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Research article

ADHERENCE TO VACCINATION AND PERCEPTION OF COVID-19 RISK AMONG POPULAITON IN THE REPUBLIC OF BELARUS

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Our research goal was to examine health risk perception and adherence to vaccination against COVID-19 among various social and demographic population groups in Belarus.

To achieve this goal, a cross-sectional study was accomplished via using an online poll. The obtained results reveal that perception of health risks caused by COVID-19 is quite significant among people living in Belarus since only 9.9 % of the questioned do not consider COVID-19 a dangerous disease. Higher levels of risk perception have been detected among medical personnel, older age groups, and people with chronic pathologies.

Most respondents believe vaccination is among the most efficient anti-COVID-19 measures; however, people are rather poorly aware about provided opportunities to get vaccinated. 33.6 % among respondents who are not vaccinated don't plan to do it with; their basic reasons for this refusal are lack of trust, both in vaccines being safe and efficient and overall trust in preparations suggested for vaccination. Having analyzed answers given by respondents who were medical workers we revealed that a greater share of them were vaccinated but reasons for refusing from vaccination were the same. 20.1 % respondents from all groups and 21.2 % medical workers who took part in the questioning stated that they needed additional information about vaccination.

When developing communication strategies aimed at raising awareness among population, we should bear in mind that lower perception of COVID-19-related health risks and refusal from vaccination are more widely spread among people younger than 40; people who don't have higher education; people with minor children in their families. Prevalence of lower COVID-19-related health risk perception is also greater among men; people who don't live in the capital; people with elderly relatives in their families. Internet resources, data provided by the WHO and Public Healthcare Ministry, and medical personnel are considered the most reliable sources of information by population in Belarus.

Key words: *poll, coronavirus, COVID-19, pandemic, risk perception, vaccination, hesitation regarding vaccination, social and demographic factors, population awareness, population health.*

According to data provided by the World Health Organization (hereinafter WHO), a new coronavirus infection (COVID-19) caused more than 194 million disease cases including more than 4 million deaths over one and a half years that have passed since the first case was registered in Wuhan, China. Despite all strict quarantine measures introduced in many countries all over the world, COVID-19 morbidity has started to grow again after a short-term fall. In Belarus 440,708 disease cases have been confirmed with laboratory tests over the whole registration period and 9,934 among them were deaths; a number of daily registered disease cases remains stably high (according to data as of July 26, 2021) [1].

This ongoing COVID-19 pandemic in spite of all strict quarantine measures indicates that wide-scale vaccination is required; given that, development and implementation of ef-

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fective vaccines is considered among the most promising strategies to overcome the pandemic [2]. According to some data, frequency of COVID-19 contagion may start to decline just as a share of people with acquired immunity to SARS-CoV-2 will go above 67 % in a given population [3]. However vaccination rates remain too slow in many countries worldwide. On July 25, 2021 672,114,822 people worldwide were completely vaccinated; 1,435,332,186 people got one vaccination dose [1]. As it was stated by the WHO Regional European director, average number of people covered by vaccination in Europe amounted to only 24 % in spite of recommended 80 % [4]. In Belarus, 753,276 people got both vaccination doses (8.1 % of the total population); 1,240,581 people got one vaccination dose (13.3 % of the total population) [1].

Vaccine availability, capabilities of a public healthcare system to organize a vaccination campaign, etc., are among significant factors influencing vaccination rates. However, certain population groups remain hesitant regarding vaccination against COVID-19 and it is another serious problem for public healthcare in many countries [5–11]. "Vaccine hesitancy" is seen by the WHO as one of ten basic threats to global health [12]. Given that, it seems extremely vital to examine factors that determine motivation to get vaccinated against COVID-19 [13].

How a person perceives a risk of a communicable disease is a most significant indicator that determines a person's attitudes towards vaccination; this indicator, in its turn, depends on multiple individual, social, and cultural factors that, among other things, can have national peculiarities. The aforementioned should be kept in mind when mass vaccination programs are developed and implemented [14, 15].

Therefore, vaccination against COVID-19 is a first-priority task to be solved by public healthcare; development of effective vaccination strategies requires national surveys that include complex studies on influence exerted by socio-demographic factors on adherence to vaccination among various population groups, a level of awareness, monitoring over trust in vaccination efficiency and better insight and assessment of how population perceives COVID-19 risks.

Our research goal was to examine health risk perception and adherence to vaccination against COVID-19 in various socio-demographic population groups in Belarus.

Data and methods. To achieve the research goal, we performed a cross-sectional study by using an online poll. The online poll was accomplished by filling in a specifically designed questionnaire made up of 26 questions and placed on the official web-site of the Scientific Practical Centre of Hygiene.

The questioning included social and demographic profiles of respondents (age, sex, marital status and family members, a place and a region of living, education, job, and activity sphere); questions regarding health including chronic diseases and COVID-19 in case history and probable reasons for contagion; questions related to perceiving health risks of COVID-19 (how dangerous the disease was for a person and other people, a probability to get infected during next 6 months); questions on priority of various prevention activities regarding COVID-19, awareness about vaccination and authority of different sources that provided information about vaccines; questions about factors that could influence a person's decision to get vaccinated against COVID-19, vaccination status of a respondent at that time, any reasons to refuse from vaccination, and any circumstances that could make vaccination in future possible as well as readiness to get vaccinated for a fee.

Respondents were offered a multiple choice (3 answer options) to answer questions about factors that made for contagion, priority prevention activities, sources of information about vaccination, factors influencing a decision to get vaccinated, reasons for refusing from vaccinations and circumstances for giving consent to it.

From May 19, 2021 to July 07, 2021 1,310 people took part in the online poll. Prior to filling in the questionnaire respondents had to confirm that they were older than 18 at that moment; they were also informed that their personal data would be used in scientific re-

search in compliance with principles of anonymity and confidentiality.

All obtained data were statistically analyzed with STATISTICA 13 software package. Data analysis involved calculating absolute and relative frequencies. 95 % confidence interval was calculated for extensive parameters according to Wilson score method and data were given as P (95 % *CI*).

We applied Pearson's chi-square test χ^2 to analyze influence exerted by social and demographic factors on perception of COVID-19-related health risk and vaccination scope among various population groups. Respondents who had contra-indications to vaccinations were excluded when a share of non-vaccinated people was determined in a specific respondents' group. To estimate effects produced by a given factor, we calculated prevalence ratio (*PR*) and its 95 % confidence interval (95 % *CI*).

Research results were considered authentic and differences between parameters significant, when probability of a correct prediction was not lower than 95.5 % ($p < 0.05$).

Results and discussion. Having analyzed social and demographic profile of the sampling, we revealed the following. 67.0 % respondents were women (64.4–69.4) and 33.0 % were men (30.6–35.6). Most respondents, or 82.7 % (80.6–84.7) were older than 31. Age distribution was as follows: people who were 20 years old and younger accounted for 1.9 % (1.3–2.8); people aged 21–30, 15.4 % (13.5–17.4); people aged 31–40, 29.6 % (27.2–32.2); people aged 41–50, 26.1 % (23.8–28.6); and people aged 51 and older, 27.0 % (24.7–29.5).

