA workers; as for the remaining 2112 children, only mothers worked at Mayak PA. The comparison group included 10,427 individuals: 5301 boys (50.8 %) and 5126 girls (49.2 %).

Comparability of the groups was achieved by the fact of birth in the CATU, balanced sex ratio, common birth period which supposed the same level and quality of medical care and the same climatic and geographical living conditions for children.

The cause-of-death assessment was carried out according to the 'International Statistical Classification of Diseases and Related Health Problems'.

Fetal and infant losses were estimated as a ratio of the number of children who were stillborn and died before the age of one year to 10^3 of births, both live and stillborn. The stillbirth rate was estimated as a ratio of the number of stillborn children to 10^3 of births, live and stillborn. During the study period 1949–1973 the following criteria for stillbirth were accepted in USSR: birth after 28 weeks of gestation; the fetal size is at least 35 cm and weight is at least 1,000 g; lack of self-sustained breathing. In future, these criteria for stillbirth have changed significantly [15]. Infant mortality rate (from birth to 12 months) is calculated as the number of death cases at the first year of life to 10^3 of live born children.

The FILs analysis was carried out taking into account sex, 5-year periods of childbirth, 5-year age categories of mothers and fathers at the date of childbirth, dose ranges of external gamma-radiation exposure to ovaries before conception.

The software package STATISTICA Version 10 (StatSoft, USA) was used for statistical analysis of the data. When comparing the rates by frequency, nonparametric statistical methods (Fischer's exact test and Pearson's chi-squared test) were used; the differences in the groups were considered as statistically significant at $p < 0.05$. The relative risk (RR) was calculated with 95 % confidence interval (CI).

Results and discussion. A total of 217 death cases that could be regarded as FILs were registered in the main group in the period 1949–1974: 132 cases (60.8 %) were boys, 85 cases (39.2 %) were girls. This number in the comparison group reached 404: 224 cases (55.4 %) were among boys, 180 cases (44.6 %) – among girls. Analysis of FILs frequency in the groups is presented in Table 1.

Generally, no statistically significant differences of FILs frequency were registered in the groups: 44.5 for 10^3 in the main group, and 38.7 for 10^3 in the comparison group, $\chi^2 = 2.79$, $p = 0.95$. A lower frequency of FILs was registered among the offspring with both parents working at Mayak PA compared to the comparison group (30.7 vs 38.7 for 10^3, respectively). Significant differences in this analysis were obtained for girls as FILs frequency in the main group was lower: 22.3 vs 35.1 for 10^3, $\chi^2 = 5.6$, $p = 0.02$.

Table 1

Frequency of fetal and infant losses in the groups

Offspring sex	Main group						Comparison group, $n = 10427$	
	Exposed mother only, $n = 2112$		Both parents exposed, $n = 2768$		Total number of offspring of mothers, $n = 4880$			
	Absolute	for 10^3	Absolute	for 10^3	Absolute	for 10^3	Absolute	for 10^3
Stillbirths								
Both sexes	33/2112	15.6[2]	26/2768	9.4	59/4880	12.1[2]	82/10427	7.9
Boys	22/1131	19.4[2]	15/1421	10.6	37/2552	14.5[2]	41/5301	7.7
Girls	11/981	11.2	11/1347	8.2	22/2328	9.4	41/5126	8.0
Infant mortality[1]								
Both sexes	99/2079	47.6[2]	59/2742	21.5[2]	158/4821	32.8	322/10345	31.1
Boys	55/1109	49.6[2]	40/1406	28.4	95/2515	37.8	183/5260	34.8
Girls	44/970	45.4[2]	19/1336	14.2[2]	63/2306	27.3	139/5085	27.3
Fetal and infant losses								
Both sexes	132/2112	62.5[2]	85/2768	30.7	217/4880	44.5	404/10427	38.7
Boys	77/1131	68.1[2]	55/1421	38.7	132/2552	51.7	224/5301	42.3
Girls	55/981	56.1[2]	30/1347	22.3[2]	85/2328	36.5	180/5126	35.1

Note: [1] – calculated against the number of live born children; [2] – statistically significant differences.

Meanwhile, comparing FILs frequency among the offspring with only mothers working at Mayak PA a significant increase was found in comparison to the comparison group as a whole: 62.5 vs 38.7 for 10^3, $\chi^2 = 24.2$, $p < 0.00001$; when considering these values taking sex into account, 68.1 and 42.3 for 10^3 among boys, $\chi^2 = 13.94$, $p = 0.0002$; 56.1 and 35.1 for 10^3 among girls, $\chi^2 = 9.8$, $p = 0.002$.

A detailed comparative analysis of structural components of FILs in the groups revealed the following characteristic features. Stillbirth rate in the main group was statistically significantly higher compared to the comparison group for boys (14.5 vs 7.7 for 10^3, $\chi^2 = 8.02$, $p = 0.005$) and for both sexes (12.1 vs 7.9 for 10^3, $\chi^2 = 6.5$, $p = 0.011$). More significant differences were obtained when comparing mortality rates of the offspring with only mothers exposed to occupational radiation: for males (19.4 vs 7.7 for 10^3, $\chi^2 = 13.2$, $p = 0.0003$) and for both sexes (15.6 vs 7.9 for 10^3, $\chi^2 = 11.64$, $p = 0.0006$).

Divergent results were obtained when assessing infant mortality. No statistically significant differences were found for the entire main group ($\chi^2 = 0.29$, $p = 0.59$), infant mortality was higher in the comparison group than in the group of offspring with both parents working at Mayak PA ($\chi^2 = 7.1$, $p = 0.008$). At the same time, the rate of infant mortality was significantly higher than in the comparison group among children with only mothers exposed to occupational radiation: for both sexes, 47.6 vs 31.1 for 10^3, $\chi^2 = 14.4$, $p = 0.0002$; for boys, 49.6 vs 34.8 for 10^3, $\chi^2 = 5.58$, $p = 0.018$; for girls, 45.4 vs 27.3 for 10^3, $\chi^2 = 9.03$, $p = 0.003$.

Finally, among the offspring with only mothers working at Mayak PA analysis of mortality rate at age less than 1 year revealed a significant increase of stillbirths, infant mortality and FILs as a whole.

Assessment of relative risk of FILs indicated statistically significant differences with the comparison group for the offspring with only mothers working at Mayak PA: risk was higher for boys (1.61; 95 % CI: 1.25–2.07), for girls (1.6; 1.2–2.14) and for both sexes (1.61; 1.33–1.95). Similar results for the offspring with only mothers exposed to occupational radiation were obtained when analyzing structural components of FILs that indicated higher risk assessments for stillbirths: for boys (2.52; 1.5–4.2) and for both sexes (1.99; 1.33–2.97); and higher risk of infant mortality: for boys (1.43; 1.1–1.9), for girls (1.66; 1.2–2.3) and for both sexes (1.53; 1.23–1.91).

FILs dynamics by 5-year periods of childbirth is presented at Figure 1.

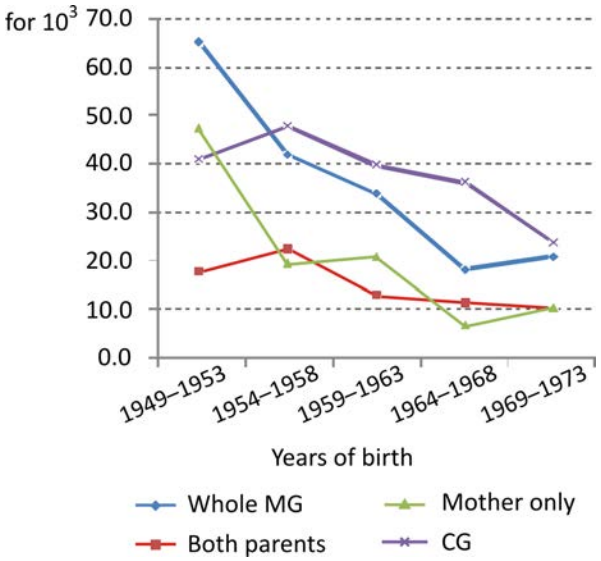

Figure 1. FILs dynamics by periods of childbirth: MG – main group, CG – comparison group

FILs frequency in both groups was the highest in the first years of the follow up period with a rather sharp decrease by 1969–1973 that was especially characteristic for the main group, from 64.4 to 20.5 for 10^3. A more gradual curve was seen in the comparison group with mortality decrease from 47.2 to 23.5 for 10^3. FILs rate among the offspring with both parents working at Mayak PA ranged from 22.1 to 10.3 for 10^3 with a peak in 1954–1958. At the same time, FILs rate dynamics was parallel to the rates of the whole main group among the offspring with only mothers working at Mayak PA and was within the range from 46.8 to 6.7 for 10^3.

Assessment of FILs relative risk in relation to periods of birth of the children (Table 2) indicated statistically significant differences in 1949–1953 when mortality rate was higher in

Table 2

Fetal and infant losses taking into account periods of childbirth

Period	Types of mortality	Main group		Comparison group		RR	[95 % CI]	Significance level, *p*
		Abs.	for 10^3	Abs.	for 10^3			
1949–1953	Stillbirth	25/1645	15.2*	15/3884	3.9	3.94	2.08–7.45	< 0.0001
	Infant	81/1620	50.0*	142/3869	36.7	1.36	1.04–1.78	0.023
	FILs	106/1645	64.4*	157/3884	40.4	1.59	1.25–2.03	0.0001
1954–1958	Stillbirth	22/1718	12.8	29/2500	11.6	1.1	0.64–1.92	0.73
	Infant	49/1696	28.9	89/2471	36.0	0.8	0.57–1.13	0.21
	FILs	71/1718	41.3	118/2500	47.2	0.87	0.66–1.17	0.3
1959–1963	Stillbirth	12/779	15.4	13/1429	9.1	1.69	0.77–3.69	0.18
	Infant	14/767	18.3	43/1416	30.4	0.6	0.33–1.09	0.095
	FILs	26/779	33.4	56/1429	39.2	0.85	0.54–1.35	0.49
1964–1968	Stillbirth	–/446	–	14/953	14.7	–	–	-
	Infant	8/446	17.9	20/939	21.3	0.84	0.37–1.9	0.68
	FILs	8/446	17.9	34/953	35.7	0.5	0.24–1.1	0.08
1969–1973	Stillbirth	–/292	–	11/1661	6.6	–	–	-
	Infant	6/292	20.5	28/1650	17.0	1.21	0.51–2.9	0.67
	FILs	6/292	20.5	39/1661	23.5	0.87	0.37–2.05	0.76

Note: * – statistically significant differences.

the main group not only regarding FILs rates as a whole (1.59; 95 % CI: 1.25–2.03) but for separate structural components as well: for stillbirths (3.94; 2.08–7.45) and infant mortality (1.36; 1.04–1.78).

A detailed analysis of the period 1949–1953 indicated a higher risk of FILs and its components among the offspring with only mothers working at Mayak PA compared to the comparison group: FILs (1.91; 1.46–2.51), stillbirths (3.06; 1.41–6.65), infant mortality (1.81; 1.35–2.42). It should be emphasized that the period of 1949–1953 years refers to the development stage of Mayak PA and could be characterized by high doses for personnel due to the standards of that period, imperfect means of individual protection and extreme deadlines for state order completion.

The structure of fetal and infant losses didn't differ a lot within the groups (Figure 2). The main causes of death included "Certain conditions originating in the perinatal period" (16.2 for 10^3 in the main group, 15.3 for 10^3 in the comparison group, $\chi^2 = 0.15$, $p = 0.69$), infectious diseases (11.9 and 10.2 for 10^3 respectively, $\chi^2 = 0.93$, $p = 0.34$), and "Respiratory diseases" (6.4 for 10^3 in the main group, 6.6 for 10^3 in the comparison group, $\chi^2 = 0.04$, $p = 0.85$). No statistically significant differences were obtained when comparing these structural types.

In the class "Pregnancy, childbirth and the puerperium" as an element of FILs an early fetal death was statistically significantly more often registered in the main group: 3.48 vs 1.34 for 10^3 in the comparison group, $\chi^2 = 7.54$, $p = 0.006$. This result corresponds to an earlier research [16] that indicated domination of certain conditions originating in the perinatal period and early fetal death in the structure of stillbirths among the offspring of mothers working at Mayak PA.

Frequency of congenital malformations as a cause of death at age under 1 year was insignificantly higher in the comparison group than in the main group: 2.5 vs 1.23 for 10^3, $\chi^2 = 2.55$, $p = 0.11$. Congenital malformations of the circulatory system, mostly of cardiac chambers

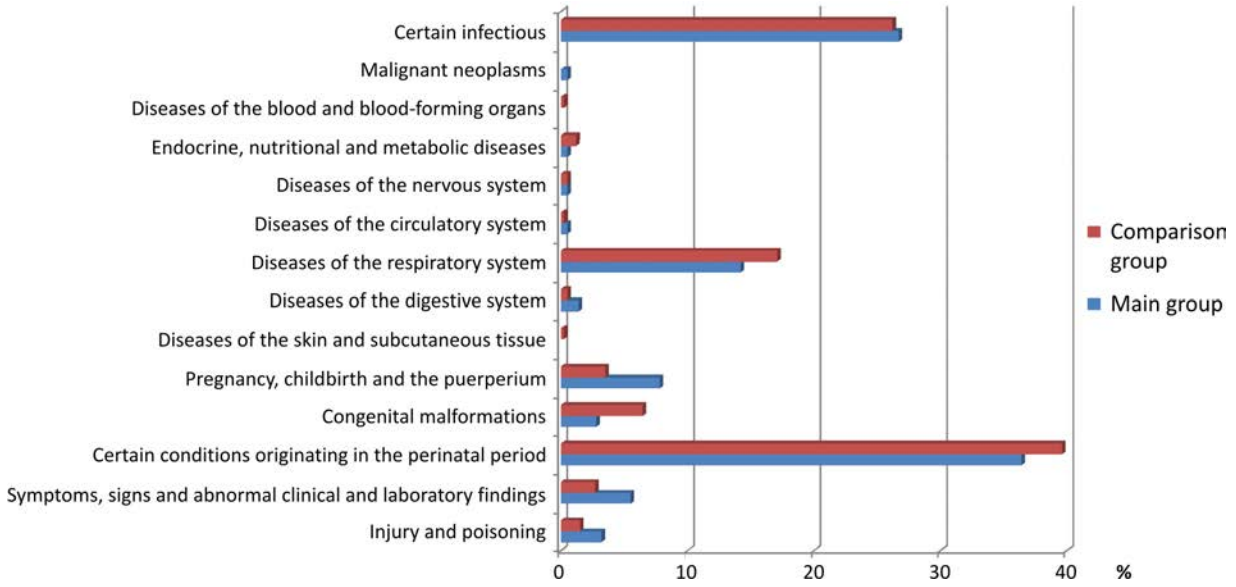

Figure 2. Structure of fetal and infant losses in the groups

and connections, were most often registered in both groups; single cases of congenital hydrocephalus and multiple congenital malformations were indicated.

Death case of a 3-month boy due to leukemia of unspecified cell type was observed in the main group; his parents were workers of a radiochemical plant of Mayak PA: external gamma-exposure dose to the ovaries prior to conception made 5.08 mGy, dose to the testicles – 385.8 mGy. An earlier analysis in the cohort of the offspring of Mayak PA workers indicated "no statistical relation between the factor of parental preconception exposure and oncological and hematological pathology in their offspring" [17]. No malignant neoplasms as FILs causes were registered in the comparison group.

Other classes of diseases as main causes of death in the FILs structure were observed sporadically with no significant differences.

Assessment of fetal and infant losses in relation to maternal and paternal age at childbirth is presented in Table 3.

Maternal age of 21–25 was the most representative in both groups: 37.4 % (1827 children) in the main groups and 39.9 % (4167) in the comparison group; as for paternal age, 26–30 years was the most representative: 39.3 % (1917) and 35.3 % (3675), respectively. The highest rate of FILs in the main group was observed among girls born to the youngest mothers, 85.0 for 10^3; in the comparison group, for girls born to mothers aged 36 and older: 59.9 for 10^3.

As for paternal age, the highest FILs rate was observed in the main group among young fathers (69.8 for 10^3), in the comparison group, among fathers aged 31–35 years (46.9 for 10^3). Statistically significant differences were found only for the youngest mothers of the main group; FILs risk among them was more than twice higher than in the comparison group: 2.42 (1.25–4.67) for girls and 2.16 (1.37–3.4) for both sexes. It should be noted that all FILs cases among live born girls in this maternal age category were observed in the families where only mothers were Mayak PA workers. No statistically significant differences were registered in other categories of maternal and paternal age.

Characteristics of preconception occupational exposure of the main group parents are presented in Table 4.

It was indicated that FILs cases related to the range of accumulated doses of preconception exposure of mothers in the range of 0–3006.3 mGy and 0–3987.5 mGy of fathers while the range of preconception doses in the whole main group was much wider: 0–4075.6 mGy to the ovaries, 0–5440.9 mGy to the testicles. FILs were most often accompanied by

Table 3

FILs risk in relation to parental age at childbirth

Parental age	Offspring sex	Main group, $n = 4880$			Comparison group, $n = 10{,}427$			RR	95 % CI
		Abs.	Number of children in the group[1]	for 10^3	Abs.	Number of children in the group[1]	for 10^3		
Maternal age									
20 years and younger	Boys	11	166	66.3	30	750	40.0	1.66	0.85–3.24
	Girls	13	153	85.0[3]	23	655	35.1	2.42	1.25–4.67
	Total	24	319	75.2[3]	53	1405	37.7	1.99	1.25–3.18
21–25 years	Boys	45	947	47.5	92	2093	44.0	1.08	0.76–1.53
	Girls	30	880	34.1	70	2074	33.8	1.01	0.66–1.54
	Total	75	1827	41.1	162	4167	38.9	1.06	0.81–1.38
26–30 years	Boys	48	941	51.0	56	1495	37.5	1.36	0.93–1.98
	Girls	25	859	29.1	49	1510	32.5	0.89	0.56–1.44
	Total	73	1800	40.6	105	3005	34.9	1.16	0.87–1.56
31–35 years	Boys	20	387	51.7	35	649	53.9	0.96	0.56–1.64
	Girls	10	330	30.3	21	603	34.8	0.87	0.42–1.83
	Total	30	717	41.8	56	1252	44.7	0.94	0.61–1.44
36 years and older	Boys	8	111	72.1	11	314	35.0	2.1	0.85–4.98
	Girls	7	106	66.0	17	284	59.9	1.1	0.47–2.59
	Total	15	217	69.1	26	598	43.5	1.59	0.86–2.94
Paternal age[2]									
20 years and younger	Boys	3	43	69.8	5	142	35.2	1.98	0.49–7.96
	Girls	1	48	20.8	5	131	38.2	0.55	0.06–4.5
	Total	4	91	44.0	10	273	36.6	1.2	0.39–3.73
21–25 years	Boys	29	773	37.5	77	1776	43.4	0.86	0.57–1.31
	Girls	26	729	35.7	57	1683	33.9	1.05	0.67–1.66
	Total	55	1502	36.6	134	3459	38.7	0.95	0.69–1.3
26–30 years	Boys	47	1017	46.2	73	1802	40.5	1.14	0.79–1.63
	Girls	22	900	24.4	60	1873	32.0	0.76	0.47–1.24
	Total	69	1917	36.0	133	3675	36.2	0.99	0.75–1.32
31–35 years	Boys	15	424	35.4	44	938	46.9	0.75	0.42–1.34
	Girls	9	380	23.7	40	869	46.0	0.52	0.25–1.05
	Total	24	804	29.9	84	1807	46.5	0.64	0.41–1.003
36 years and older	Boys	2	126	15.9	25	641	39.0	0.41	0.097–1.7
	Girls	4	143	28.0	18	557	32.3	0.87	0.29–2.52
	Total	6	269	22.3	43	1198	35.9	0.62	0.27–1.45

Note:[1] – number of children in the group born to the parents of this age; [2] – unknown paternal age (in the main group – for 297 children, in the comparison group – for 15 children); [3] – statistically significant differences.

lower average doses than in the whole main group; however, focusing on structural components of FILs demonstrated diverse results. While infant death cases were usually observed regarding lower average doses than in the whole main group, stillbirth cases were associated with higher average preconception exposure doses to ovaries and testicles.

Distribution of the offspring in relation to the categories of occupational doses of female workers (Table 5) indicated that 26 % of the whole group (1270 children) fell into the category of 100.1–500 mGy; almost a quarter of the offspring (24.5 % – 1194 children) fell into the category of "zero" doses: their mothers were Mayak PA workers but no preconception doses of occupational exposure were registered prior to childbirth; mothers of 8.6 % (419) of the children were exposed to accumulated radiation doses over 1 Gy.

Table 4

Parameters of preconception external gamma-exposure of the gonads, mGy

Parameter	Number of offspring	Average doses ± average square deviation	Median and interquartile range [25–75 percentile]	Dose range
Radiation exposure of the ovaries				
Fetal and infant losses	217	307.2 ± 522.4	58.2 [0–306.3]	0–3006.3
Stillbirths	59	390.1 ± 630.9	98.8 [5.7–466.1]	0–3006.3
Infant mortality	158	276.2 ± 474.1	55.3 [0–303.8]	0–1930.4
Main group as a whole	4880	288.0 ± 473.2	74.5 [0.51–368.2]	0–4075.6
Radiation exposure of the testicles				
Fetal and infant losses	85	530.2 ± 769.7	206.4 [3.67–799.7]	0–3987.5
Stillbirths	26	672.8 ± 789.4	292.8 [24.4–1052.2]	0–2993.2
Infant mortality	59	467.3 ± 759.1	171.1 [0–494.4]	0–3987.5
Main group as a whole	2768	545.2 ± 760.9	221.7 [30.8–756.3]	0–5440.9

Table 5

FILs risk regarding dose categories of maternal preconception exposure

Dose ranges, mGy	Offspring sex	Main group, n = 4880			Comparison group, n = 10,427			RR	95 % CI
		Abs.	Number of children in the group[1]	for 10^3	Abs.	Number of children in the group[1]	for 10^3		
Radiation exposure of the ovaries									
= 0	Boys	28	597	46.9	224	5301	42.3	1.11	0.76–1.63
	Girls	27	597	45.2	180	5126	35.1	1.29	0.87–1.91
	Total	55	1194	46.1	404	10427	38.7	1.19	0.9–1.57
from 0.1 to 20.0	Boys	16	309	51.8	224	5301	42.3	1.23	0.75–2.0
	Girls	10	283	35.3	180	5126	35.1	1.01	0.54–1.88
	Total	26	592	43.9	404	10427	38.7	1.13	0.77–1.67
from 20.1 to 50.0	Boys	14	235	59.6	224	5301	42.3	1.41	0.84–2.38
	Girls	5	184	27.2	180	5126	35.1	0.77	0.32–1.86
	Total	19	419	45.3	404	10427	38.7	1.17	0.75–1.84
from 50.1 to 100.0	Boys	17	258	65.9	224	5301	42.3	1.56	0.97–2.51
	Girls	5	198	25.3	180	5126	35.1	0.72	0.3–1.73
	Total	22	456	48.2	404	10427	38.7	1.25	0.82–1.9
from 100.1 to 500.0	Boys	34	663	51.3	224	5301	42.3	1.21	0.85–1.73
	Girls	20	607	32.9	180	5126	35.1	0.94	0.59–1.48
	Total	54	1270	42.5	404	10427	38.7	1.1	0.83–1.45
from 500.1 to1000.0	Boys	8	266	30.1	224	5301	42.3	0.71	0.35–1.42
	Girls	9	264	34.1	180	5126	35.1	0.97	0.5–1.87
	Total	17	530	32.1	404	10427	38.7	0.83	0.51–1.33
from 1000.1 and higher	Boys	15	224	67.0	224	5301	42.3	1.58	0.96–2.63
	Girls	9	195	46.2	180	5126	35.1	1.31	0.68–2.53
	Total	24	419	57.3	404	10427	38.7	1.48	0.99–2.2

Note: [1] – number of children in the group of mothers with given dose rate of external gamma-exposure.

Assessment of FILs relative risk in relation to dose categories of maternal preconception exposure compared to children of the same sex in the comparison group is presented in Table 5.

In most categories, FILs risk was higher than in the comparison group; however, no statistically significant differences were obtained in any of the dose categories compared to the control. At the same time, analysis of stillbirths in the groups indicated certain categories of preconception exposure of the ovaries with statistically significantly higher risk of stillbirths in the main group: 0.1–20 mGy – 2.93 (1.33–6.5) and 100.1–500 mGy – 2.15

(1.11–4.15) for boys and 1000 mGy and higher for girls – 3.21 (1.28–8.0).

P. Doyle et al. [18] performed an analysis of perinatal outcomes in a group of atomic production workers in Great Britain and indicated a higher risk of stillbirth and early miscarriage (less than 13 weeks of pregnancy) among working mothers, though no dose dependence was found. W. Gao et al. [19] performed a meta-analysis of the relation between radiation therapy and risk of reproduction health damage among women who had survived cancer in childhood. This analysis included 14 cohort studies involving radiation therapy in childhood. A significant relation between therapeutical radiation and stillbirths (1.19, 95 % CI: 1.02–1.39) and low weight at birth (2.22, 95 % CI: 1.55–3.17) was indicated. As for diagnostic irradiation of mothers, it was indicated that certain radiation diagnostic procedures (less than 50 mGy) have no relation to mortality increase (stillbirths and miscarriages), genetic damages, teratogenicity, growth pathologies, mental deficiency or infertility [20].

According to research works with their focus on the effects of parental exposure, "stochastic effects that are mostly associated with ionizing radiation exposure originate as mutations and are then expressed as hidden genomic damages into final clinical manifestation" [21]. In addition, it is worth taking into account non-radiation risk factors of unfavorable reproduction outcomes. It is well-known that health status of an infant is determined by "internal family factors as well as external environmental factors", the variety of mortality risk factors for infants include "regional, social, climatic, geographic and cultural factors as well as differences in infrastructure and in quality of medical service available for reproduction age women and newborns [22]. In this connection, it is worth noting that the observed groups include CATU Ozyorsk residents that supposes no differences in obstetrical, gynecological and pediatric aid as "medical service of the CATU along with the workers of the city-forming enterprise was provided by the FMBA of Russia in the form of medical and sanitary units and clinical hospitals" [23] that supposed the same standards of medical service and level of equipment supply in healthcare.

Potential directions of further FILs analysis include assessment of excess relative risk of FILs in the followed cohort of the offspring regarding the rate of accumulation of occupation exposure doses by the parents; assessment of the contribution of medical exposure of parents into FILs risk [24]; a standardized analysis of FILs against regional and national rates; factor analysis taking into account the range of non-radiation factors.

Conclusions. Analysis of fetal and infant losses was performed in a group of 15,307 children born in 1049–1973 in CATU Ozyorsk, mothers of 4880 of them were Mayak PA workers. It was stated that:

1) FILs as a whole didn't differ statistically between the group but among the offspring with only mother working at Mayak PA a significant increase of FILs, stillbirths and infant deaths was observed;

2) In the course of analyzing mortality dynamics it was indicated that in the period of 1949–1953 FILs, stillbirth rate and infant mortality among exposed mothers' offspring was higher than in the comparison group;

3) Significant differences in FILs structure were obtained for early fetal death that was more often registered in the main group: 3.48 vs 1.34 for 10^3, $\chi^2 = 7.54$, $p = 0.006$;

4) FILs risk among female Mayak PA workers aged under 20 was significantly higher for girls – 2.42 (1.25–4.67) and for both sexes – 2.16 (1.37–3.4);

5) Dose categories of preconception exposure of the ovaries with significantly higher risk of stillbirths were indicated in the main group.

Characteristic features of FILs indicated among the offspring of mothers working with ionizing radiation sources are important for further research of the effects of parental radiation exposure in preconception period and could be used in further epidemiological monitoring.

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Research article

MEASURING RISK PERCEPTION AND RISK COMPENSATION AMONG LOCAL RESIDENTS TOWARD COVID-19 IN THE TOURISM SANDBOX ISLANDS: COMPARATIVE EVIDENCE FROM THE PHUKET SANDBOX AND SAMUI PLUS MODEL IN THAILAND

P. Pholphirul

King Mongkut's Institute of Technology Ladkrabang International Demonstration School, 1 Chalong Krung Road, Ladkrabang, Bangkok, 10520, Thailand

Since Thailand is a tourism-dependent country, its economy suffered tremendously during the COVID-19 lockdown. In a pioneering effort to re-open the country, two islands (Phuket and Koh Samui) began to welcome fully vaccinated international visitors in July 2021 under the "Phuket Sandbox"[1] and "Samui Plus Model." Even though the sandbox programs were found to create more income and some benefits for tourism businesses, they also generated concern among local people about contracting such an infectious disease as COVID-19.

The aim of this study was to measure perception of COVID-19 infection among local residents due to contacts with international tourists.

Using secondary data from a survey of 400 local residents living on the two islands, monetary compensation under the contingent valuation methods (CVM) and risk perception scale of 0–10 were analyzed to get an indication of the level of local residents' risk perception toward COVID-19 possibly being transmitted by international tourists.

Our results show that the risk perception was found to be higher among those who believed that the coronavirus could possibly result in death. Older individuals, especially females, and those with higher incomes seemed to have a higher risk perception. Residents who were working in the tourism sector were found to have a lower risk level than those who were working in other branches.

There were also discordances in terms of education level and risk perception between residents in two islands. The paper suggests that awareness of residents' risk perception and effective communication regarding such risk perception should be put in place to ensure appropriate practices that affect local residents in such sandbox areas in the future.

Keywords: *risk perception, contingent valuation method, tourism sandbox, local residents, COVID-19, Thailand.*

The World Health Organization (WHO) first declared COVID-19 a global health emergency in January 2020, and on March 11 it declared the viral outbreak to be a pandemic, the highest level of health emergency. As infections began rising sharply in late February 2020, governments in many countries took unprecedented steps in March 2020 to lock down social activities in order to contain the spread of the pandemic. Nevertheless, due to the highly contagious nature of the virus, the pandemic was considered one of the biggest global crises, resulting in unprecedented economic and social consequences. In response to the increasing number of COVID-19 cases and related deaths, many countries all over the world implemented non-pharmaceutical physical interventions to stop the spread of the virus, such as nationwide lockdowns, restrictions on public gatherings and movements, and restrictions on the operation of certain contact-intensive sectors. While these measures slowed down the spread of the pandemic, they also caused a significant loss of income or,

Pangwan Pholphirul – student (e-mail: pangwan211@gmail.com; tel.: (66) 062-592-4222; ORCID: https://orcid.org/0009-0001-6864-0819).

[1] Editorial note: Phuket Sandbox is the program for visiting Phuket without quarantine

even worse, a complete loss of jobs for many individuals and businesses.

Research shows that the impacts of COVID-19 have been both massive and unequal across and within countries. National economies have experienced either single- or double-digit contractions depending on the number of infections, fatality rate, duration and stringency of measures to contain the spread of the pandemic, missed work and job losses, changes in consumer behavior, as well as resilience of particular economies and societies [1–3]. Severe income and job losses have been more common among lower-income population groups, low-skilled workers, low-education workers, informal workers, and workers in hard-hit sectors, especially in the tourism and hospitality sectors [4–6]. As no surprise, the pandemic has plunged millions of people into poverty [7, 8].

Thailand was the first country in the world to confirm a case outside China, with millions of people across the world affected later by the COVID-19 pandemic [9]. From a full analysis of the impacts of the COVID-19 pandemic on Thailand, Sudsawasd et al. found that Thailand's economy was hit hard by the pandemic. The country's GDP was predicted to fall by 13.66 %.