Respondents were also asked questions about their marital status, family members, and chronic diseases in case they had any since this would help more profound analysis of social-demographic factors that could be related to adherence to vaccination. Having analyzed the answers, we revealed that 70.7 % (68.2–73.1) respondents were either married or had a life partner, 39.9 % (37.2–42.5) lived together with their under-age children, and 19.4 % (17.3–21.6), with elderly relatives; 34.3 % (31.8–36.9) respondents suffered from chronic diseases of the cardiovascular and respiratory system, pancreatic diabetes, or other chronic pathology; 31.3 % (28.9–33.9) lived together with people who suffered from chronic diseases.

Respondents gave the following answers regarding their place of living: 60.9 % (58.3–63.5) lived in the capital; 15.0 % (13.1–17.0), in regional centers; 11.1 % (9.6–13.0), in centers of municipal districts; 6.7 % (5.5–8.2), in towns and urban settlements; and 6.3 % (5.1–7.7) lived in villages and agricultural settlements.

Most respondents (85.0 % (82.9–86.8)) had higher education and 64.8 % (62.2–67.4) had white-collar job (21.4 % (19.2–23.7) were middle managers, 7.5 % (6.2–9.0), senior managers); 6.3 % (5.1–7.8) were blue-collar workers. Figure 1 shows respondents distribution as per their job (occupation).

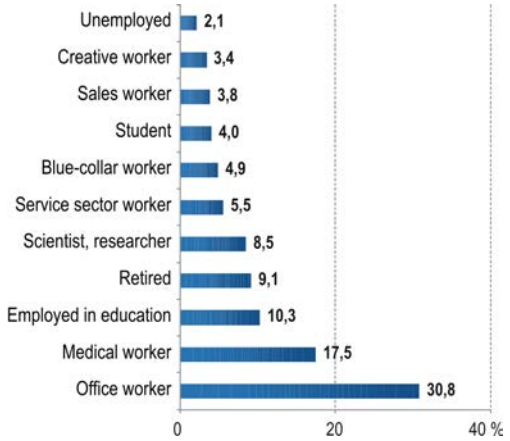

Figure 1. Respondents distributed as per job (occupation), %

Questioning results revealed that 34.7 % (32.2–37.4) respondents had COVID-19 in their case history that was confirmed with laboratory tests; 42.9 % (38.4–47.5) out of them had asymptomatic disease or disease in its mild form, 49.2 % (44.7–53.8) in average grave form, and 7.9 % (5.8–10.8), in grave form (had to be treated in in-patient hospital). We should note that a share of people with COVID-19 in their case history was considerably higher among medical workers (49.8 % (43.4–56.2)) than among other respondents (31.6 % (28.8–34.4)) ($p < 0.001$). In respondents' opinions, there were several most significant reasons for contagion including work involving contacts with many people or medical aid provision (36.9 % (32.6–41.5)); a fam-

ily member infected with COVID-19 (30.8 % (26.7–35.2)); the necessity to use public transport (20.4 % (17.0–24.4)); impossibility to keep safe distance from colleagues at a workplace (20.0 % (16.6–23.9)) (Figure 2).

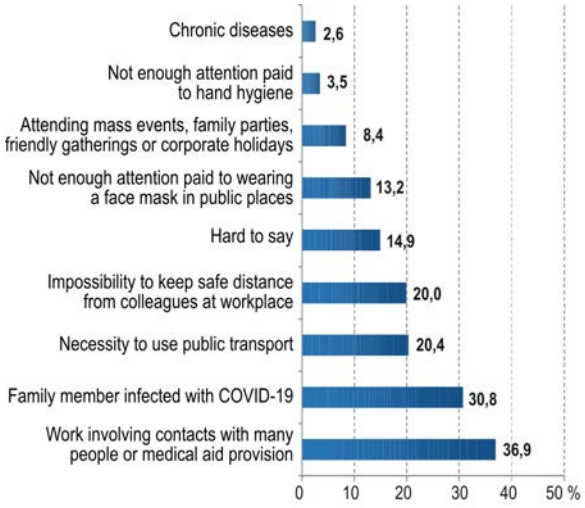

Figure 2. Respondents' answers to the question "In your opinion, what made the greatest contribution to your contagion with COVID-19?", %

Significant factors that influence adherence to prevention activities and a decision to get vaccinated include people's perceptions of hazards caused by the disease for themselves and those around them as well as subjective estimates of how probable contagion is for them given the current epidemiologic situation (perception of health risk related to pandemic spread of the infection) [14, 16]. Most respondents didn't think they could possibly get infected with COVID-19 during the next 6 months (46.9 % (44.3–49.7)). 37.7 % (34.0–41.6) out of them already had COVID-19 in case history, 36.9 % (33.2–40.8) were vaccinated. 42.1 % (39.4–44.8) respondents believed they could have the disease in its mild form during the next 6 months, and 11.0 % (9.4–12.8) were afraid they could have it in its grave form.

The poll also revealed that most respondents believed COVID-19 to be dangerous both for them and for other people (78.2 % (75.9–80.3)); 5.9 % (4.7–7.3) thought it was dangerous only for them; 6.1 % (4.9–7.5) thought it was dangerous only for others; and 9.9 % (8.4–11.6) didn't see any danger at all. 83.7 % (76.4–89.1) out of respondents who gave negative answers to this question didn't have COVID-19 in case history, 8.5 % (4.8–14.6) had asymptomatic and mild forms, and 7.0 % (3.7–12.7) were vaccinated; 78.3 % (70.4–84.5) of respondents from this groups thought they wouldn't get infected with COVID-19 during the next 6 months. Additional analysis of answers given by respondents who were medical workers revealed that they considered COVID-19 to be not dangerous much less frequently than other respondents (6.1 % (3.7–10.0) against 10.6 % (8.9–12.6) ($p < 0.05$).

Most respondents believed vaccination to be among the most effective prevention measures against COVID-19 (57.9 % (55.2–60.5). The second rank place belonged to mandatory face masks in public places (55.9 % (53.2–58.6); the third one, timely isolation of infected people and those who contacted them (54.1 % (51.4–56.8)). 51.1 % (48.4–53.8) respondents considered personal hygiene very important, 45.3 % (42.7–48.1) attributed great significance to personal prevention aimed at maintaining proper immune system functioning including healthy eating habits, proper sleep, physical activity, and giving up bad habits (Figure 3).

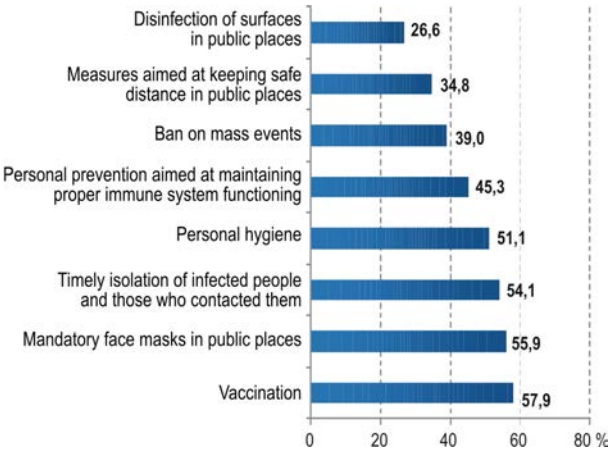

Figure 3. Respondents' answers to the question "What prevention activities against COVID-19 seem the most effective to you?", %

People's awareness about a possibility to get vaccinated is another significant factor in any vaccination campaign. Our poll revealed that only 57.5 % (54.8–60.1) respondents considered vaccine against COVID-19 to be available for all people older than 18 in Bela-

rus.17.8 % (15.8–20.0) respondents believed that vaccine was provided only for risk groups (medical workers, workers employed in education etc.), and 10.9 % (9.3–12.7) didn't think it was possible to get vaccinated against COVID-19 in the country. 13.8 % (12.1–15.8) respondents were not at all interested in vaccination against COVID-19.

The WHO experts understand that it is important to resolve issues related to infodemic which is an absence of authentic scientific data and spread of false information; it is a vital component in fight against COVID-19 pandemic [17]. Efficient communications with population require knowledge on what amount of trust people have in various sources of information. Our research revealed that most people (60.5 % (57.8–63.1)) used Internet resources to get authentic information about vaccination; data provided by the WHO and the Public Healthcare Ministry were also considered a reliable and significant source of information (Figure 4).

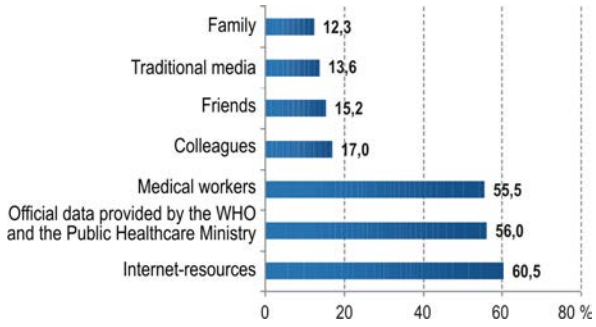

Figure 4. Respondents' answers to the question "Which source of information about vaccination seems the most reliable to you?", %

A decision to necessarily get vaccinated against this or that communicable disease depends on several objective and subjective factors including perception of risks related to a communicable disease (contagion probability, gravity of clinical course, possible complications and adverse outcomes), vaccine efficiency and duration of protective immunity, safety of a vaccine and any probable side effects or unfavorable reactions, trust in public healthcare organizations and vaccination campaign (medical personnel's' qualification, vaccines being stored properly etc.), availability of vaccine (location of vaccination points and their open hours), a possibility to choose a specific vaccine and to get vaccinated free of charge etc. Since vaccines against COVID-19 are being developed and implemented rather rapidly, assurance that vaccines are safe and effective might become the most significant factor among all the aforementioned ones during the current pandemic [11, 18]. Most respondents who took part in our research mentioned several factors that were significant for them in making a decision to get vaccinated; these factors included safety of available vaccines (65.0 % (62.4–67.6)), efficiency of available vaccines (55.0 % (52.3–57.6)), a possibility to choose a specific vaccine (34.9 % (32.4–37.5)), duration of protective immunity after vaccination (34.7 % (32.2–37.4)). 24.8 % (22.6–27.2) respondents mentioned trust in organization of vaccination campaign as a significant factor; 21.9 % (19.8–24.3), a possibility to visit other countries (Figure 5).

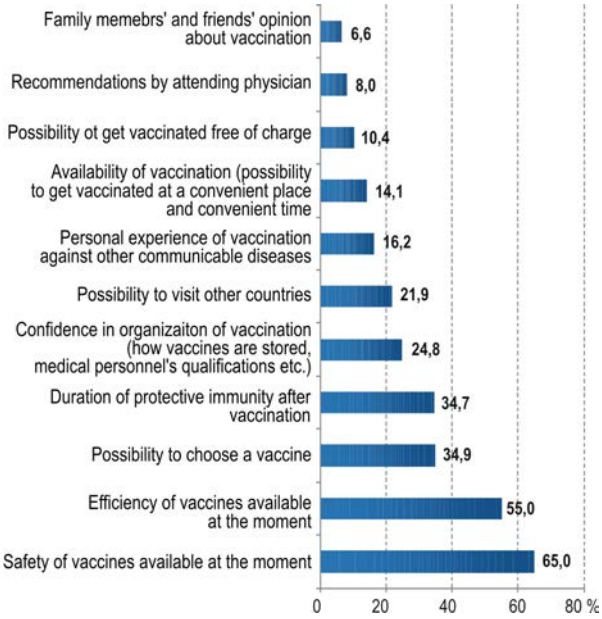

Figure 5. Respondents' answers to the question "Which factors are the most significant for you in making a decision to get vaccinated against COVID-19?", %

31.9 % (29.4–34.5) respondents who took part in our research were already vaccinated against COVID-19. 19.1 % (16.6–21.8) of those who were not vaccinated planned to do it as soon as possible; 47.3 % (44.1–50.6) said

they would probably do it a bit later; 33.6 % (30.6–36.8) stated they were not going to get vaccinated against COVID-19. There were several reasons why people refused from vaccination (were not vaccinated, were not going to, or were going to do it later); the first rank place belonged to absence of any confidence a vaccine was safe (people tended to believe that side effects and unfavorable reactions were highly probable) (64.4 % (60.8–67.8)); the second rank place belonged to absence of confidence in vaccines available at the moment (52.4 % (48.7–56.0)); it was followed by absence of confidence that a vaccine was effective (people believed that a vaccine created only weak and rather short-term protective immunity) (39.2 % (35.7–42.8)). 20.1 % (17.3–23.2) respondents said they needed additional information about vaccination and another 15.4 % (12.9–18.2) were against any vaccination as a way to prevent communicable diseases (Figure 6).

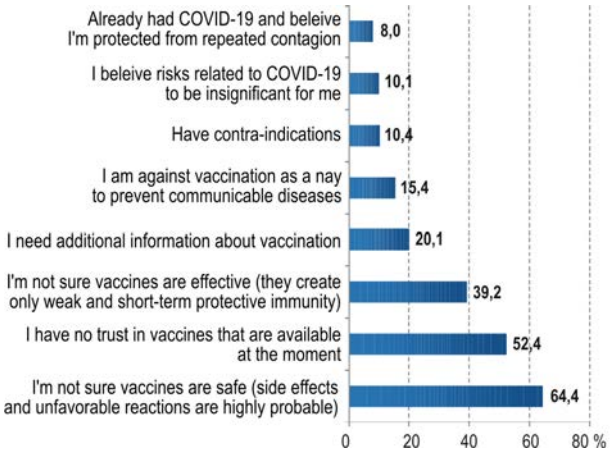

Figure 6. Respondents' answers to the question "Why do you refuse to get vaccinated against COVID-19?", %

Medical workers are a risk group when it comes down to COVID-19 contagion and a priority group for vaccination. Apart from a significant role that belongs to this group in the epidemiologic process of the virus spread, medical workers are a vital source of information about COVID-19 prevention and vaccination. Therefore, their knowledge and communicative skills are extremely important for gaining people's trust and persuading them to get vaccinated [13]. A share of vaccinated people was significantly higher among medical workers who took part in our research against people who was employed in other spheres, 45.4 % (39.1–51.9) and 29.1 % (26.4–31.8) accordingly ($p < 0.001$). Medical workers who were not vaccinated, planned to do it later or were not going to do it at all mentioned the following most frequent reasons for refusal: they had no confidence in vaccines available at the moment (56,6 % (47.4–65.4)); they didn't believe vaccines were safe (considered side effects and unfavorable reactions to be highly probable) (53.1 % (44.0–62.1); they also didn't think vaccines were effective (believed that vaccines created only weak and short-term protective immunity) (40.7 % (32.1–49.9)). 21.2 % (14.7–29.7) medical workers out of those who took part in the research stated that they needed additional information about vaccination.

A share of vaccinated among retired people was also significantly higher than among other groups (43.5 % (34.6–52.9) ($p < 0.05$).