However, with over 40 million tourists visiting each year (before the COVID-19 pandemic), Thailand is one of the world's major tourist destinations and is ranked among the top 10 countries with the highest yearly tourist arrivals. Estimates of tourism revenue that directly contributed to the GDP ranged from one trillion baht in 2013 to 2.53 trillion baht in 2016, approximately 9.00 to 17.70 % of GDP, respectively. Sudsawasd et al. also found that the most important transmission channels impacting the negative economic shock from COVID-19 came mainly from the loss of inbound tourism demand, which accounted for 61.42 % of the total impact, highlighting the importance of the tourism sector and tourism inflows on the Thai economy.

On the labor market side, the COVID-19 pandemic also led to a sharp decline in tourism activity, resulting in widespread job losses across various segments of the tourism industry in Thailand. Studies indicate that hotels, restaurants, tour operators, and other tourism-related businesses were forced to lay off or furlough employees due to plummeting visitor arrivals and revenue losses [9–11]. The pandemic exacerbated vulnerabilities among these workers, with many facing income loss, a lack of social protection, and limited access to healthcare services [12].

To mitigate the negative impacts of border restrictions, the Thai government announced the re-opening of two tourism destination islands, namely, 1) Phuket and 2) Koh Samui (Samui Island), under the names "Phuket Sandbox" and "Samui Plus", respectively.

Phuket is Thailand's largest island, located in the Andaman Sea of the west coast of Thailand. It offers a diverse range of activities, including snorkeling, diving, elephant trekking, and exploring its many temples and cultural sites.

Koh Samui is an island located in the Gulf of Thailand, known for its palm-fringed beaches, coconut groves, and luxury resorts. Both islands are popular tourist destinations in Thailand, known for their stunning beaches, vibrant nightlife, and rich cultural heritage. A map of Phuket and Koh Samui is shown in Figure 1 below.

Figure 1. Map of Phuket and Koh Samui, Thailand

In more detail, the Phuket Sandbox and Samui Plus are unique tourism destination initiatives by the Thai government to gradually re-open the country's borders to international travelers amidst the COVID-19 pandemic. The islands were opened to fully vaccinated travelers from selected countries with a low to medium level of risk of COVID-19 transmission. International visitors had to be fully vaccinated with vaccine(s) approved by the Ministry of Public Health of Thailand and had to first obtain a Certificate of Entry (COE) from the Thai embassy or consulate in their home country. Upon arrival at Phuket (or Koh Samui) International Airport, travelers had to undergo health screening, including a COVID-19 test. If the test result was negative, tourists were free to travel within the island without quarantine restrictions. After the first week, they were free to travel to other destinations in Thailand. Overall, the Phuket Sandbox and Samui Plus programs aimed mainly to revive Thailand's tourism industry, especially business operators and tourism workers, while prioritizing public health and safety of tourists and the islands' residents. The two destinations were considered "sandboxes" to provide a framework for re-opening borders in a controlled manner, allowing international tourists to enjoy the beauty of Thailand while minimizing the risk of COVID-19 infection.

Even though tourism bubbles on both islands were seen as beneficial for the tourism sector, especially among tourism businesses and related workers, concerns and anxieties regarding such an infectious disease were likely to be felt among local residents as opening up the islands could possibly lead to a larger outbreak.

There was a concern that local residents might feel anxious about the potential risk of infection due to tourism activities, especially in destinations with limited healthcare facilities or among residents that were especially vulnerable to the effects of the virus. Understanding how individuals perceived the risk associated with COVID-19 was, therefore, crucial in understanding how the government should balance economic benefits from the tourism sandbox (Phuket Sandbox and Samui Plus programs) and the costs borne by the local community from the higher risk of infection. Understanding this tradeoff between health security and economic security in such a time of crisis should provide a valuable insight into implementing effective economic and public health interventions as well as communication strategies in such times [13].

The aim of this study was to measure community risk perceptions toward COVID-19 among local people by using pieces of evidence from the Phuket Sandbox and Samui Plus programs, taking advantage of the unique situation that the sandboxes offered in terms of the tradeoff between health security and economic security.

Using secondary data from the survey of 400 local people living on both islands for the duration of one month (July 2020), two methods are used to measure the community risk perception. First, the monetary compensations under the contingent valuation methods (CVM) were computed to reflect the level of local residents' risk perception toward COVID-19 transmitted by international tourists. Second, a scale of subjective risk perception of 1–10 was used to assess residents' preferred situation regarding the tradeoff between the risk of infection and the level of openness of their island.

This research also classifies residents' risk perception by socioeconomic variables such as sex, education level, income level, working / not working in the tourism sector, and having / not having elderly or family members in the household with one of the risky comorbidities, including obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy. This analysis also classifies risk perception towards two types of case according to the severity of the COVID-19 infection, that is, "getting infected, but recovering" and "getting infected and dying".

Literature Review on Community Risk Perception of COVID-19. Risk perception plays a significant role in shaping individual behaviors and public health responses to

communicable diseases. Understanding how communities perceive the risks associated with these diseases is essential for forming effective public health interventions. This literature review aims to examine the existing research on risk perception concerning communicable diseases in general, including factors influencing perception, its impact on preventive behaviors, and implications for public health strategies. Additionally, factors associated with risk perception towards COVID-19 are explored.

Several factors influence risk perceptions regarding communicable diseases. Individual characteristics such as age, sex, education level, socioeconomic status, and health literacy contribute to variations in risk perception [14]. In many cases, individuals with higher incomes may have better access to healthcare, resources, and information about preventive measures against communicable diseases. This can lead to the perception that they are less susceptible to such diseases, thereby lowering their perceived risk. Conversely, individuals with lower incomes may have limited access to healthcare, endure poorer living conditions, and may be more likely to work in jobs with higher exposures to infectious agents. As a result, they may perceive a higher risk of contracting communicable diseases [15, 16].

Regardless of income level, education and awareness about communicable diseases significantly influence risk perception. Individuals with higher education levels may have a better understanding of the transmission routes, preventive measures, and severity of communicable diseases, leading to more accurate risk perceptions. Research by Smith et al. indicates that higher levels of education are associated with a better understanding and perception of communicable disease risks [17]. Educated individuals tend to grasp the severity and transmission dynamics of diseases, leading to more informed preventive behaviors. Studies by Berkman et al. also suggest that education enhances health literacy, enabling individuals to comprehend health information, including risks associated with communicable diseases [18]. Higher health literacy, therefore, fosters accurate risk perception and the adoption of preventive measures.

One study also suggests that age can influence risk perception towards communicable diseases, with older individuals often perceiving a higher risk due to factors such as weakened immune systems and increased vulnerability to severe outcomes. However, the relationship between age and risk perception can be complex and may vary depending on the specific disease context and individual circumstances [19].

Risk perceptions of communicable diseases can also be influenced by sex differences between males and females. For example, in terms of biological differences, hormonal differences between males and females can impact immune responses and susceptibility to certain diseases. For example, estrogen has been shown to enhance immune responses in females, potentially providing them with better protection against certain infections compared to males. Conversely, hormonal fluctuations during menstrual cycles may also influence susceptibility to certain diseases [20]. Studies consistently show that women tend to utilize healthcare services more frequently than men. This higher healthcare utilization may lead to greater awareness of communicable diseases and associated risks among females [21]. Societal norms and cultural expectations surrounding sex-specific roles may also influence how individuals perceive and respond to disease risk. For example, stereotypes about masculinity may discourage men from acknowledging vulnerability or seeking help for health concerns [22].

Additionally, cultural beliefs, media exposure, trust in health authorities, and previous experiences with infectious diseases shape how individuals perceive the risk of communicable diseases [23]. Studies have shown that perceived susceptibility and severity of the disease, as well as the perceived efficacy of preventive measures, influence risk perception [24].

The COVID-19 pandemic posed significant challenges globally, requiring effective public health responses to mitigate their impacts. Understanding community risk percep-

tion regarding COVID-19 is crucial for informing public health strategies and interventions aimed at promoting preventive behaviors and reducing transmission rates. This literature review examines existing research on community risk perception concerning COVID-19 highlighting key factors influencing perception, its impact on preventive behaviors, and the implications for public health responses.

In many recent articles, numerous factors are found to influence community risk perceptions regarding COVID-19. Similar to other commutable diseases that existed in the past, individual characteristics such as age, sex, education level, socioeconomic status, and health literacy play a role in shaping risk perception. Additionally, cultural beliefs, media exposure, trust in government and health authorities, and previous experiences with infectious diseases influence how communities perceive the risk of COVID-19 [26, 27]. Studies have shown that individuals with higher perceived susceptibility and severity of COVID-19 are more likely to engage in preventive behaviors such as wearing masks, practicing social distancing, and seeking vaccination [26].

Existing research on community risk perception of COVID-19 has focused on local residents in tourism areas, focusing on key factors influencing perceptions and the implications for public health strategies and tourism management. One such factor is that tourism-dependent communities often have a heightened risk perception due to the potential for virus transmission associated with travel and tourism activities. Residents may perceive tourists as vectors of disease transmission, leading to concerns about their own health and safety [28]. Second, the economic dependence on tourism exacerbates risk perception among local residents, as livelihoods are closely tied to the tourism industry. Fear of economic instability and job loss may conflict with concerns about public health, leading to complex risk assessments [29, 30].

Government responses to the pandemic, including travel restrictions, quarantine measures, and lockdowns, can also shape community risk perception. Residents' perceptions of the effectiveness and fairness of these policies influence compliance and attitudes toward tourism [31].

In this case, community engagement and trust-building efforts between residents, tourism stakeholders, and local authorities are essential for addressing risk perception. Transparent communication, collaboration, and involvement in decision-making processes foster trust and promote collective resilience [32, 33].

Measuring risk perception. The Contingent Valuation Method (CVM) is widely used to measure how individual perceive risk associated with hypothetical or known threats. The Contingent Valuation Method (CVM) is a popular method in which respondents (local residents) were asked to state their preferences in hypothetical or contingent situations. The CVM is widely used in economic analysis to assess individuals' willingness to accept (WTA) a particular good or service, including the valuation of intangible benefits or costs, such as environmental quality or risk reduction. While the CVM is primarily used for valuing environmental goods and services, it can also be used to measure risk perception by framing hypothetical scenarios in which individuals are asked to express their willingness to pay to mitigate or avoid certain risks or their willingness to accept compensation when an adverse outcome takes place [34].

The willingness to accept (WTA) specifically refers to the minimum amount of compensation an individual or community is willing to accept in exchange for bearing the risks associated with a hazard or threat. This literature review explores the interplay between willingness to accept and community risk perception, examining how perceptions of risk influence people's decisions regarding risk acceptance and mitigation. Willingness to accept and community risk perception are closely intertwined constructs that play a significant role in shaping individuals' and communities' responses to hazards and threats. By examining their relationship, this review highlights the importance of considering both constructs in risk management and

communication efforts aimed at enhancing community resilience[2] [13].

Another measure that is widely used to assess the subjective assessment of risk perception is the Subjective Risk-scaling. This scaling is often used to measure subjective well-being such as overall life satisfaction or the level of happiness, where the given number is subjective to individual's judgment of overall evaluation of the variable that is the result of many attributes. When used as a continuous variable, this scale is measured in "1 to 7", "1 to 10", or " 0 to 10" scale, where the latter has the mean and the median exactly at "5" [35]. Figure 2 shows the conceptual framework of this analysis.

Materials and methods. This paper uses secondary data provided by the Program Management Unit for Competitiveness, Ministry of Higher Education, Science Research, and Innovation. The survey on islands' residents was part of the integrated study on the impacts of Phuket Sandbox and Samui Plus initiatives on community, tourism businesses, and tourists. This part of the survey was a random survey of local residents during October – November 2021, after the sandboxes had been implemented for five months. Data from a total of 400 samples of local residents aged 15 and above were collected in Phuket and Koh Samui, 200 representative random samples from each island. Proportional random samples were drawn based on the district population size in Phuket and Koh Samui. The validity tests were piloted in both islands (20 samples each) to ensure quality of the questionnaires.

Questions on socio-economic backgrounds on of respondents, such as sex, age, education level, income, working / not working in tourism sectors, and having / not having elderly or members with one of the seven risky diseases, were asked in the questionnaires.

The estimated willingness-to-accept value would provide insights into individuals' risk perception and the value they place on risk reduction or mitigation. Policymakers could use this information to prioritize risk management strategies, allocate resources efficiently, and design targeted interventions to address public concerns and enhance risk communication in the two islands. With this objective in mind, two measures were used to assess local residents' risk perception, the Contingent Valuation Method (CVM) and the Subjective Risk-scaling of 0 to 10. To identify the compensation

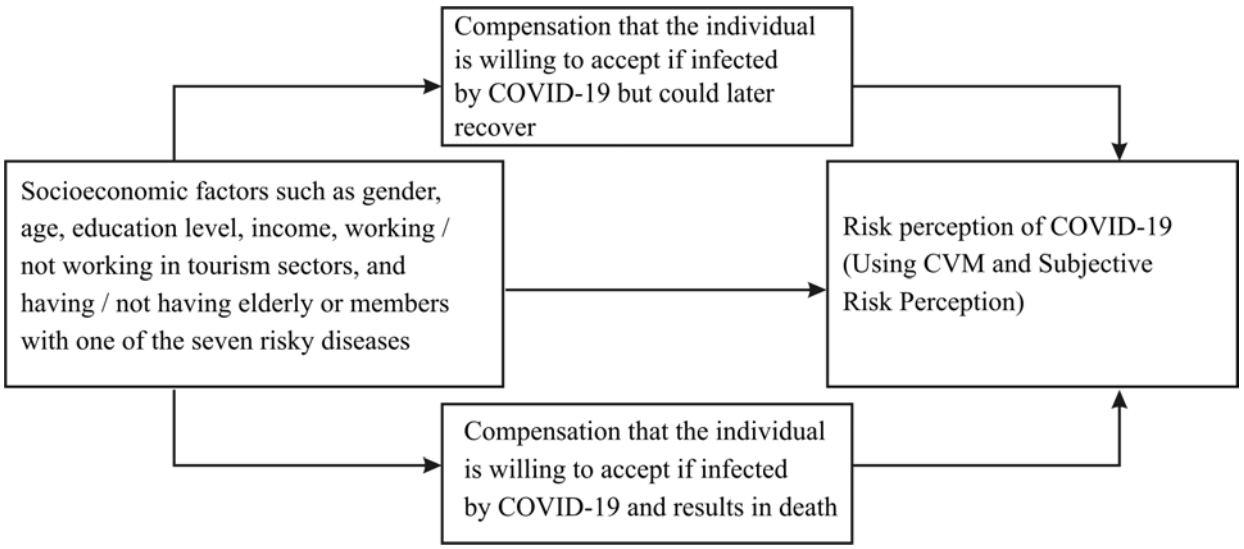

Figure 2. Conceptual framework of the conducted analysis

[2] Slovic P. Perception of risk. *Science*, 1987, vol. 236, no. 4799, pp. 280–285. DOI: 10.1126/science.3563507

that the respondent was willing to accept using CVM, local residents were presented with a hypothetical scenario and asked a series of questions to elicit their risk perception and willingness to accept in exchange for bearing the risks associated with COVID-19 infection. The participants were asked to express their demand of willingness to accept for two risk scenarios, 1) getting infected, but recovering, and 2) getting infected and dying. In the first scenario, each respondent was presented with different monetary compensation amount, starting with 20,000 Baht. If the respondent accepted the starting amount of 20,000, he / she was then presented with a lower offer of 10,000 Baht. If the respondent accepted 10,000, the risk compensating amount was then concluded to be 10,000 Baht. If the respondent did not accept the initial offer of 20,000 Bath, the offer went up to 40,000 and the process went on with the incremental amount of 20,000 Baht until the last offer of 100,000. If the respondent still did not accept the last offer, he / she was then asked to indicate the amount he / she was willing to accept. The final amount that the respondent was willing to accept from this process was then used as the respondent's 'risk value' using CVM. Similar procedure was carried out to identify the 'risk value' in the case of death, where the starting offer was 200,000 Baht and the final offer was 1,000,000 Baht.

For the Subjective Risk-scaling, residents were asked to rate their risk perception on a scale of 0 to 10. Island residents were asked to identify their preferred situation regarding the tradeoff between the risk of infection and the level of openness of the island to tourists. "0" indicated one extreme, in which the island was fully open for tourists with no health restrictions that could be extremely unsafe from COVID-19, and "10" indicated the other extreme, in which strict health rules were applied to keep everyone safe from COVID-19 with extreme limits to tourist activities. The mean and the median of this scale were "5," where both aspects were moderate.

Descriptive data were analyzed to show the differences of socio-economic backgrounds, the monetary compensation using CVM, and the subjective risk-scale of the residents in the two islands towards the two scenarios (sickness, but fully recovered and death from COVID-19). The statistical differential effects of sex, age, education level, income, working/not working in tourism sectors, and having/not having elderly or members with one of the seven risky diseases were estimated using Analysis of Variance (ANOVA), where F statistics and p-values were used to indicate the statistical differences of monetary compensations and risk levels among residents with different backgrounds for residents in both islands. Statistical confidence levels of 90, 95, and 90 % are used in the analyses.

Results and discussion. Table 1 below shows descriptive statistics of the surveyed data. It can be observed that the majority of the survey population were female (62.5 % in Phuket and 51.5 % in Koh Samui) with relatively higher levels of education (bachelor's degree and above). However, the majority of their income levels were relatively low, ranging from around 10,000 to 19,999 Baht per month. More than half (56.5 % in Phuket and 58 % in Koh Samui) worked in the tourism sector, with only a small proportion having elderly members or members with one of the risky seven diseases (21.5 % in Phuket and 17 % in Koh Samui).

Figure 3 shows the monetary compensation (classified by sickness and death) the residents in both islands were willing to accept from the government should COVID-19 be transmitted by international tourists. There are two steps of questions. It can be seen that the risk compensation is significantly higher if COVID-19 infection causes those residents to die (ranging from 56,875 baht to around 762,903 baht in Phuket and from 43,450 baht to around 367,000 baht in Koh Samui). It is also evident that the residents in Phuket report demanded higher monetary compensation for risk than those in Koh Samui. Statistical testing (F-test) conducted between both islands, as shown in Table 2, reveals that risk compensation (risk perception) is statistically different between residents in both islands, with a 95 % confidence level.

Table 1

Descriptive Sample Data Classified by Tourism Sandbox Islands
(Phuket Sandbox and Samui Plus)

Variables	Phuket (Phuket Sandbox)		Koh Samui (Samui Plus)	
	Observations	Percentage	Observations	Percentage
Total Observation	**200**	**100 %**	**200**	**100 %**
Sex				
Male	75	37.5 %	97	48.5 %
Female	125	62.5 %	103	51.5 %
Age				
18–24 years old	30	15.0 %	30	15.0 %
25–34 years old	77	38.5 %	101	50.5 %
35–49 years old	70	35.0 %	45	22.5 %
50 years and above	23	11.5 %	24	12.0 %
Current Level of Education				
Primary education and lower	18	9.0 %	31	15.5 %
Lower secondary education	23	11.5 %	21	10.5 %
Higher secondary education	50	25.0 %	29	14.5 %
Vocational education	26	13.0 %	31	15.5 %
Bachelor degree and above	83	41.5 %	88	44.0 %
Income per month (Before COVID-19 Pandemic)				
< 10,000 Baht/month	20	10.0 %	13.2	6.6 %
10,000–19,999 Baht/month	104.4	52.2 %	111.2	55.6 %
20,000–29,999 Baht/month	37.8	18.9 %	26.6	13.3 %
30,000–50,000 Baht/month	30	15.0 %	30.4	15.2 %
50,001 Baht/month and above	7.8	3.9 %	18.6	9.3 %
Worked in tourism sector				
Yes	113	56.5 %	116	58.0 %
No	87	43.5 %	84	42.0 %
Having elderly or members with risky comorbidities (obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy)				
Yes	43	21.5 %	34	17.0 %
No	157	78.5 %	166	83.0 %

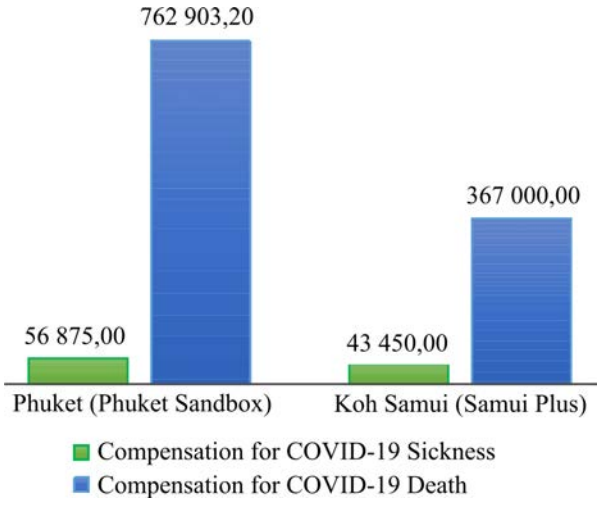

Figure 3. Monetary Compensation (CVM) classified by Tourism Sandbox Islands in Baht (Phuket Sandbox and Samui Plus)

In terms of the Subjective Risk Scale (0–10), as shown in Table 2, the average risk level is around 5.5–6.0, which is computed to be significantly different between the two islands. The F-statistics computed in Table 3 also indicates that Koh Samui residents seem to exhibit a higher risk level than those in Phuket, with a 95 % statistically significant level. This analysis suggests that socioeconomic factors (such as age, sex, education level, income, and so on) may also play different roles in community risk perception between the two sandbox islands.

Statistical testing was conducted and shown in Tables 3, 4. Table 3 displays statistical differences (F-Test) in Monetary Compensation between COVID-19 Sickness and COVID-19 Death Classified by Tourism

Table 2

Statistical Differences (F-Test) of Risk Perception toward COVID-19 classified by Tourism Sandbox Islands (Phuket Sandbox and Samui Plus)

Risk Perception toward COVID-19	Phuket	Koh Samui	F Statistics	P-value
Monetary Risk Compensation (CVM)				
– Compensation for COVID-19 Sickness	56,875.00	43,450.00	5.160	0.024**
– Compensation for COVID-19 Death	762,903.20	367,000.00	52.040	0.000***
Subjective Risk Level (0–10)	5.50	6.00	8.430	0.004***

Note: ***, **, and * denote statistical significance at 99, 95, and 90 %, respectively.

Table 3

Statistical Differences (F-Test) of Monetary Compensation between COVID-19 Sickness and COVID-19 Death Classified by Tourism Sandbox Islands (Phuket Sandbox and Samui Plus)

Characteristics	Phuket (Phuket Sandbox)						Koh Samui (Samui Plus)					
	Mean	F-Statistics	P-value	Mean	F-Statistics	P-value	Mean	F-Statistics	P-value	Mean	F-Statistics	P-value
	Compensation for COVID-19 sickness			Compensation for COVID-19 death			Compensation for COVID-19 sickness			Compensation for COVID-19 death		
Sex												
Male	54,583.33	0.090	0.761	721,739.10	0.340	0.559	39,793.81	5.080	0.0253**	328,866.00		0.029**
Female	58,250.00			787,179.50			46,893.20			402,912.60		
Age group												
18–24 years	68,000.00	0.820	0.486	892,592.60	3.310	0.021**	42,666.67	2.540	0.0578*	303,333.30	6.740	0.000***
25–34 years	61,232.88			905,555.60			40,297.03			317,821.80		
35–49 years	54,393.94			669,230.80			45,555.56			453,333.30		
50+ years	35,652.17			413,636.40			53,750.00			491,666.70		
Education level												
Primary School or Lower	32,222.22	1.510	0.201	373,333.30	2.470	0.046**	48,387.10	1.320	0.262		2.700	0.032**
Secondary School	82,173.91			472,727.30			49,523.81			419,047.60		
High School	44,583.33			826,000.00			40,689.66			389,655.20		
Diploma	50,416.67			879,166.70			38,064.52			306,451.60		
Bachelor's Degree and above	64,556.96			846,666.70			43,068.18			332,954.50		
Working in tourism sector												
Yes	59,174.31	0.210	0.651	707,692.30	1.330	0.250	40,517.24	4.780	0.0299**	315,517.20	13.480	0.000***
No	53,855.42			832,926.80			47,500.00			438,095.20		
Income level (per month)												
< 10,000 Baht	38,823.53	0.440	0.777	629,411.80	0.630	0.641	34,000.00	6.390	0.000***	150,000.00	17.870	0.000***
10,000 – 19,999 Baht	62,527.47			805,555.60			35,119.05			248,809.50		
20,000 – 29,999 Baht	53,548.39			796,875.00			45,000.00			405,000.00		
30,000 – 50,000 Baht	51,481.48			587,500.00			57,391.30			573,913.00		
50,000+ Baht	80,000.00			560,000.00			54,285.71			542,857.10		
Having elderly or members with risky comorbidities (obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy)												
Yes	52,857.14	0.130	0.715	666,666.70	0.850	0.359	47,058.82	1.050	0.306	420,588.20	2.050	0.154
No	58,000.00			788,435.40			42,710.84			356,024.10		

Note: ***, **, and * denote statistical significance at 99, 95, and 90 %, respectively.

Table 4

Statistical Differences (F-Test) of Risk Level between COVID-19 Sickness and COVID-19 Death Classified by Tourism Sandbox Islands (Phuket Sandbox and Samui Plus)

Characteristics	Phuket (Phuket Sandbox)			Samui (Samui Plus)		
	Mean	F-Statistics	P-value	Mean	F-Statistics	P-value
Sex						
Male	5.53	0.03	0.860	5.91	0.950	0.330
Female	5.48			6.09		
Age group						
18–24 years	4.87	3.36	0.020**	6.07	13.160	0.000***
25–34 years	5.31			5.57		
35–49 years	6.09			6.27		
50+ years	5.17			7.21		
Education level						
Primary School or Lower	5.94	1.67	0.159	6.87	4.740	0.001***
Secondary School	5.52			5.81		
High School	5.18			6.07		
Diploma	6.31			5.71		
Bachelor's Degree and above	5.34			5.82		
Worked in tourism Sector						
Yes	5.51	0.01	0.918	5.62	26.380	0.000***
No	5.48			6.52		
Income Level (per month)						
< 10,000 Baht	5.78	0.59	0.672	5.30	10.950	0.000***
10,000 – 19,999 Baht	5.41			5.32		
20,000 – 29,999 Baht	5.68			6.30		
30,000 – 50,000 Baht	5.93			6.04		
50,000+ Baht	6.29			7.14		
Having elderly or members with risky comorbidities (obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy)						
Yes	5.95	2.68	0.103	6.29	2.100	0.149
No	5.38			5.94		

Note: ***, **, and * denote statistical significance at 99, 95, and 90 percent, respectively.

Sandbox Islands. Comparisons between COVID-19 sickness and COVID-19 death revealed significant results for local residents in both islands if they fear that COVID-19 could cause their demise. In Phuket, variables such as age group and education level were found to be insignificantly related to the risk in the case of COVID-19 sickness, but statistically significantly related to the risk level in the case of COVID-19 death. Individuals of younger age demonstrated their higher monetary compensation in Phuket.

Only in Koh Samui, with statistical significance, female residents seemed to display higher monetary compensation than their male counterparts, implying that females have a higher risk perception of COVID-19 than males. This result is consistent with the literature review conducted by Bertakis et al. [21] and Addis and Mahalik [22], which suggested that women tended to perceive higher risks due to biological differences, societal norms, and cultural expectations. Age group appeared to be significantly related to the risk level of local residents on both islands. Especially on Koh Samui, in contrast with Phuket, individuals of older age demonstrated their wish for higher monetary compensation. This finding is consistent with the article reviewed by Van Der Weerd et al. [19], which suggests that older individuals often perceive higher risks due to factors such as weakened immune systems and increased vulnerability to severe outcomes. In Phuket, the reverse trend was

shown, which may have been due to the fact that local residents in Phuket who were in younger age groups were having higher valuation of their risk due to their involvement in the labor market which was heavily affected by COVID-19. The opportunity costs of getting infected or dying could be much higher among younger groups than that of older groups.

Adolescents and youth (18–24 years old) might not always fully comprehend the risks associated with communicable diseases, especially if they had not had direct experiences with them. The elderly (50 years and older) might be more vulnerable to the severe effects of communicable diseases, so they may have a heightened perception of risk. However, estimated results seemed to be mixed in Phuket; young adults (25–34 years old) were found to have the highest risk compensation compared to other age groups, while contrary to the case in Koh Samui, monetary compensation was found to be higher for the older-age groups. This mixed perception of communicable disease risks could be due to recent experiences with outbreaks or health-related scares, while others might feel indifferent. Peer influence, social media, and personal experiences with illness can heavily influence their perception. Age group and community risk perception of communicable diseases can vary widely based on factors such as education, socioeconomic status, access to healthcare, and past experiences [14, 23].

Additionally, for Koh Samui's residents, a higher education level seemed to be related to a lower amount of monetary compensation if those residents contracted COVID-19 and died under the Samui Plus program. Again, this result is consistent with Smith et al. [17], as individuals with higher education levels may have a better understanding of transmission routes, preventive measures, and the severity of communicable diseases, leading to lower risk perceptions. However, the effects on education level and risk perception were found to be the opposite in the case of Phuket. Individuals there with higher education levels seemed to require a higher amount of monetary compensation if they contracted COVID-19 and died. Nevertheless, this positive relationship between education and risk perception is found in a number of studies, such as those conducted by Taghrir et al. [36] and Lanciano et al. [37]. This may be because higher education might help people engage in preventative behaviors while simultaneously protecting them "from a (possible) irrational fear of being infected or dying" [36, 37].

As explained by previous research articles, for example, by Zikmund-Fisher and Sarr [15] and Myers and Goodbye [16], individuals with higher incomes should have better access to healthcare, resources, and information about preventive measures against communicable diseases. This can lead to a perception that they are less susceptible to such diseases, thereby lowering their perceived risk. However, our results from F-statistics show the contrary for the case of Koh Samui's residents. Those with higher incomes were found to have a higher anticipation for monetary compensation, implying a higher risk perception.

Another interesting result shows that if Koh Samui residents worked in the tourism sector, they seemed to require lower monetary compensation, implying a lower risk perception compared to those who did not work in the tourism sector. As mentioned above, the COVID-19 pandemic led to a sharp decline in tourism activity, resulting in widespread job losses across various segments of the tourism industry. Re-opening the country through the tourism sandbox program helped revive the tourism sector, especially for tourism businesses themselves. The anxiety and fear of the infectious disease might be expected therefore to be less of a concern among those who work in the tourism sector. However, we did not find statistically significant findings regarding having elderly individuals or members with the risky comorbidities (obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy).

In terms of the subjective risk level, as shown in Table 4, results from F-statistics are consistent with what we found for the case of

monetary compensation (as shown in Table 3). Younger individuals with higher education levels seemed to have lower risk perception in Koh Samui. Those with higher incomes seemed to show a higher level of risk perception. Additionally, local residents who worked in the tourism sector were found to have a lower risk perception level than those who did not work in the tourism sector. Individuals with higher incomes were found to have a higher scale of risk perception. Also, there were no statistically significant findings regarding having elderly individuals or members with the risky comorbidities.

It is noteworthy that this study does not estimate the likelihood of a negative event (disease or death) because it was relatively difficult for local residents to identify likelihood of them getting infected or dying from COVID-19, given many uncertainties and overflows of information during the pandemic. Rather, the estimated compensation from CVM focuses on finding how much risk they faced in terms of the value of their lives or opportunity costs in the case of infection or death from COVID-19.