There was a question about circumstances that could persuade those refusing from vaccination to use this prevention measure. The answers were as follows: 59.3 % (55.7–62.8) respondents needed additional scientific data on vaccines being safe and effective; 50.4 % (46.8–54.1) were ready to get vaccinated in case they could select from a variety of vaccines; 18.6 % (15.9–21.6) mentioned visits to other countries as a key factor in their decision-making; and 20.8 % (18.0–23.9) were not ready to get vaccinated in the nearest future under any circumstances (Figure 7).

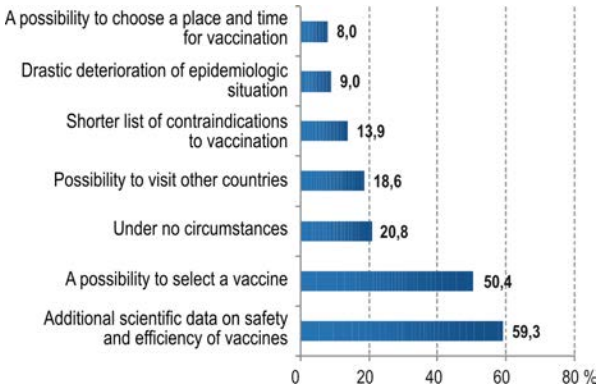

Figure 7. Respondents' answers to the question "Under what circumstances would you be ready to get vaccinated against COVID-19?", %

Table

Influence exerted by social-demographic factors on perception of COVID-19-related health risks and vaccination scope

No.	Analyzed factors	A share of people who believe COVID-19 is a dangerous disease, %	*PR*	A share of vaccinated respondents, %	*PR*
1	Age: – younger than 40 (40 included) – older than 40	 88.0 (85.1–90.3) 92.1 (89.9–93.9)*	1.53 (1.10–2.13)	 29.1 (25.6–32.9) 38.1 (34.5–41.9)*	1.14 (1.06–1.24)
2	Sex: – men – women	 87.1 (83.6–89.9) 91.7 (89.7–93.3)*	1.55 (1.12–2.16)	 31.7 (27.5–36.3) 35.0 (31.8–38.3)	–
3	Marital status: – married / have a life partner – single / do not have a life partner	 89.6 (87.5–91.4) 91.4 (88.2–93.8)	–	 35.1 (32.0–38.3) 30.9 (26.4–35.9)	–
4	Place of living: – capital/regional center – other settlement	 90.9 (88.9–92.5) 88.0 (83.9–91.1)	–	 35.1 (32.1–38.2) 30.0 (25.1–35.4)	–
5	Region of living: – Minsk – other settlement	 92.2 (90.1–93.8) 87.1 (83.9–89.7)*	1.65 (1.19–2.29)	 33.8 (30.5–37.3) 33.9 (29.8–38.2)	–
6	Education: – high – secondary/vocational/specialized secondary education	 91.1 (89.3–92.6) 84.8 (79.1–89.1)*	1.71 (1.17–2.50)	 35.5 (32.7–38.4) 24.5 (18.8–31.2)*	1.17 (1.07–1.29)
7	Job hierarchy: – top manager/middle manager – expert/office clerk/worker	 87.8 (84.2–90.8) 91.1 (89.1–92.8)	–	 34.2 (29.5–39.2) 33.7 (30.7–36.9)	–
8	Chronic diseases in case history: – yes – no	 93.8 (91.1–95.7) 88.3 (86.0–90.3)*	1.88 (1.26–2.81)	 37.7 (33.1–42.6) 32.0 (29.0–35.3)*	1.18 (1.00–1.38)
9	Underage children in a family (live together): – yes – no	 86.4 (83.2–89.1) 92.6 (90.6–94.3)*	1.85 (1.33–2.57)	 30.5 (26.6–34.7) 36.2 (32.8–39.7)*	1.09 (1.01–1.18)
10	Elderly people in a family (live together): – yes – no	 85.8 (81.0–89.6) 91.2 (89.3–92.8)*	1.61 (1.12–2.31)	 37.3 (31.5–43.6) 33.0 (30.2–36.0)	–
11	People with chronic diseases in a family (live together): – yes – no	 93.2 (90.3–95.2) 88.8 (86.6–90.7)*	1.64 (1.10–2.46)	 37.5 (32.8–42.5) 32.3 (29.2–35.5)	–

N o t e : * man differences are statistically significant at $p < 0.05$.

As for respondents being ready to get vaccinated for a fee, our research revealed that 9.3 % (7.9–11.0) were ready to pay for vaccination against COVID-19 in future; 58.9 % (56.2–61.5) were ready to pay provided there was a wide selection of vaccines they could choose from; 31.8 % (29.4–34.4) were not ready to get vaccinated for a fee.

Table contains results obtained through analyzing relations between social-demographic factors and perception of COVID-19-related health risks and adherence to vaccination.

Given that the gravest COVID-19 forms including those with lethal outcomes usually occur among elderly people, this population group is considered to be priority one for vaccination. Therefore, it seems interesting to examine how age and sex influence health risks and adherence to vaccination against COVID-19 since it helps develop more efficient national strategies of vaccination campaigns [19]. Results obtained in several studies indicate that elderly age and female sex might be associated with greater perception of risks related to COVID-19 contagion and lethal outcome due

to the disease [11, 20–22]. Our research results revealed a higher share of people who thought COVID-19 was a dangerous disease among respondents older than 40 ($p < 0.05$), and refuses form vaccination were by 1.14 (1.06–1.24) times more frequent among people younger than 40 than in the older age group. Additional analysis of risk perception by people older than 50 allowed establishing that a share of respondents older than 50 who considered COVID-19 to be a dangerous disease was considerably higher than among younger people (95.5 % (92.8–97.2) and 88.2 % (86.0–90.1) accordingly) ($p < 0.001$) (*PR* 2.62 (1.57–4.35)). We should note that having an elderly relative in a family had an inverse influence on risk perception since a share of people who thought COVID-19 to be a dangerous disease was significantly lower among people living with their elderly relatives than among respondents who didn't have elderly relatives in a family (85.8 % (81.0–89.6) and 91.2 % (89.3–92.8) accordingly) ($p < 0.01$) (*PR* 1.61 (1.12–2.31)). Underage children in a family were also a factor that caused lower risk perception (*PR* 1.85 (1.33–2.57)) and adherence to vaccination against COVID-19 (*PR* 1.09 (1.01–1.18)). Our research results revealed that women tended to have greater perception of COVID-19-related risks (*PR* 1.55 (1.12–2.16)).

Education may be a rather contradictory factor regarding vaccination. People with higher education tend to be better aware and have greater health risk perception; however, higher education may be associated with refusal from vaccination due to selective use of information about it [10, 18]. Our research established that a share of people who believed COVID-19 was a dangerous disease was higher among respondents with higher education than among those who didn't have it (91.1 % (89.3–92.6) and 84.8 % (79.1–89.1) accordingly) ($p < 0.01$) (PR 1.71 (1.17–2.50)). Refusals form vaccination were 1.17 (1.07–1.29) times more frequent among people who didn't have higher education.

We analyzed how risk perception changed depending on a place of living and established that respondents who lived in the capital (Minsk) had greater perception of COVID-19-related risks than those who lived in other settlements in the country (92.2 % (90.1–93.8) and 87.1 % (83.9–89.7) accordingly) ($p < 0.01$) (*PR* 1.65 (1.19–2.29)).

According to data obtained in research accomplished in several European countries and the USA people with chronic pathologies refuse from vaccination much rarer and are more likely to follow recommendations on how to protect their health from COVID-19 since they feel themselves too vulnerable due to additional health risk factors [22, 23]. Our research results indicated that a chronic disease in case history was a predictor of greater COVID-19 risk perception (*PR* 1.88 (1.26–2.81)) and greater adherence to vaccination as well (*PR* 1.18 (1.00–1.38)). Besides, a share of people who thought COVID-19 to be a dangerous disease was significantly higher among respondents who lived with relatives suffering from chronic pathology than among those who didn't have any relatives with chronic diseases in their family (93.2 % (90.3–95.2) and 88.8 % (86.6–90.7) accordingly) ($p < 0.001$) (*PR* 1.64 (1.10–2.46)).

Conclusions. The present research indicates that perception of COVID-19-related health risk is quite high among people living in Belarus since only 9.9 % respondents don't consider COVID-19 to be a dangerous disease; most of them have already had the infection, either asymptomatic or in mild form, or are vaccinated. Greater risk perception among medical workers and other priority risk groups (elderly people and people with chronic pathologies) is another positive fact.

Most respondents who took part in the online poll believe that vaccination is among the most effective prevention measures against COVID-19 (57.9 %); however, people are rather poorly aware about possibilities to get vaccinated: only 57.5 % respondents know that in Belarus vaccination is available to all people older than 18.

33.6 % out of respondents who are not vaccinated are not going to do it and primary reasons for this refusal are absence of confidence that vaccines are safe and effective and absence of confidence in available vaccines,

and 20.1 % people also state they need additional information about vaccination. Analysis of answers given by medical workers indicates that a share of vaccinated people is higher among them but reasons for refusal from vaccination are the same. 21.2 % medical workers who took part in the poll stated they needed additional information about vaccination.

The research results indicate that wider-scale information campaigns are necessary to spread scientifically grounded and authentic information about COVID-19 including data on safety and efficiency of vaccines. Since medical workers are a reliable and important source of information and given their insufficient awareness, it is necessary to take efforts to improve medical workers' knowledge about vaccination against COVID-19 and their communicative skills required to motivate their patients to get vaccinated.

When developing strategies aimed at raising population awareness, it should be kept in mind that lower risk perception regarding COVID-19 and higher prevalence of refusals from vaccinations are much more typical for people who are younger than 40; people without higher education; people with underage children in their family. Lower perception of COVID-19-related risks is also more frequent among men who live beyond the capital; people who have elderly relatives living with them. Internet-resources and data provided by the WHO and Public Healthcare Ministry as well as by medical workers are the most trusted sources of information for population.

Our research results can be applied to plan, implement, and assess efficiency of the national strategy aimed at vaccine prevention of COVID-19 in Belarus as well as to detect tendencies in health risk perception, knowledge, public trust and population adherence to vaccination as a priority trend in COVID-19 prevention.

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Research article

ON ESTIMATING THE ADDITIONAL INCIDENCE OF COVID-19 AMONG POPULATIONS EXPOSED TO POLLUTED AMBIENT AIR: METHODOLOGICAL APPROACHES AND SOME PRACTICAL RESULTS

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We estimate quantitatively the influence of ambient air pollution on SARS-CoV-2 spread among populations in six cities in the Russian Federation. These cities are among priority ones as per air pollution and are included in the "Clean air" Federal project (Bratsk, Krasnoyarsk, Norilsk, Omsk, Cherepovets, and Lipetsk).

Our hypothesis was that dynamic features of the infection spread would be different from an expected model of its epidemiologic process under exposure to environmental pollution. Regression and correlation analysis was performed for relationships between a daily deviation in actual incidence from a basic epidemiologic scenario and the average daily concentrations of chemicals in ambient air. The initial data were results obtained from instrument measurements of ambient air quality in the examined cities (approximately 10.8 thousand measurements covering 29 chemicals) and the daily incidence of COVID-19 from April 18, 2020 to July 31, 2021 (77,337 cases).

An authentic correlation between COVID-19 incidence and chemical concentrations in ambient air was detected in all six examined cities. The contribution of air pollution to COVID-19 incidence rate amounted to 5.0 ± 2.6 % in five cities (Krasnoyarsk, Norilsk, Omsk, Cherepovets, and Lipetsk) over the examined period. In Bratsk, this value was about 33 % and it requires additional research for either confirmation or correction. Growth in COVID-19 incidence in the examined territories is associated with particulate matter (PM_{10}, $PM_{2.5}$) and some other chemicals that can irritate the airway directly or indirectly (sulfuric acid vapors, hydrogen chloride, formaldehyde, hydrogen sulphide, etc.). Target levels were substantiated for several priority chemicals; should these levels be achieved, one would predict a decrease in COVID-19 incidence by more than 1–3 % in the examined cities.

We propose that population morbidity and mortality caused by COVID-19 require further studies, including those combined with medical and biological examination regarding efficiency of vaccination and post-vaccination immunity persistence on territories with elevated environmental pollution. This research is vital due to the considerable global medical and demographic losses during the COVID-19 pandemic and the latest research works providing evidence of a correlation between air pollution and spread of the disease, its severity, clinical course and outcomes.

***Key words**: COVID-19, ambient air pollution, chemicals, target levels.*

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The COVID-19 pandemic has resulted in grave damage to the world's population, as well as to global economies and finance. Detailed studies on reasons for the occurrence and spread of disease, its severity and modifications in its clinical course, became one of the most vital trends in scientific research in 2020–2021.

The integration of long-term epidemiologic and experimental data suggests that environmental pollution would serve to favor the spread of SARS-CoV-2 virus and negative health outcomes of COVID-19 [1–5]. Indeed, previous research focusing on respiratory viruses has provided evidence that air pollution can contribute to elevated morbidity and mortality primarily caused by communicable diseases [6–9]. Research accomplished during the first COVID-19 wave described detected direct authentic correlations between dust particles (including PM_{10} and $PM_{2.5}$), nitrogen oxides, ozone, and other pollutants contained in ambient air and COVID-19-related mortality and morbidity [10–14]. There are assumptions in some published works that air pollutants, primarily finely-dispersed dusts, can enhance coronavirus spread since they absorb the culpable virus (SARS-CoV-2) on the surface of particles [15, 16].

According to data provided by the World Health Organization (WHO), all sizes of aerosol particles carrying infectious virus can deposit directly on the mucosa of the respiratory tracts (direct contacts); additionally, the agent could contaminate surfaces or objects (fomites) and thereby be transmitted to mucosa (indirect contacts) [17]. Viable SARS-CoV-2 virus has been detected on surfaces for several hours or even several days depending on environmental conditions [18]. Therefore, when solid or liquid droplets (as a disperse phase) occur in air (as a disperse medium), there is greater potential for the virus to spread.

These assumptions have been confirmed with epidemiologic research. Using results obtained by analyzing medical and demographic statistical data collected in more than 3 municipal settlements in the USA, Wu and colleagues [19] established that an increase of 1 mg/m^3 in finely dispersed $PM_{2.5}$ dust in ambient air resulted in an 8 % increase in mortality due to COVID-19 (95 % confidence interval was 2–15 %). These authors assumed that both short-term (acute) and long-term (chronic) exposure to $PM_{2.5}$ dust would be a risk factor.