Nevertheless, the analysis in this article has some limitations. First, some attributes that could be related to risk perception were not included in our analysis. Examples of these attributes are personal factors, health status, mass media exposure, COVID-19 knowledge, political orientation, and trust in the government. These variables were not included in the questionnaires. Second, the perception of risk can vary with experience and exposure to COVID-19. Cross-sectional data can only illustrate the relationship between different attributes and the risk perception at one point in time; panel data should be obtained to study the differential impacts of different attributes studied in this paper to get more insight to understand the tradeoff between health risk and economic risk as the costs of pandemic fade.

Conclusion. As a tourism-dependent country, the Thai economy suffered tremendously during the COVID-19 pandemic. In a pioneering effort to reopen the country, two islands (Phuket and Koh Samui) began welcoming fully vaccinated international visitors in July 2021 under the “Phuket Sandbox” and “Samui Plus Model”. Even though the sandbox programs were found to create more income and some benefits for tourism businesses, concerns and anxiety regarding infectious diseases were generated among local people.

Several studies have attempted to identify how people's risk perceptions differed regarding COVID-19 infections. Using secondary data from a survey of 400 local residents living on the two islands, monetary compensations under the contingent valuation method (CVM) and Subject Risk Scale (0–10) were computed to reflect the level of local residents' risk perception toward COVID-19. Our results showed that the risk perception was found to be higher among local people in Phuket than those in Koh Samui, especially among those who believed that the coronavirus could possibly cause death.

Our results show that older individuals seemed to have higher risk perception, especially females. Those with higher incomes seemed to show a higher level of risk perception. Additionally, local residents who were working in the tourism sector were found to have a lower risk level than those who did not work in the tourism sector.

However, there were some discordances regarding education level and risk perception between residents in Phuket and those in Koh Samui. The risk perception was found to be higher among those with higher education levels in Phuket and lower among these groups in Koh Samui. However, having elderly or members with risky comorbidities, including obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, respiratory disease, kidney disease, and malignancy in the household was found to be unrelated to risk perception.

Findings from this paper clearly suggest that health risk perception can differ due to individuals’ attributes. Even though COVID-19 related issues can hardly be considered to be relevant now, our study results suggest that

these two approaches can be used to assess risk perception of other health threats that affect people of different backgrounds.

Introducing an economic scheme (such as monetary compensation for those who are infected by the disease) into a geographic area, which could trigger higher levels of risks among residents with different levels of risk perception, could have differential effects on the population.

We believe that the government should understand the differential pressures on various groups of local residents before implementing any Sandbox of this kind in the future to meet the real demand of local residents and ensure safer outcomes among them.

Other than being aware of the differential risk perception among local residents, policymakers should prepare to compensate various groups of population with different compensation packages or prepare to reduce the population's health risk with a special focus on residents who are more prone to risk or those with a high risk perception.

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Research article

PREDICTING THE RISK OF UTERINE FIBROIDS IN FEMALE COPPER SMELTER WORKERS BY CYTOGENETIC ABNORMALITIES OF BUCCAL EPITHELIUM

R.R. Sakhautdinova[1], T.V. Bushueva[1], V.G. Panov[2], V.I. Adrianovsky[1], V.B. Gurvich[1]

[1]Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, 30 Popov St., Yekaterinburg, 620014, Russian Federation
[2]Institute of Industrial Ecology, Ural Branch of the Russian Academy of Sciences, 20A Sofia Kovalevskaya St., Yekaterinburg, 620108, Russian Federation

Exposure to adverse occupational factors increases the risk of diseases of the reproductive system. Long-term studies have shown a high prevalence of uterine fibroids among all diseases of the reproductive system in women exposed to occupational hazards in industry. The occurrence of fibroids, having such complications as menorrhagia, pain in the pelvic area, and infertility, hampers performance and diminishes the quality of life. The risk of genetic mutations in cells playing a key role in the development of fibroids is raised by inhalation of industrial aerosols containing metals possessing mutagenic and carcinogenic potencies. Cytology of the buccal epithelium can be used as a method objectively indicating the genetic risk.

The purpose of the study was to develop a predictive model for assessing the risk of uterine fibroids in female workers exposed to aerosols containing metal particles at a copper smelter using buccal cytograms.

We examined 47 female workers of a copper smelter, of which 39 formed the main (exposed) group. Uterine fibroids were more frequent in the main group than in the controls (25.6 % versus 0.0 %, $p \leq 0.05$). Genotoxic indicators, such as protrusions and micronuclei, and cytotoxicity indicators were also significantly increased in this group (nuclear vacuolization and cytoplasmic vacuolation were 1.6 times and karyolysis 1.8 times higher compared with the controls, $p \leq 0.05$). We have developed two mathematical models for assessing the risk of uterine fibroids. Model 1 includes variables without restrictions of characteristics, i.e. micronuclei, perinuclear vacuoles, and cytoplasmic vacuolation (AUROC – 0.940), while Model 2 necessarily includes such genotoxic parameters as micronuclei and protrusions (AUROC – 0.883). The 4-member model (M1+M2), which includes both genotoxic (micronuclei and protrusions) and cytotoxic (binucleated cells and cytoplasmic vacuolation) indicators, has the highest significance (AUROC – 0.998).

The results indicate involvement of both genetic and cytotoxic mechanisms in the development of uterine fibroids in female workers exposed to toxic industrial aerosols. The models including both two indicators of genotoxicity (micronuclei and protrusion) and one and/or two cytotoxicity indicators have the greatest predictive value.

Keywords: *buccal epithelium, cytology, uterine fibroids, micronucleus test, cytogenetic index, genotoxicity, cytotoxicity, protrusions.*

Renata R. Sakhautdinova – Candidate of Medical Sciences, Head of the Diagnostic Laboratory Department, Research and Production Association of Laboratory Diagnostic Technologies (e-mail: sahautdinova@ymrc.ru; tel.: +7 (343) 253-14-58; ORCID: https://orcid.org/0000-0002-2726-9259).

Tatiana V. Bushueva – Candidate of Medical Sciences, Senior Researcher, Head of the Research and Production Association of Laboratory Diagnostic Technologies (e-mail: bushhueva@ymrc.ru; tel.: +7 (343) 253-14-58; ORCID: https://orcid.org/0000-0002-5872-2001).

Vladimir G. Panov – Candidate of Physical and Mathematical Sciences, Leading Researcher (e-mail: vpanov@ecko.uran.ru; tel.: +7 (343) 362-35-14; ORCID: https://orcid.org/0000-0001-6718-3217).

Vadim I. Adrianovsky – Candidate of Medical Sciences, Senior Researcher at the Laboratory of Epidemiology and Cancer Prevention of the Department of Occupational Medicine (e-mail: adrianovsky@k66.ru; tel.: +7 (343) 253-14-56; ORCID: https://orcid.org/0000-0001-7754-8910).

Vladimir B. Gurvich – Doctor of Medical Sciences, Scientific Director (e-mail: gurvich@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-6475-7753).

Reproductive health maintenance in working women has always been a priority. Exposure to industrial toxicants is considered an exogenous risk factor for diseases of the reproductive system, especially in cases of hormonal imbalance, obesity, pelvic inflammatory diseases, and low parity [1, 2]. Uterine fibroids are common growths of the womb accounting for 60–70 % of all uterine disorders. Working-age women have the highest incidence rate and the highest frequency of hysterectomies [3]. According to literature data, uterine fibroids tend to "rejuvenate" [4]. The epidemiology of the disease is greatly underestimated due to its asymptomatic stage in most patients. When studying the risks to the reproductive system, much attention is paid to the mechanisms of copper and lead reprotoxicity [5]. The pathogenetic role of industrial mutagens and carcinogens in the development of neoplasms of the reproductive system is still examined since chromosomal and gene mutations are detected in 90 % of cases [6, 7]. Chromosomal abnormalities correlate with tumor phenotypes. At present, informative non-invasive short-term tests are used for screening in both clinical practice and research. Cytological characteristics of the buccal epithelium are a biological indicator of the state of the body, which changes under adverse effects of chemical and biological factors in the industrial setting [8–14]. Functional pathological changes in the buccal epithelium correlate with indicators of impaired homeostasis and can also act as markers of precancer. In 2007, the international project known as the human MN assay in exfoliated cells (HUMN(xL)) was launched [15].

Exfoliative cytology of buccal mucosa is an *in vivo* technique of histological examination that can serve as a source of important diagnostic and prognostic information on health status, exposure to stress and adverse occupational factors; it can also be applied in oncology, dentistry, and pharmacology [16, 17]. Recent publications show that buccal cytograms are widely used to assess micronuclei in those exposed to potentially carcinogenic agents at workplaces [18–21]. According to the latest data on the leading role of the genetic mechanism in the development of uterine fibroids, substantiation of diagnostic criteria based on cytogenetic changes can be useful for early diagnosis of the disease in women exposed to industrial toxicants.

The **purpose** of the study was to develop a prognostic model for assessing the risk of uterine fibroids in female workers exposed to aerosols containing metal particles at a copper smelter using buccal cytograms.

Materials and methods. The study involved 47 female copper smelter workers, of which 39 formed the main group (mean age: 48.7 ± 1.7 years; duration of current employment: 14.8 ± 0.5 years) exposed to adverse occupational factors, the leading one being industrial aerosol of complex chemical composition containing particles of copper, lead, nickel, iron, silicon dioxide, arsenic, etc. The cases worked as crane, hydrometallurgy, and rare metal production equipment operators. According to the results of special assessment of working conditions, crane operators of the anode section of the copper smelter are exposed to the aerosol containing copper, which measured concentration (1.58 mg/m^3) is thrice as high as the maximum allowable concentration (MAC = 0.5 mg/m^3, class 3.2 of working conditions); nickel and its oxides (0.058 mg/m^3), which levels are 1.16 times higher than the MAC (0.05 mg/m^3, class 3.1), and silicon dioxide (< MAC, class 2.0). Hydrometallurgists in the copper sulfate shop of the copper section are exposed to nickel salts in the form of hydrated aerosol (0.006 mg/m^3, class 3.2), copper and sulfuric acid (< MAC, class 2). Operators of the sludge processing section in the production of rare metals of the chemical and metallurgical section are exposed to nickel and its oxides, lead and its inorganic compounds, cadmium and its inorganic compounds (< MAC, class 2), and sulfuric acid, which level (1.8 mg/m^3) is 1.8 times higher than the time-weighted average

concentration (1.0 mg/m^3, class 3.1 of working conditions). The above chemicals have proven carcinogenic potencies (cadmium, lead, nickel, and sulfuric acid) and may have an adverse effect on the reproductive system (copper and lead). The control group consisted of eight female office workers (mean age: 40.1 ± 0.92 years; duration of current employment: 9.25 ± 0.64 years) unexposed to occupational toxicants.

Exfoliated buccal epithelial cells were scraped from the inner cheeks. The material collected with the cytobrush was evenly spread onto a glass slide, dried naturally, and stained according to Pappenheim. The preparations were then analyzed using a Carl Zeiss Primo Star microscope with 16×40 and 16×100 magnifications. Cytogenetic, proliferation, and nuclear destruction indicators were counted per 1,000 cells with the results expressed in ppm (‰) [22]. To assess the risk of cytogenetic abnormalities in buccal epithelial cells in the study groups, we calculated the index of accumulation of cytogenetic damage (*Iac*) using the formula (1) representing the product of the cytogenetic index (*Ic*) and the index of proliferation (*Ip*), divided by the apoptotic index (*Iapop*):

$$Iac = (Ic \cdot Ip / Iapop) \cdot 100, \qquad (1)$$

where *Ic* is the sum of cells with micronuclei and protrusions; *Ip* is the sum of cells with two or more nuclei; *Iapop* is the sum of all cells involved in apoptosis (chromatin condensation, karyopyknosis, karyorrhexis, karyolysis, or apoptotic bodies). Based on the index of accumulation of cytogenetic damage, risk groups were classified as follows: $Iac \leq 2$ – low, $2 < Iac < 4$ – moderate, and $Iac \geq 4$ – high risk [15].

Statistica 6.0 was used for data analysis with the nonparametric Mann – Whitney test applied to assess the significance of differences. We also used ROC analysis to identify cytological features of diagnostic value.

To address the impact of accumulated cytogenetic abnormalities on the risk of uterine fibroids in women exposed to industrial aerosols, we developed a prognostic model of binary classification including logistic regression with the linear relationship with the specified variables (formula 2) or with the linear relationship with cross-terms added (formula 3):

$$y = \frac{\exp\left(b_0 + b_1 x_1 + \cdots + b_n x_n\right)}{1 + \exp\left(b_0 + b_1 x_1 + \cdots + b_n x_n\right)} \qquad (2)$$

$$y = \frac{\exp\left(b_0 + b_1 x_1 + \cdots + b_n x_n + b_{12} x_1 x_2 + b_{13} x_1 x_3 + \cdots b_{n-1n} x_{n-1} x_n\right)}{1 + \exp\left(b_0 + b_1 x_1 + \cdots + b_n x_n + b_{12} x_1 x_2 + b_{13} x_1 x_3 + \cdots b_{n-1n} x_{n-1} x_n\right)}, \qquad (3)$$

where y is the variable showing the probability of the presence ($y = 1$) or absence ($y = 0$) of uterine fibroids; $x_1, x_2, \ldots, x_n$ are independent variables (cytogenetic indicators), and b_0, b_1, … b_n are theoretical coefficients.

We considered the models with three and more and four and more variables since with the same quality of models, preference should be given to that with a smaller number of variables. The McFadden likelihood ratio index (or McFadden's pseudo-R^2 coefficient), hereinafter referred to as pR^2, was chosen as the quality criterion for models (2) or (3). For this coefficient, models with pR^2 values of 0.2 to 0.4 are already considered effective. The mandatory prerequisite for choosing the effective model was the inclusion of cytogenetic indicators (micronuclei and protrusions), without excluding introduction of cytotoxic anomalies into the calculations.

Results and discussion. In the exposed group, uterine fibroids were statistically more frequent than in the controls (25.6 % versus 0, $p \leq 0.05$). Given the facts that myoma is a multifactorial disease and the role of the genetic factor has not been definitely established so far, it is important to select cytometry variables that primarily indicate the genetic risk. To begin with, we compared changes in the cytogenetic status in all workers. Table 1 shows that the exposed women demonstrated

Table 1

Cytogram of buccal epithelium in female copper smelter workers exposed to occupational chemicals and the controls (‰)

Indicators	Controls, $n = 8$	Cases, $n = 39$
Micronuclei	1.88 ± 0.23	4.00 ± 0.12 *
Protrusions	3.13 ± 0.44	5.72 ± 0.20 *
Binucleated cells	2.63 ± 0.26	3.67 ± 0.15 *
Perinuclear vacuole	1.25 ± 0.16	1.56 ± 0.09
Nuclear condensation	3.88 ± 0.30	5.21 ± 0.20 *
Nuclear vacuolization	3.88 ± 0.40	6.59 ± 0.19 *
Cytoplasmic vacuolization	4.50 ± 0.33	7.59 ± 0.20 *
Karyolysis	2.75 ± 0.25	4.97 ± 0.19 *

Note: * $p < 0.05$.

Table 2

Quality indicators of the binary classifier based on logistic regression with three and more variables when choosing the best model based on pR^2 without variable restrictions (M_1 model) and including "micronuclei" and "protrusions" variables (M_2 model)

Quality indicators	Best fit	
	M_1	M_2
	Micronuclei, perinuclear vacuole, cytoplasmic vacuolization	Micronuclei, protrusions, binucleated cells
pR^2	0.570	0.383
AUROC	0.940	0.883
Sensitivity (TPR)	0.900	0.800
Specificity (TNR)	0.828	0.759
Matthews correlation coefficient	0.662	0.501
Diagnostic odds ratio	43.200	12.571
Youden's index	0.728	0.559

an increase in such genotoxic indices as micronuclei and protrusions by 2.2 and 1.8 times, respectively, a 1.3-fold increase in the number of binucleated cells ($p < 0.05$). They also showed changes in early and late destruction of the nucleus and cytoplasm compared to the control group. Their indices of nuclear vacuolization and cytoplasm vacuolization were 1.6 times, karyolysis – 1.8 times, and nuclear condensation – 1.3 times higher than in the controls. The above findings give evidence of a cytotoxic effect.

The index of accumulation of cytogenetic damage in the main group was 3.3 against 1.7 in the controls and corresponded to moderate risk ($2 < Iac < 4$).

To assess the genotoxic risk of uterine fibroids, we chose the prognostic model based on the cytogenetic abnormalities found.

The comparison of two models based on logistic regression with three and more variables described in Table 2 showed that the best model without choosing variable restrictions had the McFadden's coefficient of determination of 0.570 while in that with micronuclei and protrusions included as variables pR^2 equaled 0.383 with the area under the ROC curve of 0.940 and 0.883, respectively (Figure 1).

Despite the differences in the coefficients of determination, characteristics of the models with/without genotoxic indicators are quite similar, which can be considered as the aptness of mandatory inclusion of specific indicators of genotoxic effects in the model for determining the risk of uterine fibroids in the study population.

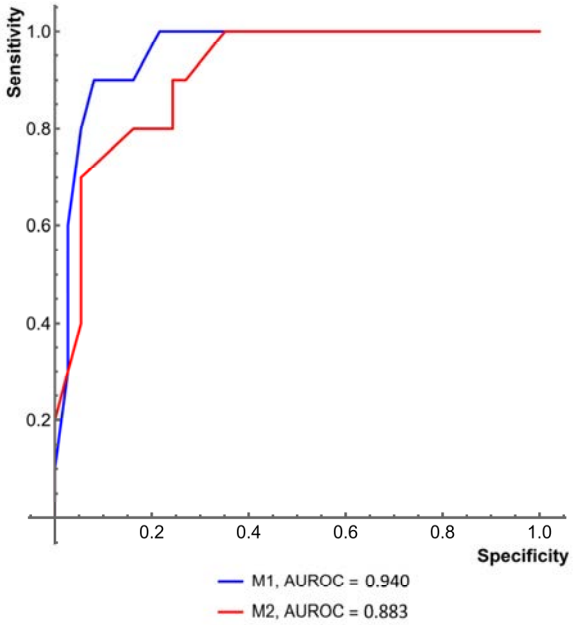

Figure 1. ROC-curves for M_1 and M_2 models from Table 2

When including four variables in the model, the coefficient of determination has little significance (pR^2 = 0.105 for the best model), but when including two genotoxicity and two cytotoxicity indicators, it improves significantly (pR^2 = 0.938 for the best model of four variables selected from all predictors). Thus, this model has the greatest diagnostic value (Figure 2). The model is described in Table 3.

One of the risk factors for female reproductive health in copper smelting is the aerosol of complex chemical composition. Lead, nickel and cadmium, which are part of the aerosol, are known to be both mutagenic and reprotoxic substances inducing chromosomal aberrations in somatic and germ cells [23]. According to the literature data, exposure to high copper concentrations causes an increase in the frequency of mitotic aberrations in cells. High exposures to nickel compounds, which also have a clastogenic effect, lead to the formation of micronuclei. The mechanism of action is based on DNA rupture and generation of DNA-protein crosslinks. An increase in the frequency of sister chromatid exchange and chromosomal aberrations is also noted. Cadmium and its compounds raise the frequency of micronuclei and chromosomal aberrations. Some mechanisms of action include inhibition of DNA repair systems, effects on cell proliferation and functioning of tumor suppressors. When exposed to sulfuric acid, binding to DNA molecules occurs, which leads to disruption of DNA chain division. Since uterine fibroids have multiple etiologies, with the molecular genetic mechanism being one of them, the study of genotoxic effects of the above chemicals on myometrium and endometrium cells remains relevant. According to foreign scientists, copper and lead "…could negatively affect endometrial functionality, compromising the decidualization

Table 3

Quality indicators of the binary classifier based on logistic regression with four variables when choosing the best model based on pR^2 without variable restrictions (M_1 model) and including the variables "micronuclei" and "protrusions" (M_2 model)

Quality indicators	Best fit
	$M_1 = M_2$
	Micronuclei, protrusions, binucleated cells, cytoplasmic vacuolization
pR^2	0.938
AUROC	0.998
Sensitivity (TPR)	1.000
Specificity (TNR)	0.966
Matthews correlation coefficient	0.937
Diagnostic odds ratio	$+\infty$
Youden's index	0.966

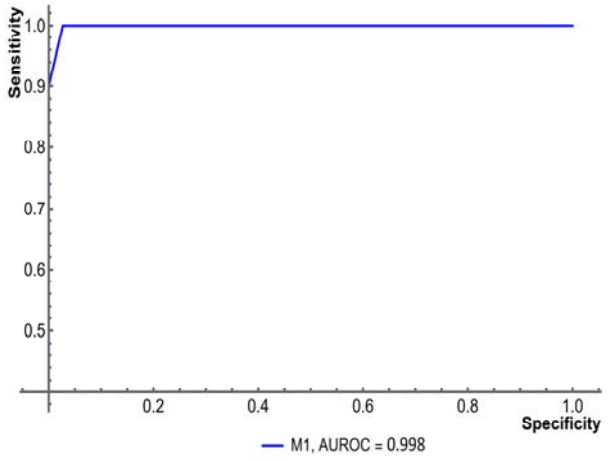

Figure 2. ROC-curve for the $M_1 = M_2$ model from Table 3

process and disrupting endometrial regeneration and embryo adhesion" [24], thus increasing the risk of endometrial cancer. The relationship between blood concentrations of cadmium and its compounds and uterine fibroid volume was also proven [25]. Nickel and its compounds affect endometrial cells and increase the risk of endometriosis. In this regard, it is worth emphasizing that the micronucleus test of buccal epithelium reveals micronuclei in cells that are formed directly as a result of DNA damage following exposure to industrial toxicants. Micronuclei are known to be formed via different pathways: as a result of DNA damage, thus representing a chromosome fragment, or damage to the spindle of division in the form of one or more whole chromosomes, which may indicate both gene and genomic disorders. Our findings showing an increase in the number of cytogenetic disorders, such as micronuclei and protrusions in the female copper smelter workers, are consistent with the results of previous studies and confirm genotoxic properties of industrial toxicants, which may be a risk factor of uterine fibroids. An increased frequency of binucleated cells in the workers is also associated with toxic effects of chemical compounds. It is also known that binucleated cells are formed as a result of nuclear division that takes place without cytoplasmic division, while their ploidy increases. The frequency of binucleated cells is an indicator of proliferative activity [26]. Thus, the variables included in the prognostic model, such as micronuclei and protrusions, can play the leading role in identifying risk factors of uterine fibroids. However, exposure to industrial chemicals can develop acquired cytotoxic effects and promote congenital genotoxic effects. The established increase in the number of cells with early and late destruction also allow us to use these variables to assess the risk of endometrial pathology. Again, since uterine fibroids have multiple potential etiologies, the role of the endocrine system in the mechanisms of their development is among the leading ones. Heavy metals within industrial aerosols have a toxic effect on the hypothalamic-pituitary system of the body. Yet, the mechanisms of genotoxic and cytotoxic effects of heavy metal aerosols have not been fully studied [27]. Our results can therefore be used to identify groups at risk of uterine fibroids in female workers exposed to occupational hazards. The presented methodology for using buccal cytograms to establish groups at risk of uterine fibroids in female workers of a copper smelter and the study results support our hypothesis that geno- and cytotoxicity of industrial toxicants in relation to buccal epithelial cells are similar in mechanism to the effects of developing pathological processes in myometrium, which allows their extrapolation. Inclusion of data on cytogenetic and hormonal status in the developed model for predicting the risk of uterine fibroids is relevant and significant for the tasks of forming groups at risk of health problems in female workers exposed to aerosols containing mutagenic and reprotoxic elements.

Conclusions:

1. Genotoxic and cytotoxic indices in buccal cytograms are significantly more frequent in women exposed to industrial aerosols containing mutagenic and reprotoxic elements compared to unexposed workers.

2. The risk of uterine fibroids can be calculated using the mathematical model that includes genotoxic and cytotoxic indices.

3. Calculation of the parameters of the model that includes genotoxic prognosis indices and, above all, such variables as micronuclei and protrusions play a decisive role in identifying risk factors of uterine fibroids.

4. It is expedient to continue the research by introducing hormonal status parameters into the models.

5. These models are relevant for the tasks of forming groups at risk of health disorders among female workers exposed to aerosols containing mutagenic and reprotoxic elements.

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Research article

ASSESSMENT OF BIOACCUMULATION AND TOXIC EFFECTS OF COBALT (II) ALUMINATE NANOPARTICLES FOR HYGIENIC SAFETY PURPOSES

M.A. Zemlyanova[1,2], M.S. Stepankov[1], O.V. Pustovalova[1], A.V. Nedoshitova[1]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya St., Perm, 614045, Russian Federation
[2]Perm State University, 15 Bukireva St., Perm, 614068, Russian Federation

Hygienic safety plays an important role in preventing health harm under chemical exposures. Hygienic regulation of levels of existing and new substances in environmental objects is the core element here carried out within experimental research aimed at establishing their toxic properties. Cobalt (II) aluminate nanoparticles ($CoAl_2O_4$ NPs) are a typical example of a new material with presumably higher toxic potential upon oral exposure as opposed to micro-sized particles (MPs). Given that, the development of safety standards requires identifying features of the negative impact of $CoAl_2O_4$ NPs, which are different from MPs upon oral exposure.

The study was performed on Wistar rats orally exposed to NPs and MPs for 20 days at the total dose of 10,550 mg/kg of body weight.

NPs have chemical composition similar to MPs, smaller size (87.11 times) and larger specific surface area (1.74 times). NPs have a more pronounced ability to bioaccumulate in the heart, lungs, liver and kidneys as compared to MPs (up to 7.54 times). Exposure to NPs resulted in more pronounced (up to 3.60 times) changes in blood indicators associated with developing redox imbalance, cytotoxic effect, liver, pancreas and kidney dysfunction, inflammatory process, and thrombocytopenia. NPs caused hemorrhagic infarcts and pulmonary edema not established upon MPs exposures. The calculated value of the tentatively permissible exposure level (TPEL) was 0.02 mg/dm^3 for these NPs content in drinking water, which is 10 times lower than the same value for MPs.

Thus, $CoAl_2O_4$ NPs upon oral exposure for 20 days at the total dose of 10,550 mg/kg of body weight have more marked bioaccumulation relative to MPs, which causes more pronounced negative effects identified by changes in blood indicators and developing pathomorphological changes. The study findings allow increasing accuracy and objectivity when developing safety standards for $CoAl_2O_4$ levels in food products and drinking water to ensure greater hygienic safety of the population.

Keywords: *hygienic safety, cobalt (II) aluminate, nanoparticles, microparticles, oral exposure, bioaccumulation, morphofunctional impairments, rats.*

New materials are being implemented in various economic activities everywhere. Given that, it is becoming especially relevant to develop essentials of hygienic safety to prevent health disorders among exposed population. Hygienic regulation of chemical levels in environmental objects is the core element; it should be based on results of experimental research aimed at investigating and estimating peculiarities of bioaccumulation and toxic effects of these new materials.

Nano-sized oxides with a spinel-like structure are a typical example of a new material, such as Al_2O_3 and CoO alloy as cobalt (II) aluminate nanoparticles ($CoAl_2O_4$ NPs). $CoAl_2O_4$ NPs are used as a pigment in various productions; as a catalyzer in chemical industry; as a component in microelectronics or

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).

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).

Olga V. Pustovalova – Senior Researcher at the Laboratory of Biochemical and Nanosensor Diagnostics (e-mail: pustovalova@fcrisk.ru; tel.: +7 (342) 237-18-15; ORCID: https://orcid.org/0000-0002-8050-7674).

Anna V. Nedoshitova – Senior Researcher at the Laboratory of Elemental Analysis Methods (e-mail: nedoshitova@fcrisk.ru; tel.: +7 (342) 237-10-37; ORCID: https://orcid.org/0000-0001-6514-7239).

production of construction materials; in casting processes in metallurgy[1] [1, 2]. At present, a possibility to use $CoAl_2O_4$ NPs in food industry is being investigating quite actively where the material can serve as supports for inulinase immobilization in sugar production by inulin hydrolysis [3] as well as a catalyzer in biofuel synthesis [4].

A wide range of possible applications in various industries and prospects of use in food industry create elevated risks of exposure to $CoAl_2O_4$ NPs for population including intake with foods. According to the data available in research literature and results obtained by our own experimental research, isolated $CoAl_2O_4$ NPs were established to mostly be able to penetrate through the gut-vascular barrier upon oral exposure and accumulate in target organs due to their smaller size against micro-sized particles (MPs) of chemical analogues [5, 6]. These NPs produce more substantial negative effects due to greater bioaccumulation and high reactivity. The results reported in these studies allow assuming that an alloy represented by $CoAl_2O_4$ NPs can be more toxic in comparison with its micro-sized chemical analogue. All this confirms the necessity to conduct experimental research aimed at establishing key peculiarities of toxicokinetics and toxicodynamics of $CoAl_2O_4$ NPs, which are different from those of MPs. The ultimate aim is to develop more precise and objective regulations of levels of the analyzed chemical in environmental objects.

In this study, our aim was to estimate peculiarities of bioaccumulation and toxic effects produced by $CoAl_2O_4$ NPs upon multiple oral exposures.

Materials and methods. $CoAl_2O_4$ NPs powder was used as a nanomaterial in experiments; it was synthesized by Giredmet JSC (Russia) by request from the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. NPs were synthesized by the sol-gel process from nitro-acid 6-water cobalt (II) ($Co(NO_3)_2 \cdot 6H_2O$) and nitro-acid 9-water aluminum ($Al(NO_3)_3 \cdot 9H_2O$). A commercial MP powder produced by Khimkraft LLC (Russia) was used as a micro-sized chemical analogue.

Physiochemical properties of both materials (MPs and NPs) were compared to confirm the alleged chemical structure of the synthesized material and possibility to consider it a nano-material. The chemical structure of the alloy component was identified by x-ray spectral microprobe analysis; particle sizes were determined by analyzing images made with scanning electron microscopy; specific surface areas were identified in accordance with the Brunauer–Emmett–Teller methodology.

Experiments were conducted on female Wistar rats with average bodyweight of 244.4 ± 7.6. All experiments on animals were performed in conformity with the requirements fixed in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS № 123). The research was approved by the Ethics Committee of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. Prior to the experiments, all rats were acclimatized to keeping conditions for 14 days.

A median lethal dose (LD_{50}) upon single administration was established to determine an initial dose of the analyzed materials upon multiple oral exposures. To do that, an experiment was conducted to simulate an acute oral exposure in accordance with the State Standard GOST 32644-2014[2]. Three groups

[1] NN1948 Cobalt Aluminum Oxide Nano Powder. *Stanford Advanced Materials*. Available at: https://www.samaterials.com/micro-nano-materials/1948-cobalt-aluminum-oxide-nano-powder.html (November 06, 2023); Cobalt Aluminate Blue Spinel Nanopowder CoAl2O4 Size: 300-500 nm. *Nanografi*. Available at: https://nanografi.com/nanoparticles/cobalt-aluminate-blue-spinel-nanopowder-coal2o4-size-300-500-nm/ (December 06, 2023).