Setti and associates revealed an authentic correlation between excessive daily PM_{10} levels and initial COVID-19 spread in 110 provinces in Italy [20]. Additionally, Chinese and British scientists used ecologic and medical data collected in 120 cities in the People's Republic of China (approximately 58 thousand COVID-19 cases) to develop multifactor mathematical models that described positive correlations between the daily number of confirmed COVID-19 cases and ambient concentrations of finely dispersed dusts ($PM_{2.5}$, PM_{10}), nitrogen dioxide, and ozone in ambient air averaged over a 2-week period [21]. The authors indicated it was vital to take necessary actions aimed at reducing air pollution since it would help minimize disease incidence.

Several studies have found that air pollution not only promotes the spread of SARS-CoV-2 but also compromises the removal of airway pathogens, diminishes overall immunity and aggravates cardiovascular or lung diseases [22–25]. Bourdrel and colleagues (2021) showed that air pollution might be related to increased COVID-19 severity and lethality in subjects with chronic diseases, such as cardiopulmonary disease and diabetes mellitus They noted that exposure to polluted air resulted in a weaker immune response that promoted viral entrance and replication in the body. Besides, viruses can persist in ambient air due to complex interactions with solid particles and gases. Such interactions vary according to chemical structure, particle electric charge, and meteorological parameters such as relative humidity, ultraviolet radiation, and temperature [26].

A wide-scale study of more than 27.6 thousand patients with COVID-19 showed that the highest mortality occurred among patients

who, prior to SARS-CoV-2 infection, suffered from cardiovascular diseases, immune and metabolic disorders, respiratory diseases, cerebrovascular diseases, or cancer [27].

All the aforementioned data are in line with results obtained by German researchers [28] who emphasize that the epidemic process, while uniform, resulted in significant differences in COVID-19 prevalence in European countries with similar and efficient public healthcare systems and similar population structures. Long-term exposure to adverse environmental factors in a period prior to the epidemic is considered a possible reason for the higher incidence of COVID-19 in some countries. Drinking water chlorination, environmental pollution with pesticides and persistent organic pollutants, among other factors, were considered to be risk factors that suppress immune function and thereby increase susceptibility to viral infection.

Results obtained via spatial analysis of fatal COVID-19 cases in 66 administrative districts in Italy, Spain, France, and Germany indicate that 3,487 of 4,443 (78 %) deaths occurred in 5 regions located in northern Italy and central Spain. Importantly, attendant orographic features compromised the efficient dispersion of airborne pollutants, such that the highest nitrogen dioxide concentrations were detected in the same five regions [29]. The authors proposed that long-term exposure to nitrogen dioxide contributed significantly to the elevated COVID-19 mortality in these regions.

Given that poor environmental quality, especially of ambient air, appears to exert both direct and indirect influences to promote COVID-19 spread and associated mortality, we employ our expertise in environmental hygiene to examine the Russian experience. Ambient air quality in many large cities in the country is rather poor due to chemical admixtures occurring in quantities that exceed hygienic standards; this includes airborne pollutants such as dusts, especially PM_{10} and $PM_{2.5}$, nitrogen oxide, and nitrogen dioxide among other pollutants that appear to elevate risks of COVID-19 spread [30–32]. The subject is of special relevance to the populations of Russian cities where ambient air is heavily polluted, including those listed within the “Clean air” Federal project, such as Krasnoyarsk, Bratsk, Cherepovets, Norilsk, and others [33–35]. Coal is a primary energy source in cities located in the Siberian Federal District in Russia where prevalent climatic and meteorological factors prevent efficient dispersion of emissions from energy-producing objects, industries, transport, and heating sources in private households. These factors combine to result in the accumulation of airborne pollutants in the bottom layers of the atmosphere, that is, the layers from which people breathe air.

The Russian Federation is currently assessing the correlation between quality of the environment and morbidity and mortality of COVID-19[1]. Here, we quantitatively assess the relationship between COVID-19 morbidity and mortality and several environmental parameters, the results of which can be used to regulate sources of air pollution. This is especially important for economic entities, notably businesses, since the COVID-19 pandemic has resulted in significant losses of working days among the employable population due to temporary inability to work. Figure 1 shows that the working population prevailed among COVID-19 patients (40.4 %), workers accounting for 27.8 % among them; medical personnel, 9.8 %; office workers, 7.7 %; and personnel employed at educational establishments, 2.8 %.

Our research goal was to quantitatively assess the influence of ambient air pollution on SARS-CoV-2 spread among selected urban populations living in Russia under unfavorable sanitary-hygienic conditions.

[1] Does air pollution influence mortality caused by the new coronavirus infection? Available at: http://cgon.rospotrebnadzor.ru/content/62/4262/ (August 18, 2021).

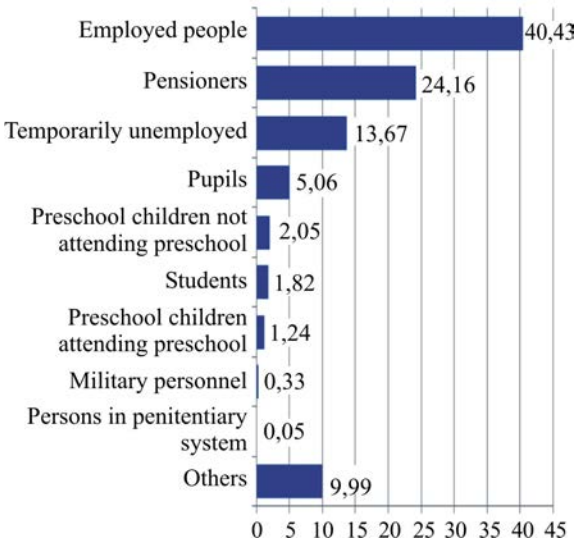

Figure 1. Composition of COVID-19 patients in Russia by occupation or social status (2020), % [36]

Research Design. The research design was built on the assumption that air pollution could change the epidemiologic process due to sorbed viruses being carried on solid particles, on the one hand, and particulate irritation of mucosa and weakened barrier functions of the respiratory organs, on the other hand. Essentially, it was assumed that air pollution created environmental conditions under which dynamic features of the infection spread were different from any expected scenario that corresponded to a standard model of the epidemiologic process.

It should be noted that an epidemiologic process model (a basic scenario of the infection spread) has a complex structure and should take into account certain peculiarities typical for a given territory or a settlement, such as population density, inter- and intra-territorial migration, peculiarities related to anti-epidemic activities organization, among other considerations. Building such a model requires special studies and is beyond the framework of the present research and its tasks.

Data and methods. COVID-19 incidence was quantitatively estimated based on an analysis of cause–effect relations between the daily growth in the number of cases and air pollution rates in six cities listed in the "Clean air" Federal project program, namely Bratsk, Krasnoyarsk, Norilsk, Omsk, Cherepovets, and Lipetsk (Figure 2).

The main sources of pollution in the studied cities are the objects of the mining and metallurgical industry, in Siberian cities (Bratsk, Norilsk, Krasnoyarsk) there are also heat power facilities operating on solid fuels.

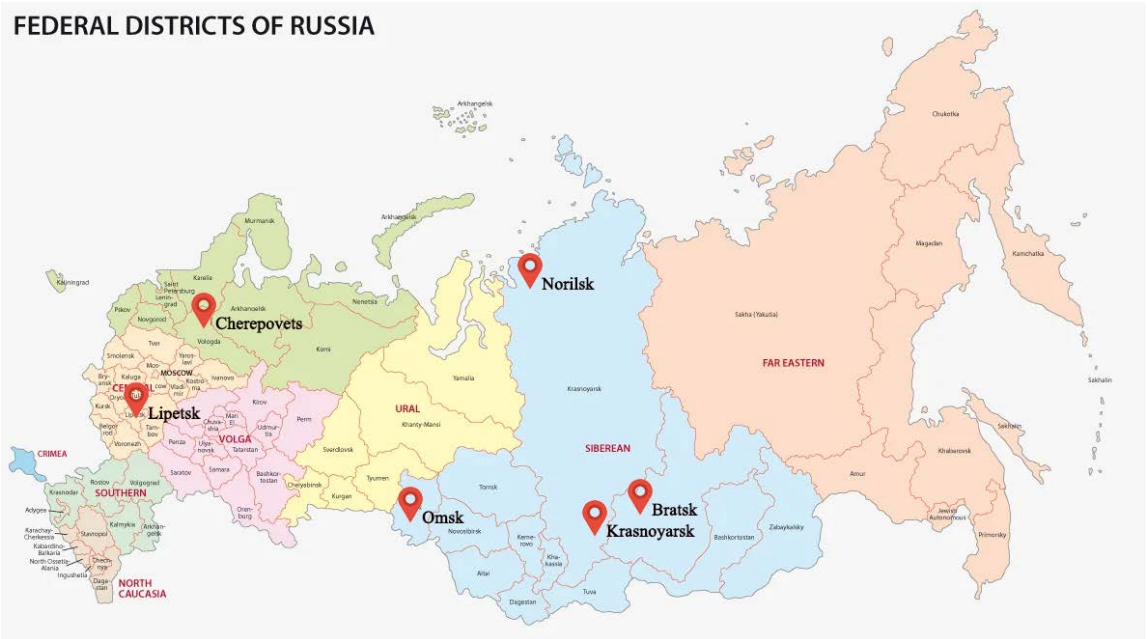

Figure 2. Location of the studied cities on the map of the Russian Federation

Results obtained via daily instrumental measurements of chemical concentrations in ambient air in these cities were taken as initial data on ambient air quality. Measurements were accomplished within social-hygienic monitoring activities by certified laboratories of the Centers for Hygiene and Epidemiology in Irkutsk region (Bratsk), Krasnoyarsk region (Krasnoyarsk and Norilsk), Omsk region (Omsk), Vologda region (Cherepovets), and Lipetsk region (Lipetsk). All monitoring posts were located in urban residential areas. Samples were taken as per the most comprehensive program (4 times a day). Comparable and certified sampling and measurement procedures were applied in each examined city.

The research covered all particulate matter and admixtures which, according to data available in relevant scientific literature, can exert a negative influence on airway function and/or the immune system. Overall, our analysis included 10.8 thousand instrumental measurements of 29 entities, including PM_{10} and $PM_{2.5}$ with non-differentiated structure, finely dispersed particles with known chemical structure (oxides of aluminum, nickel, copper, chromium, and poorly soluble fluorides) soot, benzopyrene, nitrogen dioxide, sulfur dioxide, hydrogen chloride, sulfuric acid, and aromatic hydrocarbons (benzene, toluene, xylene), among others.

Population numbers were taken from state statistics as of January 01, 2021.[2] At present approximately 3.46 million people live in the six cities examined in the present study.

Daily incidence of SARS-CoV-2 coronavirus infection was taken as the absolute number of cases over a period of time from April 18, 2020 to July 31, 2021, according to daily data provided by operative offices in the regions studied (overall, 77,337 disease cases).

Data analysis. Preliminary data preparation included the following:

– average daily concentrations of pollutants in the examined cities were computed via averaging data obtained from all daily measurements;

– gaps in initial data were filled due to averaging values of daily concentrations within a time range ± 15 days from an examined date (in case there were 5 and more computed average daily concentrations);

– relative daily incidence among populations was computed as a ratio of an absolute number of registered cases to a population number;

– disease incidence was averaged per week (to remove "weekend effects" caused by peculiarities of medical aid provision and a system of accounting a number of people's applications for medical aid to medical organizations on weekends) as per the following formula (1):

$$\tilde{y}_t^7 = \frac{1}{7}\sum_{\tau=t-3}^{t+3} y_\tau, \qquad (1)$$

where $\tilde{y}_t^7$ is incidence averaged over a 7-day period on a date t, cases/1,000; t is the present date.

To model the epidemiologic process, we simplified the daily growth of disease cases by averaging data collected over a 28-day period using a moving-average procedure (2):

$$\tilde{y}_t^{28} = \frac{1}{28}\sum_{\tau=t-14}^{t+13} y_\tau, \qquad (2)$$

where $\tilde{y}_t^{28}$ is incidence on a date t averaged over a 28-day period, cases/1,000, t is the present date.

Relationships were modeled in accordance with the basic hypothesis that a difference between detected incidence (with weekly averaging) and incidence in a basic epidemiologic scenario was due to external factors, including those related to air pollution.

To eliminate the influence exerted by a scale of a basic epidemiologic scenario in results obtained via assessing cause-and-effect

[2] 100 largest cities in Russia as per population (2021). Available at: http://www.statdata.ru/largest_cities_russia (August 01, 2021).

relations, a relative daily deviation was computed (3):

$$\delta\tilde{y}_t = \frac{\tilde{y}\tilde{y}_t^7 - \tilde{y}_t^{28}}{\tilde{y}_t^{28}}, \quad (3)$$

where $\delta\tilde{y}_t$ is a relative daily deviation in actual COVID-19 incidence from a basic epidemiologic scenario.

Computations of relative daily deviations for all six territories provided comparable standardized values such that all the data could be combined in a single data array for statistical analysis.

Modeling a system for cause–effect relations also involved correlation-regression analysis of relationships between a relative daily deviation in actual incidence from a basic scenario ($\delta\tilde{y}_t$) among a given population and the average daily concentrations of chemicals in ambient air. Models were built based on combined data from all examined territories. In addition, we searched for a time gap between changes in air pollution rates and increased disease incidence. This optimal time lag was assumed to correspond to the maximum correlation coefficient.

Statistical analysis allowed relationships to be paired between relative daily growth in incidence and air pollution rates (4):

$$\delta\tilde{y}_t = a_i x_{i,t-Li} + b_i, \quad (4)$$

where $x_{i,t-Li}$ is average daily concentration of the *i*-th chemical in ambient air on a date *t*-*Li*; *Li* is time lag; a_i, b_i are model coefficients for the *i*-th chemical.

Building models for relationships involved procedures aimed at testing statistical hypotheses regarding model parameters and model adequacy, as well as testing their biological plausibility.

Obtained models showing the relationships gave grounds for assessing a number of disease cases associated with ambient air pollution. To do that, the following values were calculated:

– daily incidence associated with ambient air pollution (5):

$$\Delta\tilde{y}_t = \sum_i \Delta\tilde{y}_{t,i} = \sum_i \left(\delta\tilde{y}_t(x_{i,t-Li}) - \delta\tilde{y}_t(0)\right)\tilde{y}_t^{28} = \sum_i a_i x_{i,t-Li}\tilde{y}_t^{28}, \quad (5)$$

where $\Delta\tilde{y}_t$ is total disease incidence associated with exposure to air pollution on a date *t*, cases/1,000; $\Delta\tilde{y}_{t,i}$ is disease incidence associated with exposure to the *i*-th chemical in ambient air on a date *t*, case/1,000;

– absolute number of disease cases associated with air pollution over a year (6):

$$\Delta Y = \frac{N}{1000}\sum_t \Delta\tilde{y}_t. \quad (6)$$

A probable contribution of air pollution to COVID-19-related mortality and morbidity among the populations studied was estimated based on the totality of data. Additionally, such a concentration of a chemical was computed that could account for permissible growth in COVID-19 incidence (target growth). The permissible (target) growth was taken as equal to 1 % and / or 3 % of a relative daily incidence for a given territory.