[2] GOST 32644-2014. OECD guidelines for the testing of chemicals. Acute oral toxicity – acute toxic class method: interstate standard, approved by the Interstate Council on Standardization, Metrology and Certification (the meeting report issued on March 28, 2014 No. 65-P). *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200115815 (March 18, 2024) (in Russian).

were made, 6 rats in each (exposure to NPs, exposure to MPs and controls). $CoAl_2O_4$ NPs and MPs were administered in a dose of 2000 mg/kg of body weight in a suspension based on bi-distilled water. The suspension was administered one time in a volume of 1 cm^3 through a probe made of stainless steel. The experimental animals were observed for 14 days after the exposure to estimate death rates in dynamics. According to survival rates, the LD_{50} value was > 2000 mg/kg of body weight.

Three groups, 10 animals in each, were created in the same manner to conduct experiments aimed at examining multiple oral exposures. Exposures were performed daily in accordance with the Methodical guidelines MU 1.2.2520-09[3] and the methodology developed by Lim with colleagues. Exposure duration was 20 days and the initial dose was 1/10 (200 mg/kg of body weight) of the established LD_{50}. The dose was increased by 1.5 times against the pervious one every 4 days. Therefore, the total NP and MP doses received by the experimental animals reached 10,550 mg/kg of body weight.

Blood samples were taken from the rats 24 hours after the last administration of the tested materials to examine their biochemical and hematological indicators. Organs were taken out to determine aluminum and cobalt levels and to identify pathomorphological changes in tissues.

Significant differences in biodistribution, bioaccumulation, biochemical and hematological blood indicators were established by the Mann – Whitney method using the U-test in STATISTICA 10. The results were considered significant at $p \leq 0.05$.

Tentative Allowable Levels (TALs) for $CoAl_2O_4$ NPs and MPs in drinking water were calculated using their toxicological hazard as a basic criterion in accordance with the Methodical guidelines MU 2.1.5.720-98[4].

Results and discussion. Comparative analysis of the chemical structure of the tested materials confirmed that they contained cobalt, aluminum and oxygen without any admixtures. This corresponds to the chemical formula stated by the manufacturers (Figure 1).

Examination of physical properties established that the nanopowder contained nano-sized particles (1–100 nanometers, 78.25 % of the total particle number), which were not found in the micropowder. NPs, as opposed to MPs, had 87.11 times smaller average size (52.12 / 4540 nanometers) and 1.74 times greater specific surface area (14.51 / 8.33 m^2/g). NP and MP images obtained with SEM are shown in Figure 2.

Upon multiple oral exposures, $CoAl_2O_4$ NPs were established to be distributed in the heart, lungs, liver, kidneys, brain and blood of the exposed rats. The total aluminum and cobalt levels were 1.57–242.69 times higher ($p = 0.002$) against the controls. Upon exposure to MPs, the total levels of the analyzed chemicals were 1.94–32.20 times higher ($p = 0.002$) in the foregoing organs and blood against the controls.

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[3] MU 1.2.2520-09. Toksikologo-gigienicheskaya otsenka bezopasnosti nanomaterialov: metodicheskie ukazaniya, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 05 iyunya 2009 g., vved. v deistvie s 05 iyunya 2009 g. [Toxicological and hygienic assessment of nanomaterial safety: Methodical guidelines, approved by G.G. Onishchenko, Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing and RF Chief Sanitary Inspector on June 05, 2009, became valid on June 05, 2009]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200074057 (March 18, 2024) (in Russian).

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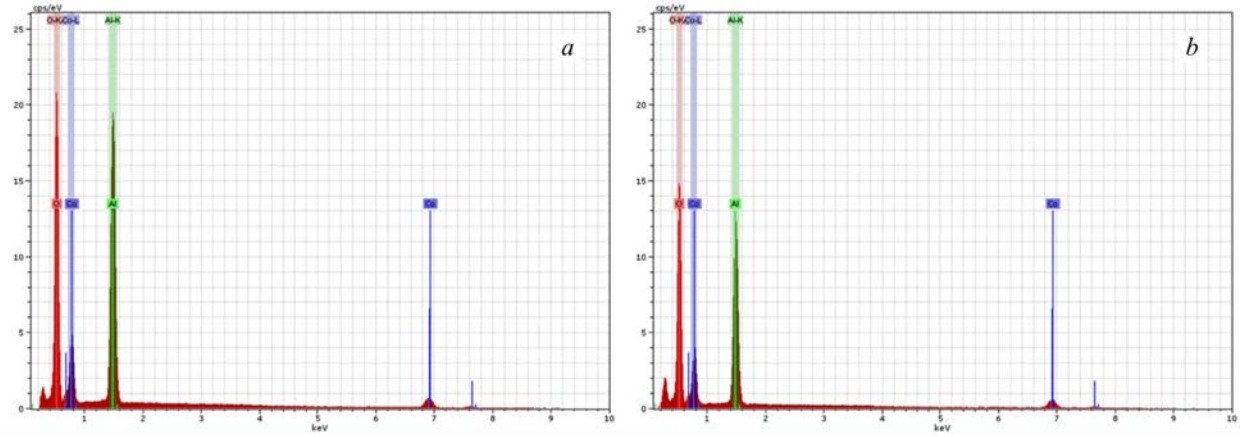

Figure 1. X-ray images of $CoAl_2O_4$ alloy samples: *a*, nano-sized; *b*, micro-sized

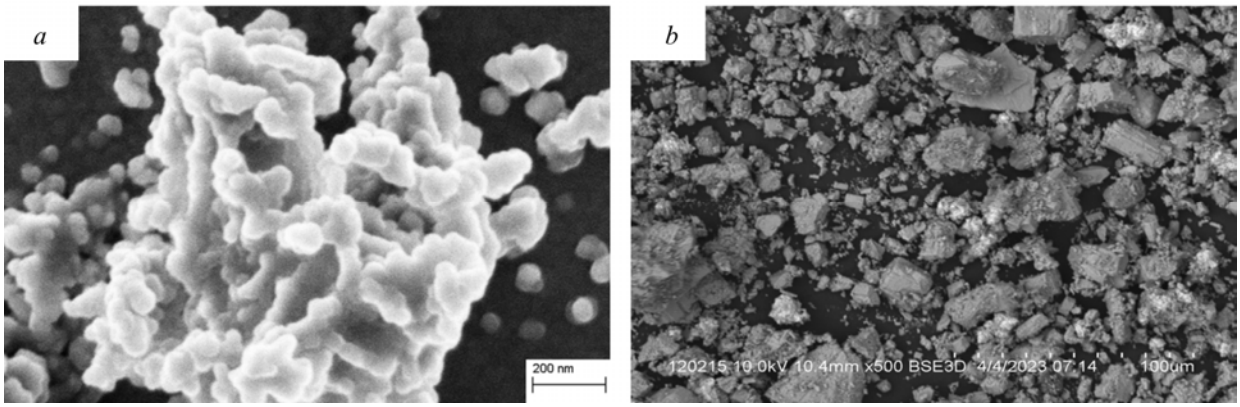

Figure 2. Images of $CoAl_2O_4$ alloy particles obtained by SEM: *a* is nanopowder; *b* is micropowder

Comparison of the obtained results established more marked bioaccumulation of $CoAl_2O_4$ NPs against their micro-sized chemical analogue judging from higher levels of aluminum and cobalt in the heart, lungs, liver and kidneys, 2.66 (p = 0.014), 7.54 (p = 0.001), 1.29 (p = 0.001) and 2.06 times (p = 0.001) respectively. The results of examining biodistribution and bioaccumulation are shown in Figure 3.

We established changes in biochemical blood indicators in the rats exposed to $CoAl_2O_4$ NPs, namely, 1.30–6.23 times higher activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), amylase, lactate dehydrogenase (LDH) and levels of creatinine, C-reactive protein (CRP) and malonic dialdehyde (MDA) (p = 0.002) as well as 1.99 times lower total antioxidant activity (AOA) (p = 0.005) against the controls. Exposure to the micro-sized chemical analogue led to 1.24–6.30 times higher activity of ALT, AST, GGT, amylase, and LDH and creatinine level (p = 0.002–0.012) as well as 2.31 times lower AOA (p = 0.002) against the same biochemical indicators in the control group. Comparative analysis of biochemical indicators established 1.37–2.25 times higher activity of ALT, AST, amylase, LDH and higher MDA levels (p = 0.001–0.014) upon exposure to NPs against MPs. The results of biochemical blood tests obtained for the experimental rats are shown in Figure 4.

Several changes were observed in hematological indicators in the rats exposed to NPs including 3.57–5.98 times lower platelet count (PLT), plateletcrit (PCT), and platelet large cell count (P-LCC) (p = 0.002) against the controls. These indicators were 1.66–1.79

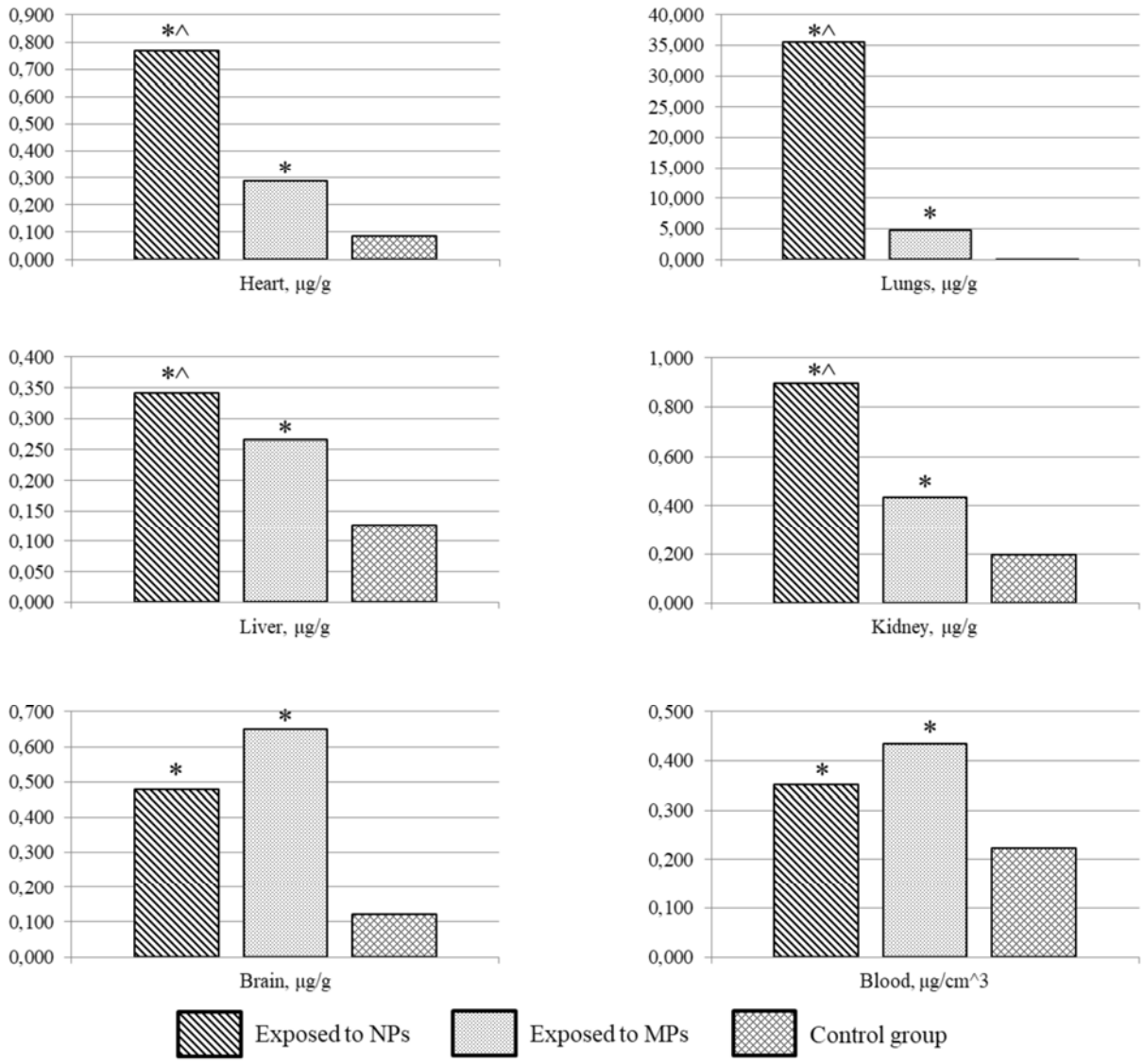

Figure 3. Total aluminum and cobalt levels in organs and tissues ($p \leq 0.05$): * means significant difference from the controls, ^ means significant difference from exposed to MPs

times lower in the rats exposed to MPs ($p = 0.045$). PLT, PCT, and P-LCC that were 2.00–3.60 times lower were a distinctive feature of effects produced by NPs on hematological blood indicators ($p = 0.001$–0.014). Figure 5 provides the results obtained by hematological blood tests.

We performed histological examination of organ tissues taken out of the rats exposed to NPs and MPs. As a result, pathomorphological changes were detected in the lungs, namely, focal interstitial pneumonia, bronchitis, vasculitis, and hyperemia; acute venous hyperemia was identified in the liver. Hemorrhagic infarctions and lung edemas were a distinctive feature upon exposure to NPs since they were not detected upon exposure to the micro-sized chemical analogue (Figure 6). We did not establish any pathomorphological changes in heart, kidney or brain tissues.

According to our calculations, TPEL of $CoAl_2O_4$ NPs amounted to 0.02 mg/md^3 and MPs 0.2 mg/dm^3 in drinking water per toxicological hazard they posed.

Analytical generalization of the results obtained by the experimental research established

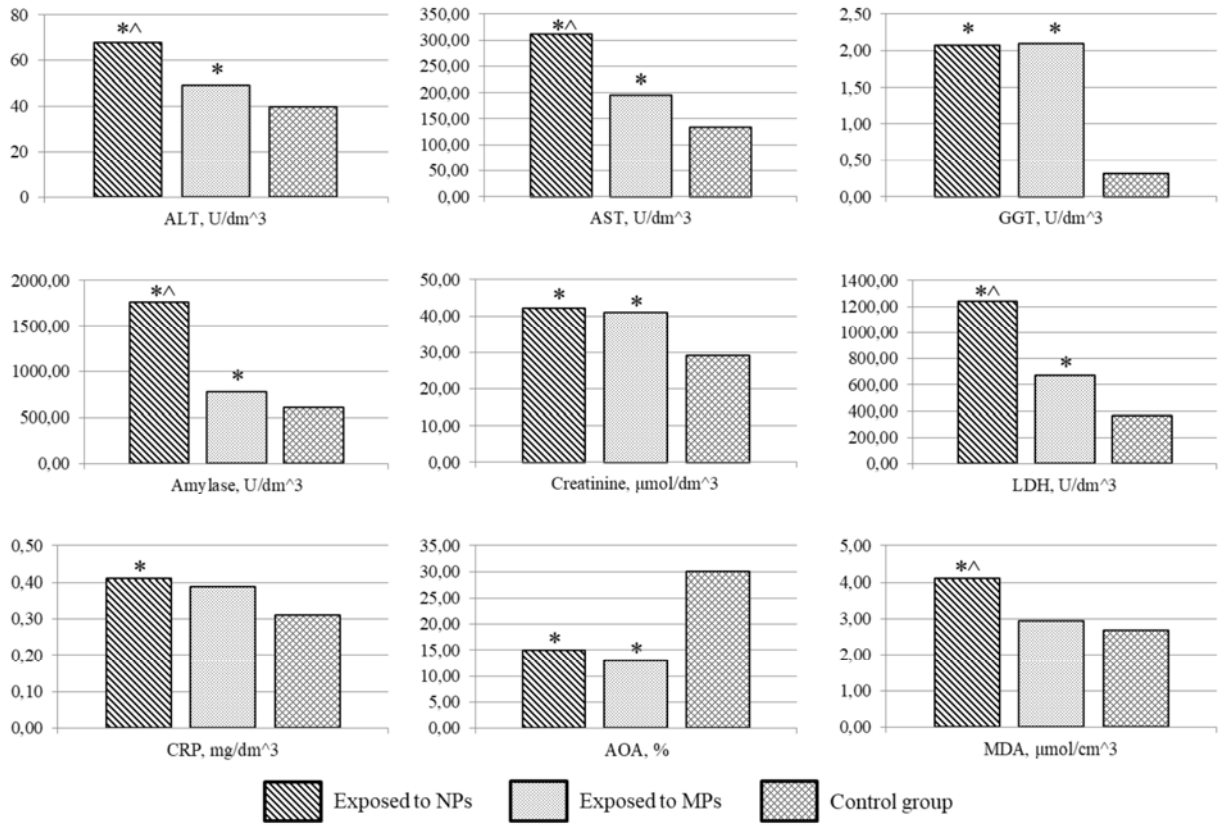

Figure 4. Biochemical indicators of rats' blood ($p \leq 0.05$): * means significant difference from the controls, ^ means significant difference from exposed to MPs

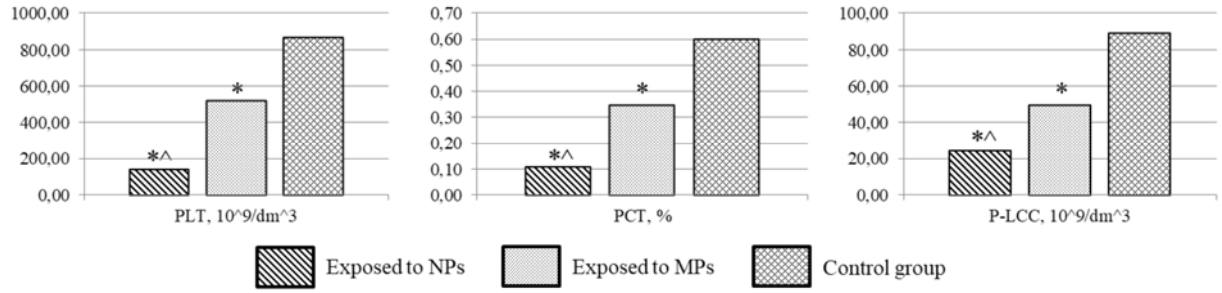

Figure 5. Hematological indicators of rats' blood ($p \leq 0.05$): * means significant difference from the controls, ^ means significant difference from exposed to MPs

that NPs of the tested $CoAl_2O_4$ powder had considerably different physical properties (up to 87.11 times) against their micro-sized chemical analogue as regards their size and specific surface area. This allows concluding that the tested nanomaterial is able to accumulate in target organs in larger quantities and induce more pronounced morphofucntional disorders [7, 8].

When considering a mechanism of $CoAl_2O_4$ NPs biodistribution and bioaccumulation upon oral administration, attention should be paid to solubility of the nanomaterial in the stomach, intestines, and blood. A study with its focus on examining cobalt bioavailability reported that levels of Co^{2+} ions did not exceed 0.1 % in artificial fluids that imitated gastric, intestine, and serum media after the tested alloy had been administered [9]. Low levels of Co^{2+} ions indicate that $CoAl_2O_4$ NPs are not soluble in the gastrointestinal tract or blood. Given that, increased aluminum and cobalt levels in organs

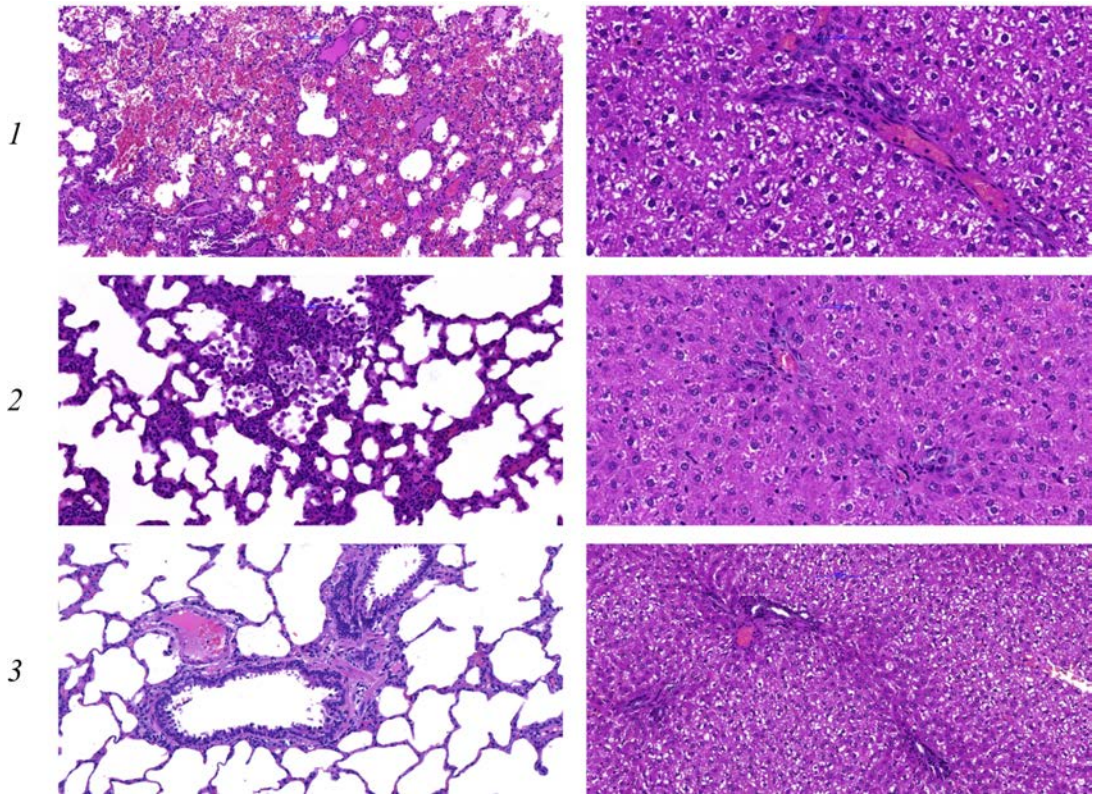

Figure 6. Microimages of tissue specimens of rats' lungs (*a*) and liver (*b*), hematoxylin and eosin stains, magnification 200x: 1 is exposed to NPs; 2 is exposed to MPs; 3 is the controls

are likely to be due to solid NPs without active participation of Al^{3+} and Co^{2+} ions in bioaccumulation.

Both $CoAl_2O_4$ NPs and MPs can penetrate through the gut-vascular barrier. This fact was evidenced by an increase in the combined aluminum and cobalt levels in the exposed rats' blood (~2 times against the controls). Blood flow carries NPs and MPs over the whole body where they are predominantly distributed in the heart, lungs, liver, kidneys, and brain (the total NP levels are up to 242.69 times higher and MP level up to 32.2 times higher against the control). NPs have a more pronounced ability to bioaccumulate (up to 7.54 times higher) in the heart, lungs, liver, and kidneys against their micro-sized analogue.

Scientific literature hardly contains any data on negative effects associated with exposure to $CoAl_2O_4$ NPs and mechanisms of their development. However, both in vitro and in vivo studies established that isolated Al_2O_3 and CoO NPs were able to stimulate generation of free radicals thereby inducing oxidative stress development [10–14]. Results reported in a study [15] also confirm this mechanism under exposure to an alloy of these two chemicals. Exposure to $CoAl_2O_4$ NPs reinforces oxidative reactions and inhibits recovery processes as evidenced by greater MDA activity and weaker AOA (~ up to 2 times against the control). Imbalance between oxidative reactions and recovery processes is more pronounced upon exposure to a nanomaterial against its micro-sized analogue (~ by 1.4 times), which may result in more marked cytotoxicity.

Greater cytolytic activity evidenced by more active LDH, ALT, and AST (up to 3.38 times against the control) indicates that cytotoxic effects develop under exposure to NPs and MPs. Changes induced by NPs are more pronounced (up to 1.85 times), which confirms their greater cytotoxicity against micro-sized materials. Changes in cell membrane per-

meability are a typical manifestation of cytotoxic effects produced by various nano-sized metal oxides [16]. An established increase in activity of such enzymes as ALT, AST and GGT in blood, which are normally localized in liver cells, can indicate increased permeability of hepatocyte membrane and, consequently, liver dysfunction [17–20]. Changes in indicators associated with hepatotoxic effects of $CoAl_2O_4$ NPs are more marked (up to 1.58 times) against influence exerted by MPs. Liver dysfunction may lead to weaker synthesis of thrombopoietin, which regulates platelet production [21]. This assumption is supported by thrombocytopenia that was established in exposed rats' blood and evidenced by lower PLT, PCT, and P-LCC (upon exposure to NPs, ~ up to 6 times against the control: upon exposure to MPs, ~ up to 2 times). Platelet indicators decreased more markedly upon exposure to NPs (up to 3.60 times against exposure to MPs). A reduction in PLT in blood can have negative effects on angiotropic, adhesive-aggregation, and fibrinolytic processes as well as immunity[5].

Cytotoxic effects caused by redox imbalance can manifest themselves as an inflammation, which is induced by pro-inflammatory cytokines released from cells due to oxidative damage done by free radicals [22]. In this study, developing inflammation was identified per growing CRP levels in blood of the rats exposed to NPs (1.3 times higher against the control) whereas exposure to MPs did not cause any significant changes in the indicator. Studies [23–25] highlight the role of inflammatory changes associated with oxidative stress in impaired functioning of the pancreas and kidneys. This is consistent with established growing activity of amylase and creatinine upon exposure to NPs and MPs (up to 2.91 and 1.41 times respectively against the controls). Effects produced by the analyzed nanomaterial on the pancreas functioning were more marked relative to effects of MPs (by 2.25 times).

$CoAl_2O_4$ NPs and MPs produce negative effects at the tissue-organ level manifested as inflammation and disrupted circulation. Exposure to NPs induces more marked disruptions of circulation in the lungs, which was evidenced by hemorrhagic infarctions and edemas. No such effects were observed in the rats exposed to MPs. This may be associated with greater NP cytotoxicity. Pro-inflammatory cytokines released from cells under exposure to NPs can increase permeability of vascular endothelium, which results in blood exudation into an organ [26, 27]. Therefore, processes that make for circulation disruptions become more pronounced as cytotoxic effects are aggravated.

The valid sanitary rules and norms[6] do not stipulate maximum permissible levels or TPEL in drinking water either for NPs or MPs of $CoAl_2O_4$ alloy. Calculated TPEL amounted to 0.2 mg/dm^3 for micro-sized particles. Considering unique physical property and more pronounced toxicity of NPs against MPs, it is advisable to reduce this calculated value by 10 times (0.02 mg/dm^3).

Conclusion. According to the results of the present study, $CoAl_2O_4$ NPs have smaller sizes (87.11 times) and greater specific surface area (1.74 times) against their micro-sized chemical analogue. Due to these properties, they have greater ability to bioaccumulate in the heart, lungs, liver and kidneys (up to 7.54 times) upon oral administration into the body during 20 days in the total dose of 10,550 mg/kg. Greater NPs bioaccumulation creates more pronounced negative effects

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such as intensified oxidation (~ 1.4 times higher), cytotoxicity (up to 1.85 times higher), liver (up to 1.58 times higher) and pancreas dysfunction (2.25 times higher), thrombocytopenia (up to 3.60 times higher), and inflammation (1.30 times higher). NPs induce more marked disruptions of circulation in the lungs manifested as hemorrhagic infarctions and edema. TPEL for the analyzed nanomaterial calculated relying on its toxicological hazard is equal to 0.02 mg/dm^3 in drinking water, which is tenfold lower than this value calculated for MPs.

The study findings allow increasing accuracy and objectivity when developing safety standards for $CoAl_2O_4$ NPs levels in food products and drinking water to ensure greater hygienic safety of the population.

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Competing interests. The authors declare no competing interests.

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Research article

ADOLESCENT SMOKING AS RISK FACTOR OF DECREASE IN ADAPTATION RESOURCES OF NEUTROPHIL METABOLISM IN ORAL CAVITY AND PROGRESSIVE RECURRENT BRONCHITIS

O.I. Pikuza[1], A.M. Zakirova[1], Kh.M. Vakhitov[1], E.V. Volyanyuk[2], E.L. Rashitova[3], A.A. Pikuza[1]

[1]Kazan State Medical University, 49 Butlerova St., Kazan, 420012, Russian Federation
[2]Kazan State Medical Academy – Branch Campus of the Federal State Budgetary Educational Institution of Further Professional Education «Russian Medical Academy of Continuous Professional Education», 11 Mushtari St., Kazan, 420012, Russian Federation
[3]Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, 1 Samory Mashela St., Moscow, 117198, Russian Federation

High prevalence of smoking among children and its early onset is an urgent problem in modern pediatrics. For proper health risk quantification, it is extremely important to understand and parameterize relationships between effects of adverse factors and physiological processes in the body, including the cellular level.

The aim of this study was to analyze how tobacco smoking influences maladaptive changes in the mucosal immunity system of the oral cavity in adolescents and to assess kinetic trends in the clinical course of recurrent bronchitis (RB) in long-term outlook.

The study included 92 patients with RB aged 16.8 ± 3.1 years. Two groups were created of them; the observation group was made of 64 patients, who admitted smoking as a habit, and the reference group included 28 people without nicotine addiction. The control group was made of 23 adolescents of the same age without any signs of the analyzed disease. Oral neutrophils (N) were selected as basic research material. N oxygen metabolism was registered by luminol-dependent chemiluminescence (LDCL) in spontaneous (sLDCL) and induced (iLDCL) variants. Functional N probing was performed using opsonized zymosan (∑) and and peptidoglycan from S. aureus strain Cowan. Concentrations of antibodies (AT) to glycolipid (GLP) (Re-mutant Salmonella Minnesota), Candida albicans and S. aureus were determined by ELISA tests.

The analyses made it possible to detect a close relationship between levels of ROS generation by neutrophilic granulocytes and the immune components of anti-endotoxic immunity. Immune changes were not established to be universal in the groups of examined patients and were shown to reflect the specificity of destabilizing effects produced by tobacco smoke. A direct moderate correlation was established in the observation group between sLDCL indicators and the concentration of anti-candidosis antibodies ($r = +0.59$, $p = 0.0382$), which reflects the level of bacterial stimulation involving dysbiotic shifts in gut microbiota. A direct correlation was detected between levels of biocidal parameters of neutorphil granulocytes and anti-glycolipid levels ($r = +0.64$ $p = 0.0417$).

Integral assessment of specific humoral immunity to glycolipid and the phagocytic link of the mucosal protection in the oral cavity reflects the degree of endogenous intoxication and kinetic trends in the RB clinical course in adolescents in subsequent years of life. These relationships may provide solid grounds for 'exposure – response' stage in the risk assessment procedure for assessing risks of respiratory diseases in smoking adolescents.

Keywords: *adolescents, smoking, recurrent bronchitis, mucosal immunity, oral neutrophilic granulocytes, endotoxin aggression, anti-endotoxic immunity, prediction.*

Olga I. Pikuza – Doctor of Medical Sciences, Professor, Professor of the Department of Propaedeutics of Childhood Diseases and Faculty Pediatrics (e-mail: pikuza.ol@yandex.ru; tel.: +7 (843) 236-06-52; ORCID: https://orcid.org/0000-0001-5332-8630).

Alfiya M. Zakirova – Candidate of Medical Sciences, Associate Professor, Associate Professor of the Department of Propaedeutics of Childhood Diseases and Faculty Pediatrics, Deputy Dean of the Faculty of Pediatrics (e-mail: azakirova@gmail.com; tel.: +7 (843) 236-06-52; ORCID: https://orcid.org/0000-0003-2976-0807).

Khakim M. Vakhitov – Doctor of Medical Sciences, Professor, Professor of the Department of Hospital Pediatrics (e-mail: vhakim@mail.ru; tel.: +7 (843) 236-06-52; ORCID: https://orcid.org/0000-0001-9339-2354).

Elena V. Volyanyuk – Candidate of Medical Sciences, Associate Professor, Associate Professor of the Department of Pediatrics and Neonatology named after E.M. Lepsky (e-mail: evolanuk@mail.ru; tel.: +7 (843) 267-61-52; ORCID: https://orcid.org/0000-0003-2342-3092).

Elina L. Rashitova – resident hematologist (e-mail: elina.rashitova@gmail.com; tel.: +7 (495) 287-65-81; ORCID: https://orcid.org/0000-0003-1450-8254).

Artur A. Pikuza – student of the Faculty of Dentistry (e-mail: pikuza74@mail.ru; tel.: +7 (843) 236-06-52; ORCID: https://orcid.org/0009-0000-5323-9929).

Environmental instability, deterioration of the economic and social situation, and acceleration of technological progress have created a qualitatively new environment around humans that places increased demands on the body [1–4]. In this regard, the load on the adaptive capabilities of the body has increased, leading to frequent disruptions of homeostatic balance. Published works emphasize the increasing frequency of tobacco smoking among children and the early onset of tobacco use [5–7]. While the prevalence of smoking among adults has stabilized in recent years, it continues to increase among adolescents and has increased 3-fold over the past 10 years [7–10].

The impact of tobacco smoke on the child's body is aggravated by deep neuropsychiatric and hormonal changes characteristic of puberty, morphofunctional rearrangement of the main regulatory systems and mechanisms, and new social loads [11, 12]. All this causes increased vulnerability and susceptibility of adolescents to occurrence of diseases, with diseases of the respiratory system occupying one of the leading places in their structure. Among them, acute diseases of the bronchopulmonary system, which have their own medical and statistical characteristics, prevail in quantitative terms. According to the recent literature, the inflammatory process in the bronchial tree is increasingly becoming recurrent with a tendency to a prolonged course [13].

Exposure to tobacco smoke has an irritating and damaging effect on the oral mucosa and causes dystrophic changes in the epithelial layer [6, 8]. Smoking can induce the development of secondary dysfunction of mucosal immunity of the oropharynx. Cigarette smoke contains free radicals that trigger oxidative stress and lipid peroxidation; it also inhibits endogenous antioxidants and dysergic shifts in immune homeostasis. Nicotine is an anthropogenic pathogenetic factor in the progression of the inflammatory process in the respiratory system and determines the formation of chronic pathology of the bronchopulmonary system [2].

In the cascade of integrated mucosal defense mechanisms, the priority role belongs to the function of the phagocytic barrier, which is carried out by neutrophil granulocytes that migrate from the vascular bed to the oral cavity [14–20]. Functionally, as effector and inducer cells, neutrophilic granulocytes closely cooperate with the coordinating role of the epithelial cover, including buccal epithelial cells [21]. They are closely interrelated with the process of colonization of bacterial pathogens, being a trigger for disrupting the integrated activity of the body in the external environment, its adaptive stability [22]. This allows us to consider neutrophilic granulocytes as cascade and network interactions that determine the development and regulation of inflammatory and immune processes [23–25].

From this point of view, great clinical and practical importance is to be found in studies that reveal the nature of maladaptation shifts on the part of the first barrier of pathogens entering the patient's body and the negative contribution of smoking to functional shifts on the part of mucosal immunity in adolescents with recurrent respiratory pathology [6, 26]. Today, the problem of high levels of smoking among children that leads to structural and functional disorders in the respiratory system is in the focus of attention of medical professionals and causes concern among health authorities both in our country and abroad [26–39]. Information on the effects of tobacco smoke in relapsing bronchitis (RB) in adolescents is extremely limited. At the same time, quantitative risk assessment requires understanding and parameterizing dependencies of the influence of hazardous factors on physiological processes in the body, including those at the cellular level. This prompted us to study this issue from the point of view of mucosal protection of the oral cavity as the first obstacle to aerogenic pathogens, with an analysis of the body adaptive resistance to the infectious process in adolescents with RB.

The goal of the study was to analyze how tobacco smoking influences maladaptive changes in the mucosal immunity system of the oral cavity in adolescents and to assess kinetic trends in the clinical course of recurrent bronchitis (RB) in long-term outlook.

Materials and methods. The study included 92 patients with RB aged 15–18 (16.8 ± 3.1) years. The examination and treatment were conducted at the children's hospital of the State Medical Institution of the Central Clinical Hospital No. 18 in Kazan, Russia. All patients signed a voluntary informed consent form before starting the study. Clinical and diagnostic measures were carried out in accordance with Russian recommendations[1]. Two groups were formed: the main group included 64 patients who confirmed their smoking habit, and the comparison group included 28 peers without nicotine addiction. The obtained data were recorded in a specially designed card and in an extract from the hospital patient's medical history. The control group consisted of 23 adolescents of the same age without signs of disease and nicotine addiction.

Criteria for including a patient:

1. A patient's age is from 15 to 18 years.

2. Informed consent signed by a patient's legal representatives.

3. Acceptable concomitant treatment: medicines used for the treatment of background diseases, except for respiratory organs.

Exclusion criteria: the presence of severe background somatic diseases in patients, including atopy, bronchial asthma, autoimmune diseases (lupus erythematosus, scleroderma), blood diseases, or hypertension.

The material for the study was oral neutrophils (N) that migrated from the vascular bed to the oral cavity. Oxygen metabolism of N was recorded by luminol-dependent chemiluminescence (LDChL). We studied spontaneous LDChL (sLDChL), which allows us to assess the endogenous activation of polymorphonuclear leukocytes in the patient's body and signs of destabilization of their functional activity, and induced LDChL (iLDChL). iLDChL was registered using two stimulants: opsonized zymosan (Σ) and S. aureus peptidoglycan of the *Cowan* strain. Registration of sLDChL and iLDChL was assessed by taking into account the light flux for 1 minute. To unify the iLDChL indicators, we recalculated them to 1000 neutrophilic granulocytes. The results were expressed in pulses-minute (imp/min).

Venous blood samples were collected from all patients with RB upon admission to the hospital, and anti-endotoxin immunity indicators were recorded by enzyme-linked immunosorbent assay (ELISA). Glycolipid was used as an antigen, which is a structural unit of endotoxin (Re-mutant Salmonella Minnesota) and determines the entire spectrum of general biological properties of endotoxin (E). The concentration of antibodies (AB) was expressed in micrograms/ml. In addition, the intensity of specific antibacterial immunity to *Candida albicans* and *S. aureus* antigens (AG) was evaluated.

Statistical analysis methods using the standard software package STATISTICA 12.0 and visualization methods based on the EXCEL package were used to interpret the study results in the observed groups of children. Methods of variation statistics were used for statistical data analysis (relative indicators were calculated with the determination of confidence limits for fluctuations in indicators with an error-free forecast probability of 95 %). To obtain objective results, an individual analysis of digital data as a percentage was applied. When analyzing quantitative indicators, we calculated arithmetic mean values and mean square deviations (M (SD)). The significance of differences in the initial values of dependent samples (i.e., within groups) was evaluated using the nonparametric Wilcoxon test, and the significance of differences in the average values of independent samples (i.e., between two groups) was evaluated using the nonparametric Mann – Whitney test.

Results and discussion. Analysis of clinical and anamnestic data of adolescents in the compared groups showed high frequency of risk factors for the development of RB that

[1] Klinicheskie rekomendatsii – Bronkhit – Deti – 2021-2022-2023 (28.09.2021), utv. Minzdravom RF [Clinical recommendations – Bronchitis – Children – 2021-2022-2023 (September 28, 2021), approved by the RF Ministry of Health]. *BU «Pokachevskaya gorodskaya bol'nitsa»*. Available at: https://gbpokachi.ru/upload/medialibrary/2c1/jei4lgfwtn7l21sl2m456zjjcshs30iw.pdf (April 27, 2024) (in Russian).

initiate repeated episodes of the disease: hereditary anamnesis burdened by bronchopulmonary pathology in 39.13 %, passive smoking in 45.65 %, incomplete family in 27.17 %, early addiction of adolescents to smoking (from 14 years) in 68.47 %. In the course of the study, it was noted that almost 28.26 % of the surveyed children who use nicotine-containing cigarettes did not have a seasonal increase in the disease. The duration of each episode ranged from 10 to 14 days or more, and patients were repeatedly admitted to the hospital. Most of them (83.69 %) complained of coughing, increased fatigue, decreased activity, emotional lability, loss of appetite, and susceptibility to ENT diseases.

Analysis of physical development showed that, deviations were 32.81 % in the main group and 28.57 % in the comparison group. According to the Quetelet index, 46.74 % of the patients in the compared groups had a lack of nutrition and 8.69 % were overweight.

We studied the indicators of mucosal immunity of the oral cavity as markers of adaptive resistance of the body and assessment of the negative effects of smoking. The phagocytic barrier function plays a priority role in the cascade of integrated mechanisms of mucosal immunity. In our research, we focused on recording the effector function of neutrophilic granulocytes and their biocidicity. Tables 1 and 2 show indicators of the functional state of neutrophilic granulocyte metabolism according to the sLDChL and iLDChL reactivity tests.

The sLDChL indicators in the acute period of RB were significantly higher as opposed to the control group and significantly exceeded the examination results obtained for the children in the comparison group: 1904.64 ± ± 72.30 uti/min (p = 0.0059) and 1152.37 ± ± 49.47 uti/min (p = 0.0486), respectively. Taking into account the obtained data, it can be assumed that the high level of endogenous

Table 1

sLDChL indicators given comparatively in the groups of examined patients

	Groups of examined patients		
	Main group (N = 64), M (SD)	Comparison group (N = 28), M (SD)	Control group (N = 23), M (SD)
sLDChL, imp/min	1904.64 ± 72.30	1152.37 ± 49.47	625.27 ± 44.08
p**	0.0059	0.0357	
p*	0.0486		

Note: p** means against the control group, p* means against the comparison group.

Table 2

The effect of cigarettes on the iLDChL indicators given comparatively in the groups examined with different methods of stimulation

Variant of functional neutrophil probing	Groups of examined patients		
	Main group (N = 64)	Comparison group (N = 28)	Control group (N = 23)
Opsonized zymosan (imp/min), M (SD)	104.12 ± 12.11	124.07 ± 14.8	118.72 ± 12.51
p**	0.0413	0.0587	
p*	0.0159		
Staphylococcus aureus (imp/min), M (SD)	87.43 ± 5.40	107.91 ± 8.6	114.90 ± 11.50
p**	0.0041	0.0368	
p*	0.0072		
p***	0.0107	0.0214	0.0723

Note: p*** means comparison between the stimulation variant, p** means against the control group, p* means against the comparison group.

activity of neutrophilic granulocytes, in addition to bacterial stimulation, was associated with the direct effect of tobacco smoke on neutrophil reactivity. Cigarette smoke is known to contain hazardous chemical compounds that initiate oxidative stress and synthesis of nitric oxide, which inhibits endogenous antioxidants in the adolescent body.

In the analysis of iLDChL (Table 2), attention was paid to the statistically significant difference in the indicators in the groups of examined patients. This was mostly observed when neutrophilic granulocytes were stimulated using S. aureus.

The production of reactive oxygen species in the zymosan-induced chemiluminescence (CL) test was less pronounced in the main group of adolescents and the indicators in the acute period of RB were 104.12 ± 12.11 imp/min, while in the comparison group – 124.07 ± 14.8 imp/min. In other words, in smokers, pronounced depression in the generation of reactive oxygen species was noticed, indicating a decrease in the potential resources of antimicrobial protection of neutrophilic granulocytes.

It should be noted that while the iLDChL values with two stimulants were almost equal to each other in healthy children, a significant decrease in them was recorded in adolescents with RB of the main group when *S. aureus* was stimulated in comparison with opsonized zymosan (p = 0.0107). Low response values were detected when using the S. aureus stimulator and amounted to 87.43 ± 5.40 uti/min (p = 0.0041) in the main group and they were significantly different from patients without bad habits, 107.91 ± 8.6 uti/min (p = 0.0072). Since the *S. aureus* iLDChL reaction requires the indispensable participation of serum opsonins for the process to progress and zymosan was already used in its opsonized form, the obtained results allow us to judge the deficiency of opsonic blood factors, which is more pronounced in RB in adolescents of the main group. The obtained results should be considered as a decrease in the antimicrobial reserve of phagocyte protection in relation to *S. aureus*, one of the most significant etiological factors of the purulent-inflammatory process.

Taking into account the fact that neutrophilic granulocytes are the main effector blood cells that emphasize endotoxin, it seemed reasonable to analyze the relationship between the indicators of antimicrobial function of granulocytes and anti-endotoxin protection (Table 3).

When analyzing the parameters of antiglycolipid antibodies, a significant decrease in their concentration in patients of the main group (5.02 ± 0.29 μg/ml) was noted not only against the control group (p = 0.0216), but also the comparison group (p = 0.0471). It is necessary to note the objective intensity of humoral antibacterial immunity to *Candida albicans* antigens, which exceeds the indicators in the comparison group by 1.5 times (7.28 ± 0.94 μg/ml vs. 4.55 ± 0.52 μg/ml). In addition, the

Table 3

Concentration of AB to glycolipid, *Candida aibicans*, *S. aureus* AG in adolescents with RB of the main group and comparison group (μg/ml)

Groups of examined patients	Glycolipids, M (SD)	*C. albicans*, M (SD)	*S. aureus*, M (SD)
Main page (N = 64)	5.02 ± 0.2.29	7.28 ± 0.94	11.28 ± 2.12
Comparison Group (N = 28)	6.21 ± 0.11	4.55 ± 0.52	6.35 ± 1.39
Control Group (N = 23)	7.86 ± 0.23	3.17 ± 0.20	4.02 ± 0.37
p***	0.0216	0.0326	0.0061
p**	0.0395	0.0475	0.0317
p*	0.0471	0.0383	0.0328

Note: p*** means between the main group and the control, p** means between the comparison group and the control, p* means between the examined patients.

intensity of humoral immunity to *S. aureus* was noticed, as evidenced by the high concentration of AB to this AG.

Taking into account the high titer of anti-*Candida albicans* antibodies, a marker of deep intestinal dysbiosis, and, consequently, colonization of gram-negative microbiota in the intestine (a source of lipopolysaccharides), we can talk about a significant deficiency of anti-endotoxin protection and signs of endotoxin aggression in the main group of patients. Registration of the intensity of humoral specific immunity to the intestinal microbiota and the phagocytic link of mucosal protection of the oral cavity expands the possibilities to assess the negative role of smoking on the adolescent body in a broader aspect.

Biological effects of nicotine are provided by a cumulative signal that includes rapid responses when nicotine cholinergic centers are activated in nerve cells. This lipophilic xenobiotic is proven to exert the effect of chronic cellular stress by penetrating into cells and reprogramming them under prolonged exposure [5, 12]. Being an anthropogenic pathogenetic factor in the progression of the inflammatory process in the respiratory organs, smoking determines the RB progression with a chronic outcome in subsequent years of life.

Neutrophilic granulocytes of the oral cavity in cooperation with buccal epithelial cells of the mucosa are known to play a leading role in regulating the functioning of colonization resistance [21, 23]. The antigenic load on colonization resistance increases initially upon exposure to smoking. At the same time, harm of smoking is manifested, on the one hand, due to the direct influence on the mucous membrane of the respiratory tract, and, on the other hand, due to the toxic effect on the functional and metabolic activity of oral neutrophils.

The analysis made it possible to test the close relationship between the indicators of generation of reactive oxygen species by neutrophilic granulocytes and immune links of anti-endotoxic immunity. The nature of immune shifts in the groups of examined patients was found to be not universal and reflect the specifics of the destabilizing effect produced by tobacco smoke. We registered a different profile of immunological protection. In smokers, a pronounced depletion of the balance of antimicrobial immunity is recorded, which is associated with dysbiotic shifts in the digestive system and proliferation of gram-negative microbiota (a source of endotoxin), *Candida albicans* and *S. aureus* fungi. Individual analysis revealed a low level of antibodies to glycolipids in the main group of subjects and a significant increase in the concentration of antibodies to *Candida albicans* and *S. aureus.*

Conclusion. From a clinical point of view, it is important to emphasize the effectiveness of using two stimulators as different methods of functional probing of neutrophilic granulocytes. In the main group, a decrease in the functional reserve of the phagocytosis system was found and a deficiency of opsonic blood factors was detected, which predisposes to a decrease in the elimination of endotoxin, its entry into the bloodstream in increasing amounts, the development of endotoxin aggression and the progressive course of the disease relapses. In the main group, a direct correlation of average strength was established between the sLDChL indicators and the concentration of anti-candida antibodies (r = +0.59, p = 0.0382), which reflects the degree of bacterial stimulation involving dysbiotic shifts of the microbiota in the intestine. A direct relationship was registered between the indicators of neutrophilic granulocyte biocidity and the concentration of anti-glycolipid (r = +0.64 p = 0.0417).

The conducted analysis allows us to take a deeper approach to the problem of smoking among adolescents with RB as a risk factor that further determines the formation of chronic pathology in the bronchopulmonary system and a decrease in the quality of life.

Key points:

1. Smoking depletes the adaptive resources of oxygen-dependent metabolism of oral neutrophils, which initiates susceptibility to a prolonged, progressive course of recurrent bronchitis in adolescents.

2. An integral assessment of specific humoral immunity to GLP and the phagocytic link of oral mucosal protection reflects the degree of endogenous intoxication and the kinetic trends in the RB course in adolescents in subsequent years of life.

These relationships may provide solid grounds for 'exposure – response' stage in the risk assessment procedure for assessing risks of respiratory diseases in smoking adolescents.

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CLIMATE CHANGE AND FOREST FIRES AS HEALTH RISK FACTORS (ANALYTICAL REVIEW)

B.A. Revich

Institute of Economic Forecasting of the Russian Academy of Sciences, 47 Nakhimovskii Av., Moscow, 117418, Russian Federation

Climate change in Russia contributes to an increase in the number of forest fires, especially in Siberia and the Far East, leading to the rise in the forest fire danger index. Increased air pollution, which is typical for a number of cities in these regions, is the reason for changes in population health due to the influx of significant volumes of smoke gases from forest fires into populated areas. Smoke from forest fires consists of aerosols and gases and contains more than 40 pollutants. During fires, frequency of calls for emergency medical care for children tends to increase due to exacerbation of upper respiratory tract diseases, including laryngitis, pharyngitis and acute respiratory infections, as well as attacks of bronchial asthma, and longer exacerbation periods of this disease. Such respiratory dysfunctions appear a few days after fires, which should be taken into account when organizing health monitoring in such situations. Among adult population, an increase in the number of deaths from cardiovascular and respiratory diseases has been proven during forest fires, and there tends to be an increase in the number of requests for medical care for COPD, bronchial asthma, myocardial infarction, coronary heart disease and other diseases. Fires in forest highlands in the Khabarovsk Krai result in deteriorating health of patients with neurological diseases.

The generalization of the results obtained by domestic and foreign studies on this issue confirms the need to improve the air pollution monitoring system during forest fires with the determination of PM for timely preventive measures by health systems, Rospotrebnadzor, FMBA and other agencies. It also seems necessary to develop modeling of pollutant spread in ambient air in settlements exposed to them, with assessments of population health risks and development of preventive measures. Given the relevance of these studies, it is advisable to hold seminars with BRICS countries within the framework of international cooperation.

Keywords: *climate change, forest fires, population mortality and incidence, diseases of the upper airways, bronchial asthma, air quality monitoring, risk factor.*

The number of forest fires is growing constantly all over the world due to variable reasons including draughts as a consequence of climate change, dried grass burning in agricultural activities, human factor etc. Approximately 54 % of the forests on the planet are located in five countries: Russia, Brazil, Canada, the USA, and China. Over the last 20 years, the number of severe forest fires has doubled across the globe. Up to 700 forest fires happen annually in European countries only, French Corsica, Greece and Italy being the leaders in this respect. In the USA, most fires occur in western states and they tend to be very strong and very long. Climate change, global warming included, long draughts and dry thunderstorms have had considerable influence on the growth in the number of forest fires due to an increasing number of heat waves and soil degradation. This has become a new and a rather serious global challenge [1–3]. It is urgent in Russia as well since forest fires are becoming more and more frequent in the country. In the period up to 2020,

Boris A. Revich – Doctor of Medical Sciences, Professor, Chief Researcher, Head of Laboratory for Environment Quality Prediction and Population Health (e-mail: brevich@yandex.ru; tel.: +7 (499) 129-18-00; ORCID: https://orcid.org/0000-0002-7528-6643).

their number grew by 10 % annually against 2016 [4]. According to various predictions, their duration can grew by 20–29 days and even more in the whole European part of Russia by 2099 [5]. In 2022–2023, a wave of fires occurred in 70 regions in the country and 4 million hectares were affected by them. The greatest forest fires were observed in the Amur, Sverdlovsk, Omsk, and Chelyabinsk regions, Zabaikalskii and Khabarovsk Krai, Jewish Autonomous Area; they also occurred in Yakutia, Magadan, Irkutsk, Tyumen, and Novosibirsk regions[1]. The Federal Forestry Agency reported that no settlement was affected by fires in 2023; still, this assessment does not consider effects of smoke gases on ambient air quality and population health in urban and rural settlements.

Global warming has become one of the basic reasons for forest fires in Siberia where temperatures were constantly above their normal climatic range. Thus, the highest air temperature above 38 °C was registered in Verkhoyansk and this has become a new temperature record to the north of the Northern Polar circle. High temperatures make the soil surface drier and more combustible, which makes for early occurrence of seasonal forest fires and a growth in their overall area [6]. In 2019, abnormal climatic conditions were observed in Siberia due to climate change. Smoke from fires in Krasnoyarsk Krai affected territories with approximately 10 million people living there in Omsk, Tyumen, Sverdlovsk, and Chelyabinsk regions and Perm Krai [7]. In summer of 2021, smokes from the record number of fires in Siberia covered more than 3000 km distance and reached the Northern Pole. The number of lightning strikes tripled to the north of 65° northern latitude, which created even greater risks of forest and tundra fires [6].

Yakutia holds the first place among RF regions per the number of forest fires almost every year. This is the largest territory in the country where the fire area reaches 8–10 million hectares over the fire season [8–10]. Fire recurrence in Siberian forests depends on their composition. For example, more fires tend to occur in shallow light coniferous forests located in the center of Eastern Siberia in comparison with its western part. More forest fires typically occur in the southern part of the mid Siberia and their frequency is growing persistently[2].

Forecast scenarios of fire frequency in Russia have been developed for more than 10 years by institutions of the Russian Academy of Sciences and Rosgidromet; this frequency is expected to grow in the Asian part of the country [11–15]. The strongest and longest forest fires should be expected in the south of this territory where temperature rise is accompanied with lower precipitations and higher values of the fire danger index. This zone includes southern areas of Kemerovo, Omsk and Irkutsk regions, Zabaikalskii Krai and some other territories. Climatologists believe the fire danger index might grow 2.5 times as high against its level in the end of the 20th century [14]. However, the number of forest fires has been growing in the European part of Russia as well. For example, in the Volga Federal District, their frequency was the highest in Nizhnii Novgorod and Samara regions in 1992–2020 [16].

The composition of forest fire smoke. Forest fires create smoke that consists of an aerosol mixture with particles of various diameters and volatile compounds. Their physical and chemical properties change as a distance from a fire seat grows and also due to dispersion in ambient air. Smoke gases created by forest fires tend to contain more than

[1] Federal'noe agentstvo lesnogo khozyaistva [The Federal Forestry Agency]: official web-site. Available at: https://rosleshoz.gov.ru/ (July 04, 2024) (in Russian).

[2] Valendik E.N., Arbatskaya M.K., Vaganov E.A., Volosatova E.N., Ivanova G.A., Levkina O.I., Ovchinnikov D.V., Shashkin E.A. Prognozirovanie chastoty lesnykh pozharov v Sibiri v svyazi s global'nymi izmeneniyami klimata [Predicting frequency of forest fires in Siberia due to global climate change]: the Report for the Russian Foundation for Basic Research grant, 1997 (in Russian).

40 chemicals including carbon oxides (CO and CO_2), methane, methanol, methyl chloride, solid aerosols (including black carbon), acetone, acrolein, and others. Acetone contents increase in smoke gases in case of peat burning [10]. There are also some data on more complex composition of smoke gases created by forest fires. If landfills with solid household wastes, some equipment or buildings with polymers in their structure are brunt in a forest fire, this may result in occurrence of free radicals, hydrogen cyanide or other hazardous chemicals in smoke gases [17].

Smoke near a fire seat contains large quantities of particulate matter (PM) sized 2.5 μm and more but as it spreads further, the number of even smaller particles grows in it. Russian toxicologists have investigated and reported toxic effects of some specific ambient air pollutants including carbon monoxide, sulfur and nitrogen oxides, aldehydes, PAHs, phenol, and heavy metals in hundreds of publications. Over the last 15 years, toxic effects produced by small-sized particulate matter have been constantly investigated by experts; new data have been reported and several reviews on the issue have been published in Russian [18]. Fine-dispersed particles with their diameter not exceeding 0.1 μm are considered gases and their carcinogenic effects are being investigated quite intensively at the moment.

Fires and ambient air pollution. The results obtained by estimating ambient air pollution with PM and other chemicals during forest fires and following days have been published only for several Russian cities. During the abnormally hot summer of 2010, forest fires occurred in the eastern part of Moscow region and fire smoke spread for more than 100 km reaching western areas of the region. The Poisson regression model for daily mortality was employed to estimate effects of exposure to PM_{10} on population mortality in Moscow during that period. Daily all-cause natural mortality was taken as the dependent variable, which conformed to the Gaussian distribution. Average daily PM_{10} levels within 53 μg/m^3 were established to have no effect on the average long-term rates of all-cause natural mortality; a gradual rise in mortality was observed under levels within 53–96 μg/m ^{3}and the peak in it was reached under levels equal to 138 μg/m ^{3} [19]. At the same time, MPLs were violated as regards such hazardous chemicals as nitrogen oxides, benzo(a)pyrene, and formaldehyde [20].

In 2010, forest fires were the reason for ambient air pollution in woody areas in many regions of the RF European part, which led to premature mortality among population. For example, mortality due to all natural causes, external ones excluded, grew by more than 1.5 times in Voronezh region (68.7 %) [21]. There was solid evidence of an authentic correlation between air temperature and carbon oxide levels in ambient air and hospital admissions due to all diseases; moderate and strong correlations were established between levels of particulate matter and the total mortality rates [22–24]. Ambient air was found to be polluted with carbon oxide, nitrogen dioxide, particulate matter, formaldehyde, and hydrocarbons during forest fires in Voronezh, Samara, Saratov and other regions. Thus, in 2018, a correlation was established in Saratov region between the fire area and dynamics of single maximum levels of nitrogen dioxide ($r = 0.47$, $p < 0.05$), particulate matter ($r = 0.57$, $p < 0.05$) and hydrocarbons ($r = 0.62$, $p < 0.05$). Probabilistic health risk due to short-term inhalation exposure was above safe HQ levels per nitrogen oxide, particulate matter, and formaldehyde [25]. The results of this study confirmed those obtained by earlier works with their focus on assessing children's health during forest fires, which established higher frequency of upper respiratory airways diseases and their longer exacerbation periods [26].

People who live in cities in Siberia and the Far East with elevated ambient air pollution levels can be considered among population groups that are the most susceptible to effects produced by forest fires. These cities are included into the Clean Air Federal project: Bratsk, Krasnoyarsk, Chita, Irkutsk and some others. In Krasnoyarsk, concentrations of ambient fine-dispersed PM_{10} were above safe

levels established in Russia (60 μg/m^3) and those recommended by the WHO (50 μg/m^3)[3]. The similar 2018 report did not contain any data on PM_{10} levels but still mentioned that levels of all particulate matter grew by 1.7–2.3 times. This means that levels of fine-dispersed particles, which pose the greatest health hazard, also grew[4]; exposure to such particles may result in a growth in the total mortality rate for urban population by 9.3–21.9 % [27].

High levels of ambient PM during forest fires are typical for foreign countries as well. PM_{10} and $PM_{2.5}$ concentrations were increased during the occurrence of large fires and megafires in Portugal, with daily concentrations exceeding the European/national guidelines in 7–14 and 1–12 days of 2017 (up to 704 μg/m^3 for PM_{10} and 46 μg/m^3 for $PM_{2.5}$), respectively. PM_{10} concentrations were correlated with total burned area ($0.500 < r < 0.949$; $p > 0.05$) and with monthly total burned area / square distance ($0.500 < r < 0.667$; $p > 0.05$) [28]. The largest forest fires in the USA usually occur in California. More than 9600 forest fires were detected in this state solely in 2020, which resulted in economic losses billions of dollars' worth. It is these fires that create up to 32–44 % of all $PM_{2.5}$ emissions in the USA. $PM_{2.5,}$ as a component in a complex mixture of solid particles and gaseous pollutants in smoke, create the most serious challenge for public healthcare due to their known health risks, high levels, and spacious dispersion during forest fires. Elevated $PM_{2.5}$ levels are hazardous both for respiratory organs and cardiovascular system.

Effects produced on human health by ambient air pollution due to forest and peat combustion gases. According to PubMed data, more than 70 articles have been published on the issue, which is hundreds and thousands of times less than on any other health risk factor. Such investigations are predominantly accomplished in Brazil with its gigantic forest fire areas in the Amazonian region; also, we should mention the USA, Canada, Greece, and some other European and South-Asian countries.

Population mortality. Russian investigations on the issue have been conducted only in several regions including Moscow, Voronezh and Irkutsk region, Chita, and Khabarovsk Krai. During the hot summer of 2010, combined exposure to abnormally high temperatures and elevated ambient air pollution due to forest and peat fires resulted in an authentic increase in mortality by 11,000 deaths in Moscow. A mathematical model, which was developed in this study, established this growing mortality to be authentically dependent on combined exposure to elevated levels of ambient PM_{10} and ozone as well as abnormally high air temperature and duration of a heat wave [29]. A highly adverse situation is observed in Chita where emissions can hardly disperse in ambient air in residential areas during forest fires due to the city being located in a hollow between mountain chains. Levels of practically all analyzed chemicals were higher than seasonal background values during forest fires and levels of particulate matter, CO, NO_2, and black carbon were several times higher than single and average daily MPLs. Calculated concentrations were confirmed by field measurements: levels of $PM_{2.5}$, total solid particles, carbon oxide, and black carbon were above their MPLs within a 5–10 km radius; acetaldehyde, up to 20 km radius. Average acute hazard quotients (HQac) were above 1 meaning elevated levels of health risk, which was also confirmed by higher calls for emergency due to diseases of the respiratory and circulatory system among children younger than 17 years and adults older than 65 years during forest fires. The

[3] O sostoyanii i okhrane okruzhayushchei sredy v Krasnoyarskom krae [On the quality and protection of the environment in Krasnoyarsk Krai]: 2017 State Report. *Krasnoyarsk Regional Ministry of Ecology and Rational Use of Natural Resources.* Available at: http://www.mpr.krskstate.ru/envir/page5849/0/id/32983 (May 17, 2024) (in Russian).

[4] O sostoyanii i okhrane okruzhayushchei sredy v Krasnoyarskom krae [On the quality and protection of the environment in Krasnoyarsk Krai]: 2018 State Report. *Krasnoyarsk Regional Ministry of Ecology and Rational Use of Natural Resources.* Available at: http://www.mpr.krskstate.ru/envir/page5849/0/id/39742 (May 17, 2024) (in Russian).

number of such emergency calls grew 1.5–4 times as high[5] [30, 31]. A database on emergency calls by population, which was created by the authors, was granted a certificate by Rospatent and this is a significant example of preparing initial medical data on effects produced on human health exactly by smoke gases created by forest fires. Such information typically contains data on forest fires, levels of pollutants in ambient air in Chita and numbers of emergency calls. Therefore, it can be used later within social-hygienic monitoring activities to objectively assess consequences of these natural disasters[6].

Another method for assessing health risks upon chemical exposures was approved by Rospotrebnadzor. It was employed by the same group of authors to estimate consequences of forest fires in Bratsk, Irkutsk region [32]. Masses of chemical emissions were determined according to The Method for Determining and Calculating Pollutant Emissions from Forest Fires; levels of pollutants in ambient air were calculated for the analyzed city; health risks were assessed and emergency calls were analyzed. Up to 82 % of the Irkutsk region area is covered with forests and the number of hospital visits usually grows during forest fires primarily due to respiratory diseases by 6.5 %, exacerbation of chronic bronchitis by 4.2 % and bronchial asthma by 5.2 % [10].

Khabarovsk region holds the first place among RF regions per areas of highland forests. Levels of particulate matter, nitrogen dioxide, phenol and formaldehyde were considerably higher than MPLs during forest fires in this region [33]. Effects produced by forest fires on population mortality were estimated in various districts of the region in 1997–2001 by comparing its rates depending on fire frequency. Unfortunately, it is rather difficult to estimate quantitative relationships between levels of pollutants and changes in population health in Khabarovsk, Komsomolsk-on-Amur and other settlements due to very few stations for ambient air quality monitoring; the list of significant pollutants also need clarifying [34]. Over the analyzed period, mortality rates grew in five districts in the region where forest fires occurred constantly but similar growth was not identified in other regions. Still, no relationship was established between this indicator and the size of fire sites [35].

A review [36] summarizes data on short-term health impacts of wildfire emissions reported in few studies on the issue that were published in the USA, Europe, Australia, some Asian countries and South American countries. We have not found any similar works with data on Russia or African countries and hope that this review can fill in this gap to a certain extent. The analysis covered such health measures as emergency department visits and hospital admissions for cardiorespiratory diseases. Despite the heterogeneity among exposure and health assessment methods, all-cause mortality, and specific-cause mortality were significantly associated with wildfire emissions in most of the reports. Globally, a significant association was found for all-cause respiratory outcomes including asthma, but mixed results were noted for cardiovascular-related effects. For the latter, estimates were only significant several days after wildfire emissions and this should be taken into account when organizing health monitoring in similar situations.

Abroad, similar investigations are mostly conducted in Brazil where premature deaths grew by 9800 cases during strong forest fires

[5] Elfimova T.A. Otsenka vliyaniya emissii ot lesnykh pozharov na ekologicheskoe sostoyanie urbanizirovannykh territorii [Assessment of effects produced by emissions due to forest fires on the ecological situation on urbanized territories]: the abstract of the dissertation … for Candidate of Biological sciences degree. Orenburg, 2014, 21 p. (in Russian).

[6] Elfimova T.A., Rukavishnikov V.S., Ivanov A.G., Efimova N.V. Kachestvo atmosfernogo vozdukha i obrashchaemost' naseleniya g. Chity za skoroi meditsinskoi pomoshch'yu v period massovykh lesnykh pozharov [Ambient air quality and emergency calls in Chita during mass forest fires]: the certificate of state registration issued for the database No. RU 2015621824 of the Russian Federation, No. 2015621350; submitted on November 02, 2015, published on December 28, 2015 (in Russian).

in 2012 [37]. Especially strong fires were observed in summer 2019 in the Amazonian region when many ecosystems were lost and smoke could be traced up to 1000 km away [38]. Approximately 10 % of excess mortality on the territory was associated with smoke gases of forest fires and directly with $PM_{2.5}$ exposure; people who lived in the leeward site suffered most. Fire activity and smoke propagation distance were estimated using both satellite data and geochemical methods based on soil analysis. $PM_{2.5}$ exposure caused 4966 premature deaths in Brazil during the 2019 fire-hazardous season. That year, mortality associated with fires grew by 74 % against its 2018 level but still was a bit below its highest levels over the analyzed period (5273 deaths in 2017). However, a meta-analysis of published data on $PM_{2.5}$ impacts established greater influence on premature deaths under low levels of such particles in regions that were far from fire sites [39]. Approximately 10 % of $PM_{2.5}$-associated all-cause mortality was contributed to forest fires all over Brazil between July and September. Since the dry season usually ends in November in the Amazon River Basin, smoke emissions of fires might have played even a greater role in 2019 than estimated in the study. Apart from mortality, fires in 2019 were associated with a greater number of hospital admissions for respiratory diseases, primarily among younger children and elderly persons [40, 41].

Some very interesting results were reported in a study that investigated impacts exerted by fires of various intensity on mortality in Athens, the city population being 3 million people [42]. Those fires affected much smaller areas than in Siberia or Far East but, even being medium-sized, they were associated with an increase of 4.9 % (95 % CI: 0.3–9.6 %) in the daily total number of deaths, 6.0 % (95 % CI: -0.3–12.6 %) in the number of cardiovascular deaths and 16.2 % (95 % CI: 1.3–33.4 %) in the number of respiratory deaths. To give an example, we would like to provide data on a growing number of deaths associated with only one large-sized fire: 49.7 % (95 % CI: 37.2–63.4 %), 60.6 % (95 % CI: 43.1–80.3 %) and 92.0 % (95 % CI: 47.5–150.0 %). Interestingly, the authors of this study did not deem these effects to be associated only with growing ambient OM levels and mentioned possible effects of other factors, for example, severe stress.

Incidence. In summer and autumn 2018, the fire-hazardous season in Khabarovsk Krai was 13 days longer than its average long-term duration and the number of large fires was 1.5 times as high than average. As a result, more than 2.5 million hectares of forests were either destroyed or severely damaged [43] and smoke pollution occurred in ambient air in Khabarovsk, Komsomolsk-on-Amur and Amursk. Single maximum levels of pollutants were 6 times higher than MPL for particulate matter and 2-6 times higher for carbon oxide and sulfur dioxide in ambient air in these cities during the strongest wildfires. This situation made it necessary to examine outcomes of forest fires for population health in Khabarovsk Krai; they were investigated by various departments of the Far East Medical University in cooperation with the Rospotrebnadzor Khabarovsk Regional office, Rosgidromet, and some scientific institutions. The results obtained by these studies are summarized in the monograph *Forest Fire Smoke and Health* [33] and other publications [44]. A rise in respiratory diseases should be expected in population exposed to forest fire smoke; this trend was identified in Nikolaevskii district, which was located in a zone with strong fires, as well as in Komsomolsk-on-Amur. Cases of ‘peculiar reverse acute pathology’, which the authors called ‘toxic bronchopneumonia’, were detected in children in Khabarovsk who spent a lot of time outdoors [45]. In addition to that, the number of hospital visits for exacerbated bronchial asthma grew among children on these territories during forest fires. A similar situation was observed in Ulan-Ude. Buryatia is also among the RF regions with the highest fire hazard. Ulan-Ude, just like Chita, is located in a hollow between mountains where dispersion of smoke gases and pollutant emissions in ambient air is very slow [46].

Growing numbers of bronchial asthma cases and cardiovascular diseases were also reported during forest fires in Portugal in 2017 [28]. The results obtained indicated that the smoke from wildfires negatively impacted children's lung function (PM_{10} exposure: increase of 320 and 648 cases of bronchitis in 2016 and 2017; NO_2 exposure: 24 and 40 cases of bronchitis symptoms in asthmatic children in 2016 and 2017) [47]. Approximately 25 % of school children in Brazil (more than 10 million people) are exposed to health risks due to high ambient air pollution created by forest fires [48]. Public healthcare spent 1 million euros on treatment of these diseases.

Smoke gas impacts on adults' health have been scarcely investigated, except firefighters and people who suffered from fires in buildings. Results obtained by foreign researchers have been reported in few publications that give evidence of COPD exacerbation during such situations. For example, a study assessed outcomes of exposure to forest fire smoke that was brought to Singapore by prevailing winds blowing from the Indonesian states of Kalimantan and Sumatra in 1997 [49]. Those fires occurred 500 km away from Singapore but still led to a rise in PM_{10} levels from 50 to 150 $\mu g/m^3$. This rise was significantly associated with increases of 12 % of upper respiratory tract illness, 19 % asthma and 26 % rhinitis. Supplementary findings from scanning the electron microscopic sizing of the haze particles showed that 94 % of the particles in the haze were below 2.5 micron in diameter. This emphasizes the significance of a pioneer study on the issue that was accomplished in Khabarovsk. It showed an authentic growth in the number of hospital visits by adults with symptoms of ARI, laryngotracheitis and acute bronchitis against the same period of the season without forest fires [33]. Another confirmation of these conclusions can be found in a work by N.V. Baranovskii with colleagues [50] about frequency of bronchial asthma attacks per questioning results obtained among adults using the ECRHS international standard [51] on three types of territories that were differently affected by forest fires[7]. Statistical analysis established an authentic increase in asthma-like symptoms on areas affected by forest fires. The authors did not mention the exact area where the study was conducted but we can assume it to be Tomsk region.

Negative impacts of ambient air polluted with forest fire smoke have been confirmed by a series of studies conducted in California; they remain significant health risk factors, especially for the most vulnerable population groups. More than 30 million hospital visits were analyzed representing a gigantic sample of data on healthcare services provided in 2008–2016 for asthma, COPD, respiratory and cardiovascular diseases including myocardial infarction and essential hypertension. As a result, higher risks were established for asthma and respiratory symptoms in comparison with cardiovascular diseases. Health risk assessment also considered a person's age and socioeconomic status and health risks turned out to differ significantly between different demographic groups.

Adults were examined in Khabarovsk Krai during forest fires. As opposed to children, they tended to have authentic impairments of the CNS and cardiovascular system as evidenced by growing numbers of hospital visits for coronary heart disease, essential hypertension and myocardial infarction [33]. Professor T.A. Zakharycheva, a co-author of this monograph, believes that 'there is practically no information about impacts of smoke gases from forest fires on the CNS' but patients with dyscirculatory and post-stroke encephalopathy who were treated in neurological hospitals in Khabarovsk and Komsomolsk-on-Amur tended to have decompensated state or transient ischemic attacks during forest fires. Patients with osteochondrosis, predominantly cervical spine one, had acute

[7] Bronkhial'naya astma [Bronchial asthma]: monograph in 2 volumes. In: RAMS Academician A.G. Chuchalin ed. Moscow, Agar Publ., 1997, vol. 1, 432 p. (in Russian).

vertebrobasilar insufficiency that manifested itself through vestibulocerebellar syndrome. The number of patients hospitalized for cerebrovascular pathology grew by 1.4–4 times ($p < 0.01$–0.001) and frequency of acute strokes grew by 1.2–4.5 times ($p < 0.01$–0.001) in a year with intensive forest fires against the previous one. The authors of this study, who are neurologists from the Far East State Medical University, believe that 'a large role in pathogenesis of cerebral ischemia is played by changes in the physiochemical state of blood resulting from exposure to toxic combustion products and having considerable impacts on cerebral hemodynamics'. In addition to that, many chemicals that are formed during fires affect various biochemical indicators, for example, lipid peroxidation [44]. This was confirmed in field conditions by research results obtained for people living in a district in Khabarovsk Krai, which was exposed to smoke from forest fires. Identification of lipid peroxidation markers and markers of antioxidant protection established signs of elevated cytolysis in one third of examined people, namely, higher levels of alanine aminotransferase (ALT) and alkaline phosphatase. The authors believe this 'might result from the lung fraction of blood entering the circulation upon inhaling toxic components in smoke able to damage cellular membranes of the respiratory airways and alveoli' [52].

A series of studies was conducted in Khabarovsk Krai to estimate effects produced by ambient air pollution due to wildfires. Among other things, it included reproductive health assessment. Pregnant women's health tended to deteriorate during periods when pollutant levels were the highest in ambient air; there was a growth in extragenital pathology detected in Komsomolsk-on-Amur during forest fires in 2018. An authentic increase in gestation and a trend towards blood hypercoagulation was established in exposed population in comparison with controls [33]. Reproductive health impairments associated with effects of long wildfires have also been reported in foreign publications [36].

Difficulties in timely extinguishing of forest fires are to a certain extent associated with closing down such an agency as the State Forest Guard. It was re-established several years later but experienced and valuable personnel had been lost. Areas with burning forests where fires were being extinguished reached 107 thousand hectares in 2019. The second problem is that some control zones are located in areas without any settlements and in case of fire only video monitoring is provided and not extinguishing. The situation with forest management is deemed 'deplorable' in the country [7] since sufficient financial support is not provided for proper forest protection and the existing agency cannot cope with the task.

In Russia, just as in any other country, several population groups are the most susceptible to effects of forest fires including people older than 65 years, people with chronic cardiorespiratory diseases, pregnant women and children who often fall sick. In cities, especially those with population above one million, special attention should be paid to elderly people and homeless people during periods of high air temperatures above the temperature threshold [19, 53] and during forest fires. Even if notification about coming heat and forest fires has been delivered in due time, some people from risky groups cannot take protective measures. Therefore, authorities, social organizations and volunteers should provide such people with drinking water, medications, and food. Demographic forecasts in Russia indicate a further growth in the share of people older than 65 years in the age structure of the country population. Given that, special attention should be paid to this population group by the whole social sector. The results obtained by studies conducted in the USA and Canada show that if short-term outcomes of PM exposures are taken into account, overall health impairments due to forest fires turn out to be even greater than expected [54–56].

This review concentrates on health risks caused by effects of forest fires. But there is another urgent issue, namely, health risk as-

sessment in situations when fires occur at household waste landfills, which typically contain huge quantities of various polymer materials. Smoke gases from fires at such objects contain volatile organic compounds, styrene, butylene, acetaldehyde, acetic acid, and other toxic chemicals. They can become pollution sources and be detected in ambient air at a considerable distance away form a fire site [57]. Smoke gases from wildfires can contain radioactive substances as well; for example, $_{127}$Cs often occurs in areas with radioactive pollution. The major part of this isotope is emitted into ambient air from burning forest floor [58]. This should be considered when planning control or scientific research on such territories.

Another serious issue is absence of any medical information for people how they should behave during abnormal heat and forest fires and either for decision-makers as regards what actions they should take in such situations. This has extremely negative impacts on human health. For example, in 2010 in Moscow, clinicians and experts in preventive medicine used to issue very controversial recommendations for population. In 1998 in Khabarovsk, people were recommended 'to use oxygen bags' during forest fires and heavy smoke pollution in ambient air. This might have enhanced negative impacts and resulted in oxidative stress caused by pollutants [33].

When analyzing the situation with forest fires in the country, some legal experts point out gaps both in the state legislation and in the very system for forest management in their publications. In their opinion, the Forest Code of the Russian Federation does not establish specific requirements to people, who extinguish forest fires; there is no effective regulatory legal base for protecting forests from fires; the state regulation in the sphere has some other drawbacks [59]. Some faults in legal documents on forest protection are also mentioned by a legal expert M.V. Oleynik [60], who insists on existence of several problems including illegal lumbering, illegal wood exports, absence of proper control over the forest fund and a unified information system. At the same time, there are some positive examples of implementing a video monitoring system around forests in Perm Krai. This made it possible for the Forest Guard to react in due time and take necessary measures aimed at localizing a fire and preventing smoke gases from spreading onto densely populated residential areas.

Experts from the Central Scientific Research Institute on Civil Defense and Emergency Situations of the Ministry of Emergency Situations of the Russian Federation believe it is necessary to implement a hardware-software complex Safe City into municipal management structures. This complex allows developing models for middle-term and long-term prediction of forest fires based on the analysis of historical data on meteorological conditions, fire hazard, forest fires, and other indicators [61]. Such a database and prediction scenarios are likely to be useful for Rospotrebnadzor regional offices within assessment of a sanitary-epidemiological situation on a given territory and development of relevant preventive activities during forest fires.

Experience gained by BRICS countries may be of interest since they regularly suffer from huge forest fires and therefore investigate health outcomes of this natural disaster and estimate economic losses caused by ambient air pollution in residential areas. In a study [62], the authors used a combination of regional and global air quality models and observations to examine the impact of forest and vegetation fires on air quality degradation and public health in Southeast Asia. They found that eliminating fire could substantially improve regional air quality by reducing the population exposure to fine particulate matter ($PM_{2.5}$) concentrations by 7 % and surface ozone concentrations by 5 %. These reductions in $PM_{2.5}$ exposures would yield a considerable public health benefit across the region averting 59,000 (95 % uncertainty interval (95 CI): 55,200–62,900) premature deaths annually. The authors conclude that reducing forest and vegetation fires should be a public health priority for the Southeast Asia region.

It is quite advisable to conduct a similar study for the most fire-hazardous Russian territories. It is necessary to reinforce monitoring of respiratory incidence among population, especially children, in action plans of healthcare organizations in areas exposed to smoke from forest fires and to include relevant preventive activities in them. Relevant agencies and institutions should organize control of ambient PM levels and develop models considering a temperature of smoke gases and PM spread as a key indicator of such gases towards densely populated residential areas.

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Review

ASSESSING THE RISK OF NEGATIVE EFFECTS PRODUCED BY ELECTROMAGNETIC FIELDS OF CELLULAR COMMUNICATION ON THE CENTRAL NERVOUS SYSTEM OF CHILDREN AND ADOLESCENTS (REVIEW). PART 2. INDICATORS OF COGNITIVE PROCESSES

N.I. Khorseva[1], P.E. Grigoriev[2,3]

[1]Emanuel Institute of Biochemical Physics of Russian Academy of Sciences, 4 Kosygina St., Moscow, 119334, Russian Federation
[2]Sevastopol State University, 33 Universitetskaya St., Sevastopol, 299053, Russian Federation
[3]Academic Research Institute of Physical Methods of Treatment, Medical Climatology and Rehabilitation named after I.M. Sechenov, 10/3 Mukhina St., Yalta, Republic of Crimea, 298603, Russian Federation

This paper continues the authors' review that dwells on modeling radiofrequency electromagnetic fields (RF EMF) and results obtained by measuring electroencephalography indicators, sensorimotor reactions, fatigue, work capacity, duration of an individual minute and the reproduction of a given rhythm in children and adolescents.

Health risk assessment is always based on data obtained by either laboratory tests or epidemiological studies. This paper analyses publications that describe effects of RF EMF exposure, including Wi-Fi, on cognitive processes in children and adolescents as well as methodical approaches to investigating this exposure. However, there are few such studies; in particular, effects produced by Wi-Fi exposure on cognitive indicators of adolescents aged 14–17 years, were found only in two publications.

Literature analysis has established that research findings do not always give an unambiguous estimation of RF EMF effects. The review covers the reasons for ambiguous interpretation of research results: a variable range of test-systems used for investigating indicators of cognitive processes; simultaneous analysis of single exposures including descriptions of 'effect of improvement' in indicators; changes in cognitive indicators registered for a group of children and adolescents in a wide age range.

Nevertheless, most results give evidence of negative changes in attention and memory of children and adolescents. Given that, longitudinal studies are becoming especially relevant since they estimate changes in various indicators in dynamics, including those induced by changes in mobile phone use. The review highlights the relevance of comprehensive investigations with their focus on health outcomes of RF EMF exposure intrinsic to 5G technologies considering their global implementation.

Keywords: *radiofrequency electromagnetic field, Wi-Fi, central nervous system, brain, attention, memory, children, adolescents.*

There are surprisingly very few studies on effects produced by radio frequency electromagnetic fields (RF EMF) on cognitive function in children and adolescents despite the issue being topical at present. This review focuses on studies that investigate changes in cognitive processes in children and adolescents, who actively use mobile communication, and primarily concentrate on chronic RF EMF exposure.

At present, authors of reviews that analyze accomplished studies in the sphere mostly highlight ambiguous and even controversial results obtained in investigating effects produced by RF EMF on cognitive function indicators in children and adolescents [1, 2].

Natalia I. Khorseva – Candidate of Biological Sciences, Senior Researcher at the Laboratory of Physical and Chemical Problems of Radiobiology and Ecology (e-mail: sheridan1957@mail.ru; tel.: +7 (905) 782-87-17; ORCID: https://orcid.org/0000-0002-3444-0050).

Pavel E. Grigoriev – Doctor of Biological Sciences, Associate Professor, Professor of Psychological Department; Leading Researcher at the Institute of Physical Methods of Treatment, Medical Climatology and Rehabilitation (e-mail: mhnty@yandex.ru; tel.: +7 (978) 767-22-10; ORCID: https://orcid.org/0000-0001-7390-9109).

T. Ishihara et al. [3] and J.-H. Moon [4] suggest a very cautious approach to assessing RF EMF exposures, including their effects on cognitive function in children and adolescents, in their works published in 2020.

In particular, having analyzed findings reported in 12 relevant studies, T. Ishihara et al. pointed out that no significant negative effects of RF EMF on cognitive function in children and adolescents were detected in 86 % of the analyzed cases. However, the authors selected very dissimilar studies for their analysis as regards both respondents' age and duration of use, mobile phones (MP) included, and analyzed parameters. We believe this to be the reason for such ambiguous conclusions.

A study by C. Sage et al. [5] presents a very nontrivial approach to investigating RF EMF effects on cognitive function in children and adolescents: analysis of epigenetic research. However, this work was criticized by D.R. Grimes and D.V.M. Bishop, who stated that overwhelming majority of available research data give evidence that microwave and radio frequencies employed in up-to-date communication devices are quite safe [6]. However, it is unclear what was used by the authors as solid grounds for such categorical statements.

On the contrary, a review by O.A. Vyatleva illustrates that intensive use of smartphones by children and adolescents results in declining cognitive function as well as slower maturation of the cortical zones responsible for speech, attention, emotion, reinforcement, and executive functions [7]. These statements are further confirmed by findings reported in the previous studies by J. Ferreira et al. and C. Fernández et al. that involved modeling of RF EMF exposures for mobile phone and tablet users [8, 9].

We believe there are several reasons for this 'ambiguity' related to negative RF EMF effects on cognitive function in children and adolescents.

First, many diverse test-systems are employed in the analyzed studies to investigate changes in indicators of cognitive processes upon RF EMF exposure.

In particular, foreign studies employ a wide range of tests to assess cognitive function upon RF EMF exposures including the Woodcock – Johnson Letter-Word and Passage Comprehension test [10]; assessment of inattention as defined for the Attention Deficit component of Attention deficit / Hyperactivity disorder (ADHD) [11]; N-back test [12]; 'Go / No Go' and 'Groton maze' learning tasks [13]; neuropsychological tests (Hanoi tower test, digit span test, K.A.S. test, stroop test, digit symbol test, Beck depression inventory test and Benton judgment of line orientation test) [14]; the Amsterdam Neuropsychological Tasks [15]; visual discrimination task and a modified Sternberg working memory task [16]; wide variety of computer test systems [17–23]; CogHealth™ specialized software package [13, 24].

Russian researchers also employed several methods in their studies including questioning [25], filling in the forms 'Arranging Numbers' and 'Memorizing Numbers' [26], instrumental tests performed on an automated psychophysiologist work station [27], LUM (local universal monitoring) software package [28].

Nevertheless, despite this wide range of test systems, few studies investigate RF EMF effects on cognitive function in children and adolescents.

Second, reviews very often cover findings reported in studies that investigate only one-time RF EMF exposure and sometimes even describe some improvement in cognitive indicators [19, 29].

Multidirectional effects were reported for primary schoolchildren of the 1^{st}–3^{rd} grade in a study by O.A. Vyatleva and A.M. Kurgansky. Some negative effects were detected but still the authors found negative correlations between attention and memory and duration of active MP use. The authors labeled these effects as 'stimulating influence', which was not associated with exposure to MP ra-

diation but rather depended on skills of MP use [25]. However, changes in cognitive function were assessed by questioning in this study and not by using special diagnostic methods.

It is worth noting that such effects of 'seeming improvement' in psychophysiological indicators were described in our works as well when we performed longitudinal studies to investigate changes in psychophysiological indicators of children and adolescents who actively used mobile communication. We believe that this effect may be caused by adaptation responses to this external exposure being rather new for children and adolescents as well as by the exposure being quite short-term (including cases of short experience of MP use) [27].

In addition to that, reviews often cover studies without any detected exposure effects reported in them. These controversial effects produced by RF EMF exposure on cognitive function in children and adolescents were previously discussed in the book [27] and monograph [28].

Third, changes in cognitive function are usually registered for groups of children and adolescents with wide age ranges; practically all studies perform age-specific analysis of using MP or other gadgets for each age group but cognitive function is typically characterrized for the whole data array without considering age-specific features. This group of studies can be tentatively divided into several basic groups:

1. Studies that investigate changes in cognitive function with simultaneous instrumental assessment of levels of exposure to affecting RF EMF. Studies by O.A. Vyatleva with colleagues [25] and S.A. Meo et al. [30] are good examples of this approach. The former work reported a 'stimulating effect' of exposure to MP RF EMF [25]; the latter reported that higher RF EMF created by base stations resulted in delayed spatial working memory and attention and affected fine and gross motor skills [30].

2. Studies that investigate combined RF EMF exposures coming from various sources. Thus, M. Guxens et al. employed a 3D spatial model to assess the relationship between residential RF EMF exposure from mobile phone base stations, residential presence of indoor sources, personal cell phone and cordless phone use, and children's cognitive function at 5-6 years of age. Yet, the study findings were rather controversial [15]. In contrast to the study by M. Guxens et al., A. Cabré-Riera et al. assessed RF EMF doses for two groups of adolescents aged 9–11 years and 17–18 years in 2 population cohorts in the Netherlands and Spain not for all analyzed sources combined but separately (mobile phones, screen activities and far-field). The study established that higher overall estimated whole-brain RF EMF doses from all RF EMF sources together and from phone calls were associated with lower non-verbal intelligence score [31].

3. Studies that focus on the relationship between indicators of cognitive processes [11, 17, 18, 21] and academic performance [32] and the MP use mode. Although authors of most publications detected certain negative changes in cognitive function, the very approach to such studies is rather questionable. In particular, M.J. Abramson et al. accomplished their study on adolescents aged 11–14 years where they established poorer accuracy of working memory and longer completion time for tasks both for adolescents who used MP most frequently and for people who sent many SMS. On this basis, the authors assumed that cognitive changes were unlikely due to exposure to radiofrequency [17]. We believe that such conclusion can be made only basing on comparison of cognitive indicators identified for students who used SMS only and those identified for students who used MP exclusively for talking. Practically the same results were obtained by S. Thomas et al. (adolescents aged 12–13 years) [18]; however, in contrast to M.J. Abramson et al. [17], the authors investigated changes in cog-

nitive function in dynamics one year after considering changes in the MP use mode, including voice calls and SMS. They detected changes in simple reaction time and working memory task but no correlation was identified between mobile phone exposure and cognitive test completion. However, the authors make the same methodical mistakes in this study as M.J. Abramson et al. did when interpreting their study findings. F. Zheng et al. detected higher inattention in those secondary school students (aged 12–20 years) who played games on their MP for more than one hour a day [11]. In this case, we believe the detected effects to be more closely associated with 'screen time' of a gadget than to its electromagnetic radiation. Finally, by using questioning, X. Liu et al. established a correlation between duration of MP use and academic performance of schoolchildren aged 12–18 years: those adolescents who actively used MP (≥ 2 hours/day on weekdays and ≥ 5 hours/day on weekends) had a considerable decline in academic performance against those who used MP less actively [32]. However, the authors did not provide any information about what adolescents used their MP for (voice calls, SMS, MMS, watching videos, etc.).

4. Studies that compare effects of MP exposure and exposure to cordless phones (CP) on students' cognitive function. M. Redmayne et al. detected poorer cognitive function in a cohort of schoolchildren aged 8–11 years due to MP and CP use; however, the authors believe that their study findings do not give unambiguous evidence of negative effects produced by MP and CP RF EMF [21]. Prospect (longitudinal: the authors' remark) studies hold a significant place here [20, 23, 33, 34], in particular, cohort studies on cognitive function of Swiss adolescents aged 12–17 years with simultaneous assessment of RF EMF cumulative dose [20, 23, 24, 33]. Yet, the obtained results are still ambiguous. For example, A. Schoeni et al. believe that RF EMF exposure affects memory since a decline in figural memory performance was more apparent in MP users against CP users [20]. Nevertheless, in another study, the same authors did not find any negative changes in attention concentration and behavior in adolescents who used MP and CP [32]. A study by M. Foerster et al. [23] compared indicators of cognitive processes in adolescents from the 7–9th grades with a cumulative individual microwave radiation dose from mass media, both associated and not associated with RF EMF (in particular, mobile phones). The authors established poorer figural memory in MP users. Similar results were reported in a prospect study accomplished in Australia on primary school students as poorer cognitive function was detected in MP users and not CP users [24]. We believe that the obtained results were so ambiguous because all these studies analyzed indicators detected for the whole data array without considering age-specific features of the analyzed groups.

5. Complex studies with their focus on psychophysiological indicators, including those describing how well cognitive function is developed in children and adolescents who are active mobile communication users. Among such works, we should mention longitudinal cohort studies involving children and adolescents aged 5–16.5 years that have been accomplished in Russia since 2006 [27, 28]. In contrast to most studies, cognitive indicators are assessed separately for each age group and any dynamics of changes in them is estimated considering MP use mode. Semantic memory has been established to decline more than volitional attention in children who are active mobile communication users; also, many indicators in them are at the bottom limit of the age-specific physiological norm or already below it. The obtained results confirm that chronic exposure to MP electromagnetic radiation can have considerable effects on psychic cognitive processes in children and affect their academic performance. It should be noted that preventive measures taken within this monitoring and

aimed at implementing safe culture of modern gadget use (first of all, MP) have been proven to be effective. Psychophysiological indicators of schoolchildren who adhered to safe user modes improved significantly and returned to their age-specific physiologic range [28, 35, 36].

At present, new technologies, Wi-Fi among them, are being implemented quite actively and are being used everywhere, including educational establishments. However, many researchers do not pay any attention to likelihood of negative effects that might be caused by this implementation. There is an established opinion that since values of electric field intensity and power density are below the threshold values stipulated by the International Commission on Non-Ionizing Radiation Protection (ICNIRP), any risks of negative effects are minimal. However, the issue is far from being resolved and accumulated experimental studies, which we presented in the previous review, are clear evidence of it. In addition to that, many countries (France, Italy, Greece, Germany – Bavaria, Belgium, Great Britain, Tajikistan, Azerbaijan, Belarus, and Bangladesh) have introduced the full prohibition on using this technology in schools or imposed considerable limitation on its use. This was done to prevent possible negative effects on children and adolescents.

The literature analysis shows that very few studies investigate influence on children and adolescents. In particular, we can mention works by K. Bamdad et al. and M.A. Hosseini et al. The former reported the adverse consequences of 2.4–2.48 GHz radiofrequency electromagnetic fields of Wi-Fi router devices on divided attention levels of female university students (aged 14–17 years) [37]; the authors of the latter did not establish any effects on cognitive function upon short-term exposure to Wi-Fi waves [38].

At present, special attention should be paid to wide-scale implementation of 5G technologies. Although there still have not been any studies on biological effects of the 5G standard [39], nevertheless, likely impacts on population health remain the most acute issue [40].

Given that, general polemics about biological effects of 5G should, in our opinion, primarily include a discussion about how this standard may affect children and adolescents. This age groups happens to not only be the most sensitive to changes in any environmental factors but also have a greater 'potential' to become vulnerable to EMF created by the 5G standard. This is because higher frequencies of the standard are intensely 'absorbed' from ambient air into the water component in human sweat and derma cells thus resulting in higher absorption [41]; since children tend to have more 'watery' skin than adults, this potentially increases their radiosensitivity.

The fact is that at present active implementation of 5G technologies is considerably ahead of hazard assessment for lifelong millimeter range exposures on skin and eye sclera, primarily in children and adolescents [42, 43].

We believe the problem requires immediate solution in order to identify potential adverse effects of such exposures.

Conclusion. A developing body is known to be especially sensitive to electromagnetic environmental factors. In particular, this concerns the central nervous system. Since most children start to use gadgets with active RF EMF already at preschool age, this calls for revision of approaches to assessing risks caused by such exposures. This means developing new methods for modeling both absorbed RF EMF doses and effects of acute and chronic exposure to RF EMF of mobile communication devices assessed per intensity of their use.

Most researchers highlight negative RF EMF effects on functional indicators of the central nervous system, cognitive function in particular. Still, some studies have established no adverse effects. We believe this is due to different methodical approaches and many

diverse tests systems employed to detect any possible effects.

At present, it is important to continue studies with their focus on possible negative effects produced by RF EMF on the central nervous system in children and adolescents. We believe it might be useful to adopt useful practices from other countries where use of some technologies, Wi-Fi for example, is limited in educational establishments. In additional to that, researchers should pay the greatest attention to establishing every possible effects caused by exposure to RF EMF created by 5G-based devices.

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Review

METHODOLOGICAL ASPECTS OF USING ALLOSTATIC LOAD ANALYSIS IN ASSESSING HEALTH OF WORKING POPULATION EXPOSED TO ADVERSE OCCUPATIONAL FACTORS (ANALYTICAL REVIEW)

G.A. Bezrukova[1], A.N. Mikerov[1,2], T.A. Novikova[1]

[1]Saratov Hygiene Medical Research Center, 1A Zarechnaya St., Saratov, 410022, Russian Federation
[2]Saratov State Medical University named after V.I. Razumovsky, 112 Bol'shaya Kazachaya St., Saratov, 410012, Russian Federation

Preventive medicine of pathogenetically based technologies for prenosological diagnostics of health risks at the stage of reversible physiological dysregulation is being introduced into practice as a relevant strategy for preserving population. It provides suitable conditions for timely prevention of chronic diseases and reducing risks of premature mortality among working age population. Using resources of the scientific information systems CyberLeninka, eLibrary, PubMed and Google scholar, the authors analyzed and summarized scientific literature data on methodological aspects and problems related to practical application of the concept of allostasis and allostatic load (AL) in assessing and predicting health risks for working population

The review focuses on the main causes of physiological dysregulation leading to AL formation under environmental exposures, including occupational ones; presents the most popular biomarkers of the functional state of the neuroendocrine, immune-inflammatory, cardiovascular and metabolic systems included in the sets of variables for determining the AL index. The review also provides the description of the most common algorithms for calculating the AL index used in preventive examinations of workers and highlights methodological approaches to the correction of AL values with regular intake of medicines. The sex-specific age dynamics of AL is presented; attention is drawn to the aggravating effect produced on AL by negative behavioral factors.

The review shows that it is still difficult to introduce this methodology into routine practices of preventive medical examinations of working population despite the proven diagnostic and prognostic significance of the prenosological diagnosis of health disorders based on AL. This is mostly due to lack of consensus on standardized approaches to creating sets of biomarker scales and a method for calculating the AL index, as well as considering the sex factor and contribution of therapeutic effects to cumulative assessment of risks of developing physiological dysfunctions.

Keywords: *homeostasis, allostasis, allostatic load, allostasis biomarkers, allostatic load index, prenosological diagnostics, working population, working conditions, adaptation, occupational stress.*

Contemporary global challenges determine priority trends in the state policy of the Russian Federation. These priorities include health protection, improvement of life quality, and growth in welfare of the country population based on birth rate growth, growth in life expectancy at birth, longer years of active life, decline in mortality and disability as well as on creating suitable conditions for timely disease prevention and formation of healthy lifestyles[1].

Results obtained by recent studies in hygiene and occupational medicine obviously indicate that existing and new occupational factors emerging in the 21st century able to affect workers' life and health are caused by

Galina A. Bezrukova – Doctor of Medical Sciences, Associate Professor, Chief Researcher of the Department of Occupational Medicine (e-mail: bezrukovagala@yandex.ru; tel.: +7 (917) 317-61-90; ORCID: https://orcid.org/0009-0009-6254-3506).

Anatoly N. Mikerov – Doctor of Biological Sciences, Head (e-mail: a_mikerov@mail.ru; tel.: +7 (495) 292-78-90; ORCID: https://orcid.org/0000-0002-0670-7918).

Tamara A. Novikova – Candidate of Biological Sciences, Associate Professor, Head of the Laboratory of Occupational Hygiene (e-mail: novikovata-saratov@yandex.ru; tel.: +7 (495) 292-78-90; ORCID: https://orcid.org/0000-0003-1463-0559).

[1] O natsional'nykh tselyakh razvitiya Rossiiskoi Federatsii na period do 2030 goda i na perspektivu do 2036 goda: Ukaz Prezidenta RF ot 07.05.2024 № 309 [On national goals of the Russian Federation development for the period up to 2030 and for the future period up to 2036: The RF President Order issued on May 07, 2024 No. 309]. *Prezident Rossii*. Available at: http://www.kremlin.ru/events/president/news/73986 (June 24, 2024) (in Russian).

exponentially growing use of new technologies, first of all, digital ones; key changes in workplace structures and design and organization of work processes as well as employment structure given the global trend of workforce ageing [1, 2]. All this is accompanied with growing biological and psychosocial occupational hazards [3] that create additional risks of somatic (essential hypertension, coronary heart disease, obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease, dorsalgia, and neoplasms) and mental (depression-like states, actual depression) health issues in workers of various occupational groups [4–6].

Given that, it is important to rely on the complex system of social-hygienic monitoring that involves analysis of effects produced by working conditions on workers' health, occupational risk assessment, prediction of progression of occupational and work-related diseases [7] as well as general somatic pathology that affects work ability. This requires farther development of a methodology for prenosological diagnostics of diseases in workers in order to achieve their early detection and provide timely personalized and group prevention [8, 9]. The World Health Organization (WHO) gives recommendations how to determine early signs of negative effects produced by occupational environment and work processes on workers' bodies. They should be determined relying on pathogenetically grounded informative diagnostic methods eligible for detecting disrupted mechanisms of adaptation to external exposures at the stage when physiological deregulation can still be reversed prior to the development of nosologic syndromes and clinically identifiable occupational and general somatic diseases [10].

Over the last decades, methodical instruments based on the concept of allostasis and allostatic load (AL) have been successfully used in complex assessments of intensity of physiological deregulation caused by occupational factors including psychosocial, physical, chemical, biological and some others [11]. These instruments allow estimating adaptability to chronic occupational stress per combined changes in the circulatory, respiratory, and immune systems, metabolic processes and anthropometric data [12]. At present, although the AL model is in great demand in foreign epidemiological studies [13–15], it still has not been actively employed in Russian hygiene and occupational medicine within prenosological diagnostics and prediction of occupational or work-related diseases.

The aim of this study was to summarize and analyze available literature data on up-to-date methodical aspects and issues of using the concept of allostasis and allostatic load in practice to assess and predict risks of diseases in working population.

Materials and methods. Russian and foreign studies were searched in relevant databases including RSCI, eLibrary, CyberLeninka, PubMed and Google scholar. Search requests included the following keywords and their combinations: 'allostasis', 'allostatic load', 'allostasis biomarkers', 'allostatic load index', 'homeostasis', 'prenosological diagnostics', 'adult population', 'working population', 'working conditions', 'adaptation', 'occupational stress', 'occupational burnout', 'age', 'biological sex'. The search depth covered the period between 1997 and 2024. The review covers 55 studies selected from preliminarily analyzed 207 foreign and Russian scientific publications.

Results and discussion. Homeostasis primarily maintains dynamic internal stability of the body. In contrast to that, allostasis is a process aimed at effective regulation of adaptability to new emerging environmental challenges (stressors) through change that makes the body more resistant to new environmental conditions [16]. Over time, 'wear and tear' of this compensatory-adaptive mechanism of response to stressor exposures may lead to subclinical accumulation of dysfunction in some regulatory systems in the body. This state is known as allostatic load [17]. Homeostasis is maintained by each involved functional system in the body performing

physiological control of its indicators through negative or positive feedback providing metabolism stability. In contrast, allostatic reactions are regulated by the central nervous system that makes constant corrections in changes of the body physiological state occurring due to stressor exposures [18]. I.N. Karatsoreos and B.S. McEwen believe allostasis to be a dynamic, integral and adaptively plastic mechanism that combines sensory perception and cognitive assessment of risks related to stressor exposures and initiates a cascade of typical stress-mediated responses [19].

B.S. McEwen described four models of body responses to changes in the environment, allostatic load or even overload being their outcome: 1) frequent or permanent exposures that induce chronic stress and repeated physiological agitation; 2) absence of stable adaptation to persistent stressors; 3) persisting elevated levels of compensatory-adaptive reactions after a stressor exposure has ended; 4) inadequate and / or insufficient adaptation mechanisms that should fight against a stressor. These four types of overactive or ineffectively managed compensatory-adaptive reactions can exist separately or in their combinations [11, 20]. Any chronic or frequently repeating stressor exposures are able to induce AL growth when body adaptive reserves are depleted and compensatory-adaptive pathways of basic allostasis-regulating systems (the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system, immune-inflammatory system) become non-coordinated. This can ultimately manifest itself as stress-related disease [21, 22]. Therefore, under persisting stressor exposures, AL-related adaptive changes at best mitigate induced metabolic and functional disorders; at worst, they lead to chronic diseases, accelerated ageing and premature all-cause mortality [11, 23].

Environmental factors, occupational ones included, produce certain effects on the body that are determined by genesis and levels of stressor exposures. Identification of their impacts, either cumulative or combined ones, involves certain difficulties associated with multiple variable existing pathogenetic ways of disease progression and their interplay as well as with individual susceptibility and adaptability to the environment [24]. At the same time, results obtained by foreign epidemiological studies indicate that use of allostatic load as a poly-system indicator of chronic physiological dysfunction allows investigating multiple stressors in their integrity and quantifying their influence on population incidence and mortality [11, 25].

Initially, the allostatic load index (AL index) was suggested by T.E. Seeman with colleagues as an instrument to measure subclinical health impairments[2]. It was calculated based on biomarkers presented by primary mediators of stress-related responses, including dehydroepiandrosterone sulfate (DHEA-S), cortisol, adrenalin and norepinephrine, and biomarkers of secondary chronic stress outcomes that manifest themselves in the cardiovascular (systolic and diastolic blood pressure, total cholesterol, high density lipoproteins) and metabolic (waist-to-hip ratio, glycated hemoglobin) systems. Later the authors admitted that this set made of 10 indicators was not meant to become a standard AL index scale and could not be considered comprehensive. The reasons are that it was based on biological data available to its developers and therefore it can be altered and added with other markers of body regulatory and functional systems depending on specific research aims [26].

At present, more than 70 biomarkers of various functional systems have been suggested to determine the allostatic load index. The neuroendocrine, cardiovascular, metabolic and immune-inflammatory systems are the basic ones used in allostatic load calculation [15, 27, 28] (Table 1).

[2] Seeman T.E., Singer B.H., Rowe J.W., Horwitz R.I., McEwen B. Price of adaptation – allostatic load and its health consequences. MacArthur studies of successful aging. *Arch. Intern. Med.*, 1997, vol. 157, no. 19, pp. 2259–2268.

Table 1

Biomarkers employed to calculate allostatic load

Functional system	Biomarkers
Neuroendocrine	adrenalin, cortisol, dehydroepiandrosterone sulfate, epinephrine, norepinephrine, testosterone, thyrotrophic hormone
Parasympathetic	heart rate variability
Immune-inflammatory	erythrocyte sedimentation rate, white blood cell count, C-reactive protein, fibrinogen, interleukin 1 receptor antagonist, beta interleukin, interleukin 6, interleukin 8, interleukin 10, interleukin 12p70, tumor necrosis factor alpha, E-selectin, intercellular adhesion molecule type 1, total immunoglobulin E, insulin-like growth factor 1, herpes I and II antibodies
Cardiovascular	resting heart rate, pulse wave velocity, systolic and diastolic blood pressure, pulse pressure
Metabolic	total cholesterol, high density lipoproteins cholesterol, low density lipoproteins cholesterol, CS-HDL / TCS ratio, triglycerides, apolipoprotein A1, apolipoprotein B, fasting glucose, glycated hemoglobin, insulin, HOMA-IR, adiponectin, leptin, body mass index, waist circumference, waist-to-hip ratio, waist-to-height ratio
Respiratory	peak expiratory flow rate, the forced expiratory volume in 1 second to forced vital capacity ratio
Urinary	creatinine, cystatin C
Hepatobiliary	albumin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamate aminotransferase, alkaline phosphatase

In various epidemiological studies, multi-system models for AL assessment may include, as a rule, between 5 and 26 physiological biomarkers to describe health; on average, 9 indicators [29]. In practice, AL scales have often been added with highly correlated variables such as systolic and diastolic blood pressure; total cholesterol (TCS) and low density lipoproteins cholesterol (LDL-CS); fasting glucose in blood serum and glycated hemoglobin (HbA1c); body mass index and waist-to-hip ratio. Another widespread practice is to include primary mediators of stress reactions into analysis that reflect recent stress events and not a cumulative effect of stressor exposures [13]. Some researchers believe that such practices are able to reduce the statistical significance of prognostic AL models due to increasing measurement inaccuracies [30, 31].

It is noteworthy that despite growing numbers and diversity of biomarkers included into allostatic load assessment by different researchers, a consensus has not been reached yet as regards what biomarkers are the most eligible for such assessments [18, 19, 29]. C. McCrory with colleagues aimed to create the most effective set of AL biomarkers. To do that, they took data obtained by 13 cohort medical and preventive examinations of working population in Western European countries, the USA and South Africa and performed comparative meta-analysis of strength of correlations between 40 biomarkers describing the functional state of the neuroendocrine, parasympathetic, immune-inflammatory, cardiovascular, metabolic, respiratory, urinary, hepatobiliary and antioxidant systems and general health measures of 67,126 examined participants such as walking speed [32], handgrip strength [33] and self-rated health. The study results showed significant correlations between integrated health measures and only 9 out of 40 analyzed biomarkers: DHEA-S, heart rate variability (HRV), C-reactive protein (CRP), resting heart rate (HR), peak expiratory flow rate (PEFR), high density lipoproteins cholesterol (HDL-CS), waist-to-height ratio (W/H), HbA1c and cystatin C. In addition to that, the

authors established that the AL index based on only five biomarkers (CRP, HR, HDL-CS, W/H and HbA1c), which were present in each analyzed cohort study, had a significant correlation with high mortality risk, just as more complex sets of AL biomarkers [15]. It is noteworthy that three variables, namely CRP, HbA1c and HR, which were analyzed in a cohort of Great Britain's National Child Development Study (NCDS) (7981 participants were examined at the age of 44–45 years and 54–55 years), had an authentic correlation with elevated risks of cardiovascular mortality and premature all-cause mortality over a 10-year period whereas the cortisol level did not have any prognostic value [23, 34].

It should be noted that not only validity of variables that are suggested for inclusion in an AL scale is considered significant in accomplishing periodical check-ups of working population. Other important aspects are economic costs related to testing AL indicators as well as correctness and standardization of taking biosamples within a mass outpatient examination, first of all, those aimed at analyzing primary stress mediators (cortisol, adrenalin, noradrenalin, dopamine, and DHEA-S) [12, 35]. At the same time, relying on population data analyses, researchers more often believe it is possible to exclude these primary indicators from an AL scale since they have a lower prognostic value as compared to biomarkers of the immune, metabolic and cardiovascular physiological systems [36–38]. D. Mauss with colleagues took workers employed at industrial enterprises in Germany as an example to develop a simplified approach to AL index assessment. It was based on five routine indicators including diastolic blood pressure, HbA1c, LDL-CS, WC and HRV and showed a strong correlation with workloads in a model that described an imbalance between efforts and remuneration [35].

Apart from selecting relevant variables, another significant issue is to select a method for AL index calculation, which should be relevant to research aims [31]. At present, there are approximately 15 algorithms for AL index estimation. The method suggested by T.E. Seeman with colleagues[3] is the most popular. According to this algorithm, intensity of cumulative health impairment is determined by total dichotomous manifestation of disease risk through risk quartiles. Biomarker values that fall within the high risk quartile (the upper 75 % percentile) are assigned score 1 whereas all the others are estimated as zero (low risk). The only exclusion are some indicators since even their low values (the lower 25 % quartile) create high risks of physiological dysregulation, for example, HDL-CS or DHEA-S. After code values have been assigned to all biomarkers, an individual AL index is calculated by simply summing up the scores assigned to each biomarker [39]. Similar to the foregoing algorithm, the AL index can be calculated based on values of biomarkers falling within the upper (90 % percentile) and lower (10 % percentile) decile of high subclinical health risks [40]. Experts believe that regardless of a combination and quantity of variables employed to calculated AL, its total index as possible predictor of disease in long-term outlook is better than any other biomarkers when analyzed separately [15, 27].

Use of quartiles / deciles to identify high risks of biomarkers included into an AL scale means that each of them makes an even contribution to this multisystem model. However, each physiological system is described with different numbers of variables, and this, especially in a situation when all variables are strongly correlated, can lead to inaccuracy in AL index calculations [41]. As a rule, the metabolic and cardiovascular systems [15, 27] are described with a greater number of indicators. To eliminate any shifts towards them, a weighted estimation of average system risk was suggested. It allows uniform presentation

[3] Seeman T.E., Singer B.H., Rowe J.W., Horwitz R.I., McEwen B. Price of adaptation – allostatic load and its health consequences. MacArthur studies of successful aging. *Arch. Intern. Med.*, 1997, vol. 157, no. 19, pp. 2259–2268.

Table 2

Clinically relevant cut points for high-, moderate-, and low-risk categories of specific biomarkers by E.J. Rodriquez et al. [31]

System	Marker	Risk categories		
		High	Moderate	Low
Cardiovascular	Systolic blood pressure, mmHg	≥ 150	120–149	< 120
	Diastolic blood pressure, mmHg	≥ 90	80–89	< 80
	Total cholesterol, mg/dL	≥ 240	200–239	< 200
	HDL cholesterol, mg/dL	< 40	40–59	> 60
	Total/HDL cholesterol ratio	> 4.0	3.0–4.0	< 3.0
Metabolic	Glycated hemoglobin, %	≥ 6.5	between 5.7 and < 6.5	< 5.7
	Waist-hip ratio (women)	≥ 0.85	between > 0.80 and < 0.85	≤ 0.80
	Waist-hip ratio (men)	≥ 1.0	between 0.95 and < 1.0	≤ 0.95
	Body mass index, kg/m^2	≥ 30	between 25 and < 30	between 18 and < 25
	Albumin, g/dL	< 3	between 3 and < 3.8	≥ 3.8
Inflammatory	C-reactive protein, mg/L	> 3	1–3	< 1

of all functional systems in the ultimate AL index regardless of how many biomarkers were assessed for each of them [42].

Another way to calculate an AL index is an algorithm based on clinically established reference threshold values of biomarkers. It is primarily implemented within mass preventive medical check-ups of adult people in outpatient clinics and relies on the following scale that estimates risks of physiological 'wear and tear' on the body: low risk, 0 score; moderate risk, 0.5 score; high risk, 1 score [43] (Table 2).

It is noteworthy that the AL calculation method based on clinical measurements has some limitations. They reduce its potential eligibility as an indicator of subclinical health impairment, first of all, due to absence of established risk category ranges for most biomarkers included into extended sets of AL index variables. Another reason is identification of high risks of dysfunction at values corresponding to a clinical phenotype, for example, metabolic syndrome [43]. Given that, it does not seem advisable to employ clinical measurements in allostatic load assessment when examining people from specific occupational groups (military, rescue workers, firefighters, and law enforcement personnel) since their occupational activities make high demands of their physical and mental health [18]. As a rule, these occupational groups have lower incidence rates and levels of physiological deregulation as opposed to general population due to strict tests taken before entering an occupation [44].

Selection of a method for calculating the AL index sensitive to system prenosological changes in health becomes important in comparative examinations of physiological adaptability to environmental factors in different occupational groups. Most researchers believe the z-score analysis to be the most informative in this respect [18, 39]. The z-scores are assigned to AL biomarkers depending on the number of standard deviations from the relevant average in a sample identified for each variable; deviations above the average are considered positive and below the average, negative. For biomarkers, low levels of which correspond to high risks of physiological deregulation, an additive inverse value of an indicator is included into the AL index. Higher total scores per selected continuous values of variables correspond to the greatest physiological deregulation [45]. This method allows estimating allostatic load for various population groups even when different thresholds of its subclinical risks are employed in each analyzed sample [18]. This makes for wide use of the z-score analysis in longitudinal studies [46].

Regular drug administration remains an open question in allostatic load assessment. This concerns therapies with drugs aimed at stabilizing the cardiovascular system (hypotensive drugs, beta blockers, and calcium blockers), lipid (statins) and carbohydrate (hypoglycemic drugs) metabolism. The expert society has not yet reached any consensus on the matter [31, 39]. In earlier research, the prevailing opinion was that administration of drugs that normalize functional state of the body systems and reduce risks of chronic pathology does not require any changes in a current AL index assessment [47]. Later on, more and more researchers started to believe that even though supportive drug therapy reduces individual health risks its use already indicates existing physiological deregulation in the compromised systems. This deregulation cannot be considered completely reversible if constant drug therapy is needed [42].

Different approaches are employed to adjust the AL index for people who take drugs aimed at maintaining values of diagnostic biomarkers within their reference ranges. Most frequently, relevant data on drug administration are included into AL index calculations, the cardiovascular system, glucose and lipid metabolism being the primary targets for drug therapy. System risk for those drug-taking patients who participate in epidemiological studies is assigned into the quartile of high health risk regardless of actual values identified for indicators of the systems supported by drug therapy [48]. E.J. Rodriquez and others offer a compromise that involves adding a half-score to the total indicator of a functional system supported by drug therapy [31]. We should also mention high prevalence of blood pressure (BP) indicators in clinical measurements of the AL index. Given that, T. Robertson and E. Watts recommended adjusting systolic and diastolic BP for patients who take hypotensive drugs by adding 10 and 5 mmHg respectively to their actual levels. This allows more accurate prediction of subclinical risks [49].

Epidemiological studies usually cover established hygienic (physical, ergonomic, chemical, and biological) and psychosocial occupational factors that promote chronic occupational stress [21]. In addition to them, it is recommended to consider several indicators able to independently influence regulatory allostasis systems when examining working population. These indicators are conventionally considered secondary determinants of adaptation processes [44]. Age and sex are two non-modifiable factors that play the most significant role in use of the allostatic load model within preventive medical heck-ups of adult population [30, 50].

The allostasis theory considers the AL model as a general physiological mechanism of cumulative body ‘wear and tear’ due to accumulated stressor exposures that occur through the lifetime [18, 21, 23]. On this basis, most researchers consider the AL index a universal indicator that describes age-specific changes in functional systems and has a strong correlation with biological ageing [39, 49]. Age dynamics in allostatic load is a nonlinear process; the rate at which system dysfunctions accumulate in the body has a positive correlation with risks of adverse vital outcomes, primarily, all-cause mortality [17]. Results obtained by epidemiological studies in various occupational groups show a slow rise in AL starting from the age of 20–25 years with a subsequent drastic rise in its growth rate at the age of 35–65 years. Next, the plateau is reached and after that an insignificant decline is possible during a period with the highest mortality risks (people older than 90 years) [13]. Stabilization of AL population values at the end of lifetime is assumed to be due to selective premature deaths at working age when people with the highest AL do not live long enough to reach late maturity or old age [18]. On the other hand, peculiarities of AL age dynamics emphasize how important it is to monitor it throughout the whole period of working, even in workers who do not have any health-related complaints. This opens an almost 40-year long

'window of opportunity' to correct physiological dysfunctions by using medical and preventive technologies and commitment to healthy lifestyles [13].

Biological sex and sex dimorphism of cognitive assessment of biopsychosocial risks can influence AL, either independently or in synergy. Multiple studies performed in various occupational groups and adult population in general report that higher AL is typically detected in men younger than 45–50 years than in women of the same age. Sex-specific differences in AL tend to smooth out with age [11, 13, 51]. Sex-related variability in AL can be caused by multiple factors. The most significant ones include immune-, cardio- and neuroprotector effects of estrogen as well as higher biological sensitivity to stressor exposures, which is typical for men [52]. Sex dimorphism in AL is mostly considered within the approach to its assessment based on clinical measurements due to sex-specific differences in reference ranges established as physiological norms for certain biomarkers of chronic stress. In case other methods are used to identify the AL index, sex-dependent risk of physiological dysfunctions is analyzed rarer despite its significance and a necessity of such analysis is usually determined by research aims [36, 39, 49].

Apart from age and sex, some other well-known modifiable risk factors of chronic diseases have significant influence on AL dynamics. Primarily, we should mention behavioral ones (unhealthy diets, low physical activity, smoking, and alcohol intake) [53], which increase AL levels and growth rates [11, 18, 24]. The latter, together with working conditions, is recommended to be considered when analyzing current health of working population, predicting vital impairments and premature mortality as well as when developing health recovery programs and preventive and therapeutic measures to prolong healthy and active working life.

Conclusions. The review has presented the results obtained by retrospective and instant studies that focus on practical use of the allostasis concept in prenosological health assessment in various occupational groups and adult population in general. These results show that allostatic load is an effective marker of cumulative physiological deregulation at the multisystem level and can be used as a predictor of poly-morbid states and premature all-cause mortality. At the same time, despite its verified diagnostic and prognostic significance, it would be difficult to integrate AL into the standard of preventive medical check-ups for working population since there is still no consensus as regards standardized approaches to creating a biomarker scale or selecting a method for AL calculation. Other unresolved issues are related to considering sex factor and contributions made by therapeutic interventions into cumulative assessments of physiological dysfunctions.

The most disputable issue is whether any primary mediators of stress-related responses (adrenalin, noradrenalin, or cortisol) should be mandatorily included into an AL scale. However, results obtained by population studies clearly show that these neuroendocrine biomarkers tend to have daily fluctuations in their activity caused by routine daily stresses. They correlate with risks of subclinical health impairments and adverse outcomes of occupational stresses to a lesser extent than biomarkers of various functional systems (cardiovascular, metabolic, or immune-inflammatory). This allows excluding these biomarkers from AL panels without any reduction in their diagnostic and prognostic value.

At present, the analysis of the continuous z-scores is considered by most experts to be the most informative methodical approach to allostatic load quantification. At the same time, this method involves additional statistical unification of initial data just as AL index calculations based on quartiles / deciles of the highest risks of multisystem dysfunctions. Hence, they allow stratifying health impairments only within one selected population and exclude comparison with population groups, for which other sets of AL biomarkers were

employed. Given that, it seems more advisable to employ the AL estimation method, which is based on clinical measurements, when conducting mass preventive medical check-ups of working population. This method relies on using verified sex-specific reference ranges of routine clinical-laboratory, functional and anthropometric indicators that correspond to low, moderate and high risks of physiological dysfunctions.

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Review

THE ROLE OF GENETIC FACTORS IN LIKELIHOOD OF TYPE 2 DIABETES MELLITUS

E.S. Ageeva, Iu.I. Shramko, A.V. Kubyshkin, I.I. Fomochkina, A.A. Zhukova, C.O. Tarimov, S.G. Nastoyashchii, M.B. Zaurova

Crimea Federal University named after S.I. Georgievsky, 5/7 Lenina blvd, Simferopol, 295000, Russian Federation

Type 2 diabetes mellitus (DM2) is the ninth leading cause of worldwide mortality. To predict likelihood of the disease and its unfavorable clinical course with developing complications, it is necessary to consider genetic and molecular factors of DM2 pathogenesis. Therefore, the aim of this review was to analyze the role that belongs to genetic factors in molecular mechanisms of DM2 development and to establish the most significant single nucleotide polymorphisms (SNP) in DM2 pathogenesis.

The authors have analyzed literature sources found in such databases as CYBERLENINKA, E-library, and National Center for Biotechnology Information over the last 10 years as well as the integrated database GeneCards.

By now, Genome-Wide Association Studies (GWAS) have identified about 100 genes and more than 700 polymorphisms that influence DM2 risks and its likelihood. Classifications of candidate genes with their effects and expression being limited by external and internal transcription factors are rather tentative. Literature analysis has established certain ambiguousness of the role that belongs to genetic markers in DM2 pathogenesis. It is advisable to add new genetic markers of the PPARγ, TLR4, IRS and IL-6 genes to the existing test-systems. This will increase the likelihood of detecting hereditary predisposition to diabetes mellitus according to the key molecular-genetic mechanisms of its development and ensure implementation of relevant measures aimed at preventing the disease and early identification of genetic risk groups.

Keywords: *diabetes mellitus, hereditary predisposition analysis, genetic markers, single nucleotide polymorphisms, signaling pathways.*

Type 2 diabetes mellitus (T2DM) is a polycomponent disease primarily caused by insulin resistance (IR) [1]. Worldwide, it is estimated that 425 million adults (20–79 years) have diabetes, projected to reach 629 million by 2045. Also, DM is the ninth leading cause of worldwide mortality [2]. Personal human genome (individual allele combinations, single nucleotide polymorphisms or SNP included) and impacts of various risk factors are combined in T2DM pathogenesis [3–5]. By now, genome-wide association studies (GWAS) have identified approximately 100 genes and more than 700 polymorphisms able to modify T2DM risk [6]:

1. Genes associated with β-cell function: *ABCC8, IGF2BP2, IRS1, CDKAL1, KCNJ11,*

Elizaveta S. Ageeva – Doctor of Medical Sciences, Professor, Head of the Department of Medical Biology (e-mail: ageevaeliz@rambler.ru; tel.: +7 (978) 506-55-01; ORCID: https://orcid.org/0000-0003-4590-3580).

Iuliana I. Shramko – Doctor of Medical Sciences, Associate Professor of the Department of General and Clinical Pathophysiology (e-mail: julianashramko@rambler.ru; tel.: +7 (978) 752-96-73; ORCID: https://orcid.org/0000-0003-4946-7317).

Anatoliy V. Kubyshkin – Doctor of Medical Sciences, Professor, Head of the Department of General and Clinical Pathophysiology (e-mail: kubyshkin_av@mail.ru; tel.: +7 (978) 028-01-11; ORCID: https://orcid.org/0000-0002-1309-4005).

Iryna I. Fomochkina – Doctor of Medical Sciences, Professor, Head of the Department of Basic and Clinical Pharmacology (e-mail: fomochkina_i@mail.ru; tel.: +7 (978) 731-67-80; ORCID: https://orcid.org/0000-0003-3065-5748).

Anna A. Zhukova – Candidate of Biological Sciences, Associate Professor of the Department of Medical Biology (e-mail: biologkrim@mail.ru; tel.: +7 (978) 708-41-35; ORCID: http://orcid.org/0000-0002-0725-2089).

Cyrill O. Tarimov – Assistant of the Department of General and Clinical Pathophysiology (e-mail: kirito.k@yandex.ru; tel.: +7 (978) 752-15-29; ORCID: https://orcid.org/0000-0001-6246-3551).

Sergey G. Nastoyashchii – post-graduate student of the Department of Propaedeutics of Internal Medicine (e-mail: kirito.k@yandex.ru; tel.: +7 (978) 752-15-29).

Mariam B. Zaurova – post-graduate student of the Department of General and Clinical Pathophysiology (e-mail: zaurova.mariam@bk.ru; tel.: +7 (978) 031-56-06).

KCNQ1, SLC30A8, C2CD4A, WFS1, TCF7L2, GCK [5].

2. Genes that participate in formation of insulin resistance: *PPARG*, *IRS1*, *ADIPOQ*, *ADIPOR2* [6].

3. Genes associated with glucose metabolism and participating in glucose level regulation: *G6PC2*, *GCK*, *GCKR*, *OCT3* [6].

4. Genes that have some associations with T2DM but their exact molecular pathways have not been established yet: *ACHE*, *PLS1*, *PCNXL2*, *PAPL*, *CR2*, *LPIN2* [7, 8].

There are only tentative classifications of candidate genes, action and expression of which are limited by external and internal transcription factors. Therefore, **the aim of this review** was to analyze significance of certain genes and their SNPs with an important pathogenetic role in basic molecular mechanisms of T2DM development.

Materials and methods. The authors analyzed relevant databases including CYBERLENINKA, Elibrary, National Center for Biotechnology Information, as well as the integrated database GeneCards[1].

Genes associated with β-cell function. Most identified variants of the genes from this group influence insulin secretion by pancreatic β-cells and not insulin effects [9].

Human sirtuin SIRT1, NAD^+ histone deacetylase, modulates insulin secretion and regulates activity of transcription factors and their co-regulators [10, 11]. A study conducted on a Chinese population investigated 5 SNPs that cover 100 % of common genetic variations (minor allele frequency ≥ 0.05) within the SIRT1 gene. A subsequent analysis of insulin resistance risk and T2DM risk in the examined population established their different roles [12]. The homozygous combination per the allele A of *SIRT1* gene polymorphism rs10509291 was associated with high T2DM risk in a Chinese Han population [p = 0.002; OR (odds ratio), 95 % CI (confidence interval) = 1.551 (1.179–2.04)]. Linear regression analyses adjusting for age, gender, and body mass index (BMI) showed that HbA1c and HOMA-IR in subjects with rs10509291 AA genotype were higher than those with TT genotype in T2DM group (p = 0.045, p = 0.035, respectively).

GCK (Glucokinase). The *GCK* gene plays an important role in glucose metabolism and glucose-stimulated insulin secretion. It makes for glucose absorption and its transformation into glycogen in the liver [13]. Rare mutations in GCK cause Maturity-Onset Diabetes of the Young 2 (MODY 2). This 3'UTR SNP was also established to be associated with diabetes (OR = 1.36, 95 % CI = [1.11–1.65], p = 0.002), where the allele A (allele frequency 0.05) was associated with the highest T2DM risk [14].

HNF1A and *HNF4A (Hepatic nuclear factor)* encode a transcription factor that participates in the development and functioning of pancreatic β-cells. Heterozygous mutations lead to *HNF* function loss and induce a decrease in glucose phosphorilation into glucoso-6-phosphate (G6P). The reaction blocks G6P from entering the glycolytic pathway thereby leading to non-progressing fasting hyperglycemia [15]. Family inheritance of diabetes was significantly more common in patients with the p.I27L TT genotype in a Turkish population (β = 1.45; 95 % CI = [1.2–4.2]; p = 0.036) [16].

Mutations rs757110 of the *ABCC8 gene (ATP Binding Cassette Subfamily C Member 8)*, which encodes the sulfonylurea receptor, might be associated with hyperinsulinemia [15].

SLC30A8 (solute carrier family 30 member 8, encodes the zinc efflux transporter protein). The protein that is encoded by this gene is a zinc transporter. *SLC30A8* is expressed in the pancreas, namely, pancreatic islets. The encoded protein is co-localized with insulin in granules of INS-1 cells secretory pathway. Mice with zero *SLC30A8* levels have lower insulin secretion (GSI) and glycemia. Homo-

[1] GeneCards®: The Human Gene Database. Available at: https://www.genecards.org/ (March 14, 2024).

zygous rs13266634 TT genotype of the *SLC30A8* gene, according to E.S. Melnikova with colleagues, is associated with T2DM risk for women in all age groups in a Novosibirsk population (OR = 1.51; 95 % CI = [1.11–2.05]; p = 0.008). In contrast, rs13266634CC genotype of the *SLC30A8* gene is associated with protector effect as regards T2DM (OR = 0.57; 95 % CI = [0.35–0.92]; p = 0.026) [17].

PAM (*Peptidylglycine Alpha-Amidating Monooxygenase*) encodes α-amidase, which is localized on secretory granule membranes and participates in wrapping insulin granules and their release from β-cells [18]. Two GWAS-identified SNPs in the *PAM* gene (rs78408340, c.1616C>G, OR = 1.47, MAF = 7.3) and (rs35658696, c.1688A>G, OR = 1.23, MAF = 0.045), influence T2DM risk [19]. Both polymorphisms are associated with decreased insulinogenic index (which reflects glucose-stimulated insulin secretion) and this allows assuming their effects to be mediated by changes in β-cell functioning and associated with T2DM risk [18, 19]. H.J. Yoo with colleagues [20] compared glucose levels in carriers of various allele combinations of rs13175330 polymorphisms of the *PAM* gene. They established that rs13175330 allele G carriers in the hypertension without therapy group had significantly increased levels of insulin, insulin resistance, and oxidized low-density lipoproteins (LDL) and significantly decreased LDL-cholesterol levels and LDL particle sizes compared to the AA carriers (OR = 1.607; 95 % CI = [1.220–2.116]; p = 0.001).

C2CD4A and *C2CD4B (Calcium Dependent Domain C2, Containing 4A, B).* This is a locus present on the 15q chromosome and identified by GWAS [21]. It is associated with proinsulin levels and T2DM risk. The allele A (rs7172432 *VPS13C/C2CD4A/C2CD4B*) associated with T2DM disrupted glucose-mediated insulin secretion GSI in a diabetes-free Danish population (n = 5722) [22]. The fasting glucose lead SNP (rs11071657) is located between the C2CD4A and C2CD4B genes that are located near the VPS13C gene encoding the lipid transportation protein [22]. The risky allele is associated with lower glucose-stimulated insulin reaction and changes in the insulinogenic index, which gives evidence of its relationship with T2DM risk.

KCNQ1 gene *(potassium voltage-gated channel KQT-like subfamily, member 1)*. The *KCNQ1* gene product is expressed in pancreatic islets and participates in regulating insulin secretion by pancreatic β-cells. The rs2237892 polymorphism is associated with T2DM risk. Iranian patients with the CC genotype (rs2237892) had 30 % lower T2DM risk against healthy donors and those differences were trend-like (p = 0.475).

Adenosine triphosphate-sensitive potassium channel (K_{ATP}) plays an important role in regulating glucose-mediated insulin secretion by pancreatic β-cells through the connection between the cellular membrane potential and cellular metabolism. A K_{ATP}-channel pore is a heterodimer that consists of four tetramers of the (Kir6.2) potassium channel and highly affine sulfonylurea receptor 1 (SUR1). SUR1 is encoded by the *ABCC8*, which is located next to *KCNJ11*. *KCNJ11* has 219 SNPs; of them, six have been paid more attention due to their association with diabetes. The polymorphism (rs2237892 allele 23Lys OR = 1.62; p = 0.019) of the *KCNJ11* gene is believed to be associated with elevated T2DM risk in a Kyrgyz population [23]. The authors analyzed the relationship of this gene in combinations with other genes and then assumed the *KCNJ11* gene to be significant for T2DM risk in various populations with different likelihood (Mauritanian population: OR = 2.08, 95 % CI = [1.09–3.97], p = 0.026; Chinese Han population: OR = 1.25, 95 % CI = [1.04–1.50], p = 0.017) predominantly through IR formation [24].

A strong correlation was found between the rs5219 polymorphism and T2DM susceptibility in Caucasians and in some Asian populations (OR = 1.376 (1.085–1.745), p = 0.008) [24]. The allele A (valine to isoleucine substitution in position 250) reduces K_{ATP} sensiti-

vity, which makes the channel hyperactive and results in subsequent inhibition of insulin secretion. Insulin secretion is influenced more significantly in AA genotype carriers against GA genotype carriers. The rs5210 and rs5215 polymorphisms of the *KCNJ11* gene can also cause neonatal diabetes and inborn hyperinsulinemia [24].

TCF7L2 (*transcription factor 7 like 2*) encodes a transcription factor, which, as a Wnt signal pathway component, participates in expression of certain genes. Their products are involved in growth and development of pancreatic β-cells. IVS3C>T (rs7903146), which is located in the 3rd intron, has two alternative allele variants, C and T. The allele combination C/T (rs7903146) is known to impair the capability of pancreatic islets to produce insulin as a response to being stimulated by the insulinotropic intestinal hormone or glucose. Its frequency and associations with T2DM risks differ between various populations. Thus, for example, in Caucasians, the minor allele T was found more frequently, in 22–36 %, and was associated with high T2DM risk. But it was much less frequent in Asians (3–6 %) and was not associated with the disease. However, the gene was shown to have pathogenic influence in combinations with other genes. In a Kyrgyz population, IVS3C>T of the *TCF7L2* gene was not significantly independently associated with T2DM risk; however, its predisposing influence was found in combinations with some genotypes of the *ADIPOQ* (G276T/CC OR = 1.97 at 95 % CI between 1.07 and 3.61; p = 0.04) and *KCNJ11* genes (Lys23Lys/CC OR = 2.65 at 95 % CI between 1.12 and 6.28; p = 0.042 and Glu23Lys/ CT OR = 3.88 at 95 % CI between 1.27 and 11.91; p = 0.027) [23].

WSF1 (*Wolfram syndrome 1)* is a wolframin gene. It participates in regulating homeostasis of calcium channels. GWAS established not less than 19 polymorphisms of the *WFS1* gene to be associated with T2DM risk. The heterozygous TC genotype (rs1801214) in a Saudi Arabia population was authentically associated with pre-diabetes susceptibility (OR = 0.60; 95 % CI = [0.44–0.80]; p < 0.01) [25]. Another most important SNP, rs1046322 WFS1, is significantly associated with BMI and waist circumference in South-Eastern Asian population. Quantitative deficiency of the *WFS1* gene makes carriers of the single functional WFS1 column susceptible to diabetes and metabolic syndrome as well as to environmental exposures. However, frequency of this SNP varies in different populations, from 7.2 % in Chileans to 50 % in Siberians [26].

IGF2BP2 (*insulin like growth factor 2 mRNA binding protein 2*) belongs to a protein family binding to the insulin-like growth factor 2 (IGF-2) for further transfer of insulin signals. GWAS established the *IGF2BP2* rs4402960 and rs1470579 polymorphisms to be associated with T2DM risk in Asians. In the case-control study, the carriers of TT genotype at rs4402960 had a higher T2DM risk than the G carriers (TG + GG) (adjusted odd ratio (AOR) = 1.962, 95 % CI = [1.065–3.612], p = 0.031); CC carriers at rs1470579 were more susceptible to T2DM than A carriers (CA + AA) (AOR = 2.014, 95 % CI = [1.114–3.642], p = 0.021) [27].

IRS-1 is Insulin Receptor Substrate-1. The *IRS-1* gene is located in the 2q36-37 locus and has several known polymorphisms. This gene encodes a protein, which is phosphorylated by insulin receptor tyrosine kinase and thus plays a critical role in the insulin signaling pathway. Such polymorphisms as Gly972Arg, Pro170Arg, Ala513Pro and Met209Thr of the *IRS-1* gene make for the inhibited activity of phosphatidylinositol 3-kinase (PI3K) and developing insulin resistance and obesity. Among all examined mutations, glycine-to-arginine substitution in the codon 972 (2963G>A, p.Gly972Arg, rs1801278, G972R) is most frequently considered in relation to T2DM risk. This polymorphism reduces levels of IRS-1 phosphorilation and stimulates its inhibiting impacts on tyrosine kinase thereby reducing the speed of

the insulin signaling pathway in glucose transportation, translocation of glucose and glycogen transporter [28, 29], especially in obese patients [30]. The GA, GA+AA genotypes increase IR in patients with T2DM with severe hyperglycemia (OR = 2.536; 95 % CI = [1.030–6.249]; p = 0.043) [31]. Marked reduction in IRS-1 expression was observed in visceral as compared to subcutaneous adipose tissue of morbidly obese patients [32].

IRS-2 participates in insulin-stimulated glucose absorption, metabolic regulation in the liver, and lipid metabolism [33]. Glycine-to-aspartate substitution in the locus 1057 of the gene (Gly1057Asp) is a commonly known polymorphism of the *IRS-2*, which is associated with lower sensitivity to insulin and impaired glucose tolerance as well as with elevated obesity risk [34]. People with BMI < 27 kg/m^2 tended to have GD and DD genotypes more frequently in the control group against the cases. However, allele D carriers prevailed in the group of T2DM patients with BMI > 27 kg/m^2. Therefore, the authors concluded that the minor allele D was associated with T2DM risk although the difference in allele frequency was not significant (OR = 1.55, 95 % CI = [0.961–1.41]; p = 0.108). The combined genotype analysis showed that the difference in the allele and genotype frequencies reached statistical difference between the cases and the controls as well as the odds ratio substantially increased when the R allele (G972R) was present together with D allele (G1057D) in any combination. The GG genotype was much more frequent among obese patients, especially against the control group. This may indicate that having the allele D protects people with normal body weight from T2DM [34].

CDKAL1 is an inhibitor of the cyclin-dependent kinase (CDK5) [35]. It influences β-cells functioning by inhibiting the activity of CDK5 and tRNA-modifying enzymes. An association between multiple polymorphisms of the *CDKAL1* gene (rs7754840, rs4712524, rs10946398, rs7756992) and T2DM in various populations was confirmed by several studies. Thus, rs10440833, rs10946398, rs4712523, rs4712524 and rs7754840 of the *CDKAL1* were associated with susceptibility to GDM in Central China [36] and Pakistan population [37]. The strongest association was revealed between T2DM risk and the rs10946398 polymorphism of the *CDKAL1* gene [38], which was established by the authors of this meta-analysis in various populations. In an Asian population, the differences were statistically significant (p < 0.01) for dominant genetic model of the rs10946398 *CDKAL1* (AA against AC+CC) (OR = 0.75, 95 % CI = [0.64–0.88], p = 0.0003); this was not detected for non-Asian populations [38].

HHEX gene *(hematopoietically expressed homeobox)* is responsible for somatostatin secretion in pancreatic δ-cells. A study accomplished on a population sample from southeastern Iran (n = 250) aimed to estimate possible correlations between polymorphisms of the *HHEX* gene and T2DM risks. The finding revealed the all measured inheritance models of rs1111875G/A and of rs5015480C/T variants dramatically increased the risk of T2D while another polymorphism (rs7923837A/G) was not associated with risk/protective role in T2D [39].

The MTNR1B gene (melatonin receptor 1B) is associated with risk of gestational DM; however, findings are rather controversial. Allele G combinations of the rs10830963 polymorphism of the *MTNR1B* gene were established to be associated with fasting hyperglycemia, disrupted insulin secretion and T2DM. Risk analysis established risky genotypes in Caucasians: CG (OR = 1.40; 95 % CI = [1.16–1.70]; p < 0.001) and GG (OR = 2.21; 95 % CI = [1.54–3.17]; p < 0.001) and Asians: CG (OR = 1.15; 95 % CI = [1.02–1.28]; p = 0.020) and GG (OR = 1.52; 95 % CI = [1.23–1.89]; p < 0.001) [40].

The *UCP2* and *UCP3* (*uncoupling protein 2, 3*) genes. They uncouple oxidation and oxidative phosphorilation in mitochondria of skeletal muscles, cardiac muscle and brown

adipose tissue; disrupt insulin production; inhibit fatty acids metabolism. In the *UCP2* gene, the Ala55Val polymorphism in the 4th exon has been studied most profoundly (rs660339, with C to T substitution in the position 164 of the transcript). This leads to a substitution in amino acid sequence from alanine to valine. This substitution is associated with elevated obesity risk and higher obesity and T2DM prevalence [41]. However, this risk is population-dependent: the SNP is associated with elevated insulin secretion in Caucasians and lower T2DM risk in Asians. The mutation -866G/A (*rs659366*) of the *UCP2* gene disrupts normal insulin production by pancreatic cells. In Asians, the allele combinations AA (OR = 1.254; 95 % CI = [1.022–1.540]; $p = 0.031$) and AG (OR = 1.198; 95 % CI = [1.047–1.371]; $p = 0.009$) play the key role in T2DM pathogenesis [42].

Genetic variants in genes of *glutathione synthetize (GSS)* and gamma-*glutamyl transferase 7 (GGT7)* are markers of T2DM susceptibility. They potentially increase the disease risk by disrupting glutathione metabolism and contribute to the disease pathogenesis through intracellular glutathione deficiency. I. Azarova with colleagues believe [43] the *GSS* G/A-A/A genotypes per rs13041792 to be authentically associated with elevated T2DM risk (OR = 1.21; 95 % CI = [1.04–1.42]; $p = 0.046$). Meanwhile, the SNP rs6119534 (OR = 0.73; 95 % CI = [0.53–0.99]) and rs11546155 (OR = 0.42; 95 % CI = [0.22–0.80]) of the *GGT7* gene were established to have significant associations with reduced T2DM risk.

Genes that participate in formation of insulin resistance. *ADIPOQ (the adiponectin gene).* Several SNPs of the adiponectin gene including rs17300539, rs1501299, rs266729, and rs2241766 are the most significant concerning diabetes pathogenesis [44, 45]. These polymorphisms have been established to be associated with insulin resistance, dyslipidemia and atherogenesis. Some studies also established associations between alleles of the rs17366743 locus and hypoadiponectinemia in people with disrupted tolerance to carbohydrates [45–48].

Associations between polymorphic locuses of the *ADIPOQ* gene and T2 DM have been established in various ethnic and geographic groups across the globe [45]. Thus, as association between rs1501299 and obesity was revealed in a Spanish population [49]. D.L. Brovin with colleagues [50] established a relationship between rs2441766 and rs266729 and levels of total and high-molecular serum adiponectin in females with abdominal obesity and metabolic syndrome in Saint Petersburg. H. Kaur and others [51] showed *ADIPOQ*-3971A>G (rs822396) and +276G>T (rs1501299) polymorphisms to be risk factors of obesity and metabolic syndrome in a North Indian Punjabi population. Frequency of the GG (-3971A>G) and TT (+276G>T) genotypes was higher among patients with obesity ($p = 0.008$ and $p = 0.035$ respectively). Both genotype variants (-3971GG and +276TT) created authentically elevated obesity risk (OR = 1.52; $p = 0.03$ and OR = 1.58, $p = 0.04$ respectively). The G-T haplotype model (possessing -3971G and +276T alleles) was shown to provide approximately 3-fold risk towards the obesity susceptibility (OR = 2.69, $p = 0.009$) and metabolic syndrome risk (OR = 2.73, $p = 0.009$) [51].

Some polymorphisms of 45 T>G (rs2241766) of the *ADIPOQ* gene have been investigated in a Russian population. They are associated with risks of obesity, insulin resistance and T2DM[2]. The allele combinations TG and GG (rs2241766) increase T2DM risk (OR = 3.81; 95 % CI = [1.79–8.09] and OR = 10.0; 95 % CI = [2.25–44.7]).

It is noteworthy that studies with their focus on associations between the given polymorphisms and T2DM have yielded

[2] Vakhromeeva K.A. Polimorfnye geneticheskie markery sakharnogo diabeta 2-go tipa i ikh assotsiatsii s kliniko–metabolicheskimi pokazatelyami v russkoi populyatsii [Polymorphic genetic markers of type 2 diabetes mellitus and their associations with clinical metabolic indicators in a Russian population]: the abstract of the dissertation … for Candidate of Medical Sciences degree. Tyumen, 2015, 23 p. (in Russian).

somewhat controversial results in several populations [52, 53].

ADIPOR1, ADIPOR2. The ADIPOR1 and ADIPOR2 receptors located in various tissues bind to adiponectin and thus support its biological functions. The receptor to adiponectin 1 (*ADIPOR1*, located on the 1q32 chromosome) is synthesized in the human body predominantly in skeletal muscles whereas the receptor to adiponectin 2 (*ADIPOR2*, located on the 12p13.33 chromosome) is predominantly expressed in the liver. D.S. Khodyrev and others [54] established an association between the polymorphic marker rs11061971 of the *ADIPOR2* gene and T2DM in a Russian population ($p = 0.004$ when comparing allele frequencies, $p = 0.011$ when comparing genotype frequencies). According to their findings, the allele A and AA genotype reduced T2DM risk (OR = 0.76 and 0.75 respectively) whereas the allele T and TT genotype increased T2DM risk (OR = 1.31 and 1.63 respectively). A study [55] established the allele GA of the +795 G/A (rs16928751) polymorphism of the *ADIPOR2* gene to be associated with high BMI in T2DM patients in a Crimea population. Carriers of the TG genotype of the −102 T/G (rs2275737) polymorphism of the *ADIPOR1* gene had higher risk of elevated HbA1c levels (1.36 higher against TT carriers TT, $p < 0.05$). Carriers of the TT genotype of −106 T/C (rs2275738) polymorphism of the *ADIPOR1* gene were found to have higher glucose levels in blood (1.14 times higher against CC carriers, $p < 0.05$).

LEP (the leptin gene) encodes a protein that is secreted by white adipocytes into the circulation and plays a major role in the regulation of energy homeostasis. Circulating leptin binds to the leptin receptor (*LEPR* gene) in the brain, activates lower signaling pathways that prevent excessive food intake and makes for energy consumption. Several mutations associated with T2DM were registered for the leptin gene *LEP* (rs7799039 (-2548 G/A)) and the leptin receptor *LEPR* (rs1137101 (Gln223Arg)) [56–58]. Special attention is paid to influence exerted by the SNP rs1137101 of the *LEPR* gene on obesity. C.O. Mărginean and others [59] showed AG+GG for the *LEPR* 223 gene ($p = 0.0001$) and GA+AA for the *LEPR* 1019 gene ($p = 0.0001$) to be the most frequent genotypes of polymorphisms of the *LEPR* 223, 1019, 492 and 976 genes in obese children. Such polymorphisms of the *LEP* gene as rs7799039 and rs2167270 were associated with obesity in a Taiwanese [60] and Arabian [61] population (OR = 1.85; 95 % CI = [1.37–2.5]). At the same time, some studies dispute impacts of combined polymorphisms of adiponectin and leptin receptor genes [62, 63]. The allele combination GG of the polymorphism (rs7799039) of the *LEP* gene was shown to be associated with high systolic blood pressure in Crimean T2DM patients [64].

PPARγ (PPARG) encodes the protein PPARG, a nuclear receptor that regulates gene transcription (*LPL*, *FATP* and others). The *PPARγ* gene is closely involved in lipid metabolism regulation, mostly due to being expressed in adipose tissues and the liver [65]. Mutations of the *PPARG* gene can disrupt these regulatory mechanisms thereby resulting in metabolic disruptions and elevated cardiovascular risk [66]. E. Pokushalov with colleagues [67] revealed specific polymorphisms in the PPARG promoter, in particular, rs10865710 and rs3856806, which have significant associations with glucose levels in DM patients. Analysis in sub-groups established considerable differences in lipid profiles that were associated with the PPARG- polymorphism. A decrease in levels of low density lipoproteins was 11.7 % higher in people with *PPARG* polymorphisms than in their peers without them (95 % CI from -19.3 to -4.0 %; $p < 0.01$).

The Pparg2 Pro12Ala and IL6-174G>C polymorphisms regulate body weight and potentially have a role in obesity risk worldwide [68]. Bearing this in mind, we can consider them promising for associative investigations of obesity phenotypes.

FTO (fat mass and obesity) encodes 2-oxoglutarate-dependent dioxygenase of nucleic

acid, which may be associated with growing fat depots resulting in obesity. The gene is located in the 16q12.2 chromosome and can have variations in its intron areas. In a study [69], mRNA FTO expression was established to be lower in obese patients and patients with T2DM against the controls ($r = 0.401$, $p < 0.001$). The role of several polymorphisms of the *FTO* gene including rs9939609, rs141115189, rs9926289, rs11075990, rs1121980, rs17817449, rs3751812, rs9939609, rs9940128, rs9941349 and rs9939609 was investigated as regards risks of T2DM associated with obesity and other components of metabolic syndrome (high fasting levels of glucose and triglycerides, low levels of high density lipoproteins) [70–76]. Minor homozygous genotypes of the rs11075990, rs1121980, rs17817449, rs3751812, rs9939609, rs9940128, rs9941349 polymorphisms of the *FTO* gene were shown to be associated with T2DM risk (OR = 2.20–2.78; $p < 0.05$) [75].

IL-6 (interleukin-6) is a pro-inflammatory cytokine, which is mostly synthesized by macrophages. Its role in insulin resistance development is basically determined by a possibility to increase glucose absorption during physical activity with occurrence of muscle hypertrophy, myogenesis and oxidation of fatty acids in skeletal muscles [77, 78]. The *IL-6* gene is located on the 7p21 chromosome and includes seven exons. The most widely spread SNP is located in the promoter zone –174 of the IL-6 gene and is associated with G-C substitution (rs1800795) [79]. Homozygotes per the allele C tend to have a lower plasma IL-6 level associated with a lower concentration of glycated hemoglobin in blood and fasting insulin against the allele G carriers. At the same time, some studies report absence of any association between the allele combinations CC and GC and T2DM risk ($p = 0.039$) [79].

AKT encodes a protein, which consists of 480 amino acid residuals and includes three domains. AKTs are divided into three isoforms (AKT1, AKT2 and AKT3) based on differences in amino acid residuals of serine / threonine. AKT2 is found in tissues sensitive to insulin (liver, muscles and adipose tissue) and is encoded by a gene located on the 19q13.1 chromosome. Serine / threonine kinase AKT2 is an effector protein in the insulin signaling pathway and is associated with metabolic impacts of insulin [80]. Variations of the *AKT2* gene are associated with T2DM, fasting hyperglycemia and postprandial hyperinsulinemia. The *AKT2* mutation p.Pro50Thr related to a rare allele was associated with peculiarities of metabolic syndrome, lipodystrophy and T2DM risk in a Finnish population (OR = 1.05; 95 % CI = [1.0–1.1]; $p < 0.001$) [81]. The p.Pro50Thr mutation of the *AKT2* gene causes a reduction in glucose absorption in insulin-sensitive tissues and is associated with elevated fasting insulin levels in blood and T2DM risk ($p = 0.038$). The (p.E17K) mutation of the *AKT2* gene may disrupt the insulin signaling pathway, which results in lower levels of insulin in plasma, ketone bodies, fatty acids and glucose [81–83].

Genes associated with glucose metabolism. *GCKR* (glucokinase) determines the threshold of glucose-stimulated insulin secretion (GSI), controls gluconeogenesis and glycogen synthesis in hepatocytes. There are two known polymorphisms of the *GCKR* gene, rs780094 and rs1260326. The AA genotype (rs780094) was shown to be associated with hypertriglyceridemia in DM patients (OR = 5.335; 95 % CI = [1.779–15.99]; $p = 0.003$). The TT genotype (rs1260326) is associated with levels of HOMA-IR, FPG and triglycerides (OR = 4.523; 95 % CI = [1.458–14.03], $p = 0.009$). At C-T substitution, proline is replaced with leucine in the amino acid sequence of the encoded protein. Protein structure modification changes its function and is associated with decreasing glucose-6-phosphatase levels and growing levels of GCK, phosphofructokinase and fatty acid synthase. These metabolic changes can potentially explain lower glucose levels in plasma and higher levels of triglycerides [84, 85].

G6PC2. Glucose-6-Phosphatase Catalytic Subunit 2 (G*6PC2*) competes with pancreatic

GCK activity for glucose use. Two SNPs, rs560887 and rs563694, are associated with hyperglycemia in European populations. Any significant sex- and age-dependent differences have not been established among dominant and recessive models of the *G6PC2* gene polymorphisms ($p > 0.05$) [85].

The role that belongs to genotype in molecular mechanisms of T2DM development. Defects of the insulin signaling pathway are a probable common mechanism that explains elevated insulin resistance as well as glucose and insulin levels. This fact is confirmed by accumulated data [86], which are also supported by our findings [55, 64].

Insulin-stimulated glucose transport goes by the *PI3K/AKT-dependent signaling pathway* (phosphatidylinositol 3-kinase /protein kinase B), which results in GLUT4 (glucose transporter 4) moving onto the plasmatic membrane to attach glucose and activate glycogen synthase. Being stimulated by insulin, *PI3K* phosphorilates membrane phospholipids and turns PIP2 (phosphatidylinositol 4,5-bisphosphate) into PIP3 (phosphatidylinositol (3,4,5)-trisphosphate). This complex phosphorilates / activates *PDK1* and *PDK2* (phosphoinositide-dependent kinases), which, in its turn, activates *AKT/PKB* and *PKC* (protein kinase C) phosphorilation for moving *GLUT4* onto the plasmatic membrane from the intracellular space. Carriers of rs2494746 CG/GG or rs2494738 GA/GG genotypes in AKT1 have higher T2DM risk against homozygous carriers [87].

Insulin resistance in T2DM patients is characterized by several defects in the cascade of insulin signaling transfer. This is confirmed by changes in expression of genes that encode metabolic pathways in T2DM patients (*IR*, *IRS*, *PI3K*, *AKT* and *GLUT4)* [88].

In addition to insulin signal transfer by PI3K, insulin can activate the *mitogen-activated protein kinase (MAP) ERK*, which results in expression of kB (IkB) and NF-kB and inhibitors of the *Janus kinase/signal transducers and activators of transcription (JAK/STAT)* thereby initiating transcription of pro-inflammatory genes, in particular *IL-6* [89]. In addition, phosphorilated STAT3 induces expression of SOCS3, which acts as a feedback inhibitor for the leptin signaling pathway thus influencing appetite [90]. Alimentary hyperglycemia reinforces fatty acid synthesis, which activates *PPARγ* genes inducing a descending cascade of expression of genes that reinforce lipogenesis. As a result, a new cycle of *PPARγ* expression starts and *TLR4* expression occurs as well [91]. This activates genes encoding multiple inflammation mediators, including the CRP gene promoter that interacts with IL-1 and IL-6 [92]. Pro-inflammatory mediators enhance lipolysis by direct and indirect damage to cellular membranes thereby increasing levels of free fatty acids. This creates the final loop in genetically determined vicious circles of T2DM pathogenesis [93].

Genetic information can be used in practice to predict T2DM risk using the Cambridge Risk Score (CRS) and Framingham Diabetes Risk Model (FDRM). They both consider age, sex, sugar-reducing therapy, family history of T2DM, BMI, smoking, as well as levels of high density lipoproteins, triglycerides and fasting glycemia [94]. Investigation of a genetic status, including such key genes as *PPARγ, TLR4, IRS* and *IL6*, can become a marker eligible for early T2DM detection, especially in patients who have family history of the disease.

Conclusion. Multiple polymorphisms associated with T2DM development have been established in various geographic and ethnical groups across the globe. SNP markers of gene involved into key molecular-genetic T2DM mechanisms, in particular *PPARγ, TLR4, IRS* and *IL6*. This will increase the likelihood of detecting hereditary predisposition to diabetes mellitus and ensure early identification of genetic risk groups aimed at developing targeted prevention activities.

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