A target concentration was computed as per the following formula (7) that was obtained via transformation from the regression equation (5):

$$x_i^N = \frac{\delta\tilde{y}^N}{a_i} \quad (7)$$

where x_i^N is a target concentration of the *i*-th chemical, mg/m^3; $\delta\tilde{y}^N$ is a target relative growth in disease incidence taken as equal to 0.01 (or 1 %) or 0.03 (or 3 %).

The suggested approaches were tested in some cities that were included in the "Clean air" Federal project (Bratsk, Krasnoyarsk, Norilsk, Omsk, Cherepovets, and Lipetsk). Correlations between ambient air quality and COVID-19 spread were considered separately for each examined city due to specific chemicals occurring in ambient air in each of them and, accordingly, different programs for instrumental measurements that were applied there.

We also attempted to reveal any common regularities by analyzing data combined for all six examined cities.

Basic results. Figures 3 and 4 show average weekly disease incidence for the examined territories. While similarities in the overall temporal pattern of disease incidence was evident in the six cities, individual cities showed variances one with the other.

Assessment of a correlation between these deviations and air pollution rates in different cities allowed the development of several reliable mathematical models; Tables 1–3 contain their parameters for some cities (in these tables: n is a number of daily observations, R^2 is determination coefficient).

Reliable models were obtained for a correlation between daily fluctuations in the

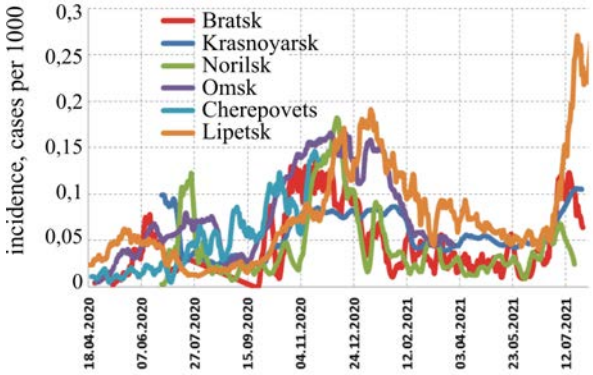

Figure 3. Daily incidence of SARS-CoV-2 on the examined territories averaged over a 7-day period

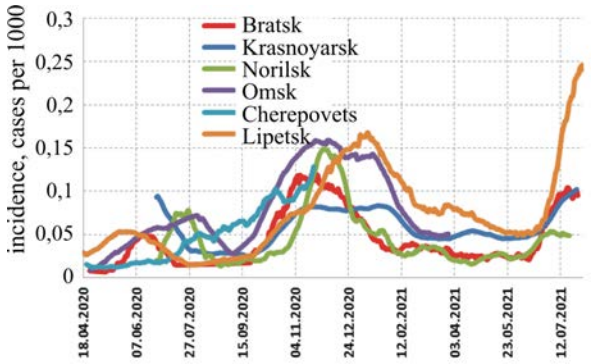

Figure 4. Daily incidence of SARS-CoV-2 according to a basic epidemiologic scenario (average over a 28-day period)

Table 1

Parameters of relationships between a value $\partial\tilde{y}_t$ and average daily concentrations of chemicals in ambient air ($p < 0.05$) in Bratsk

Chemical	a_i	b_i	n	R^2	A range of daily concentrations over the examined period, mg/m^3
Benzopyrene	41,600	–0.0384	316	0.0225	[0*; 1.42E-05]
Particulate matter PM_{10}	31.63	–0.00356	321	0.0230	[0; 0.036]
Particulate matter $PM_{2.5}$	20.87	–0.00452	319	0.0165	[0; 0.123]
Hydrogen chloride	9.19	–0.027	316	0.0394	[0; 0.06]
Dimethylbenzene	2.26	–0.0935	309	0.0363	[0; 0.35]
Methylbenzene	3.76	–0.0817	309	0.0355	[0; 0.17]
Carbon (soot)	2.24	–0.0250	313	0.0199	[0; 0.19]
Ethylbenzene	17.35	–0.0719	309	0.0171	[0; 0.022]

Note: 0* – here and further on – means “lower than limit of detection”.

Table 2

Parameters of relationships between a value $\partial\tilde{y}_t$ and average daily concentrations of chemicals in ambient air ($p < 0.05$) in Cherepovets

Chemical	a_i	b_i	N	R^2	A range of daily concentrations over the examined period, mg/m^3
Particulate matter PM_{10}	6.04	–0.0432	209	0.0597	[0; 0.0707]
Particulate matter $PM_{2.5}$	5.28	–0.0331	209	0.0349	[0; 0.061]
Nickel oxide *	48.63	–0.0465	209	0.0267	[0; 0.00733]

Note: * occurs in ambient air as fine-dispersed solid particles.

Table 3

Parameters of relationships between a value $\partial\tilde{y}_t$ and average daily concentrations of chemicals in ambient air ($p < 0.05$) in Lipetsk

Chemical	a_i	b_i	n	R^2	A range of daily concentrations over the examined period, mg/m^3
Nitrogen dioxide	0.593	–0.0229	382	0.0104	[0; 0.135]
Ammonia	2.78	–0.0208	382	0.0207	[0; 0.0506]
Hydrogen sulphide	119.6	–0.00945	377	0.0104	[0; 0.001]
Manganese and its compounds*	122.8	–0.0122	375	0.0125	[0; 9.75E-04]
Ozone	1.76	–0.0223	382	0.0188	[0; 0.0765]
Prop-2-en-1-al (acrolein)	57.4	–0.0127	380	0.0104	[0; 0.00263]

Note: * occurs in ambient air as fine-dispersed solid particles.

growth of COVID-19 cases and daily fluctuations in the airborne concentrations of benzene (a_i = 0.088; b_i = –0.0143; R^2 = 0.02) and hydrogen sulphide (a_i = 82.1; b_i = –0.063; R^2 = 0.32) in Krasnoyarsk; daily fluctuations in concentrations of nitrogen dioxide (a_i = 2,18; b_i = –0.0912; R^2 = 0.02) and sulfuric acid (a_i = 21,5; b_i = –0.099; R^2 = 0.11) in Norilsk; and daily fluctuations in concentrations of ethyl benzene (a_i = 2,96; b_i = –0.039; R^2 = 0.02) in Omsk.

Table 4 models the relationship between specific airborne pollutants using data from all examined cities and $\partial\tilde{y}_t$, which were considered authentic ($p < 0.05$).

Based on these mathematical models, we tentatively assessed the contribution made by chemical factors to the incidence of COVID-19 among populations in the examined cities (recalculated as per annual incidence) (Table 5).

Estimated contributions to COVID-19 incidence from airborne pollutants varied from 1.6 % (Krasnoyarsk) to 8.9 % (Norilsk) in five of six examined cities, results that compare well with those reported from studies outside the Russian Federation. Results obtained for the city of Bratsk fall outside this range since the computed contribution for chemical factors amounted to almost one third over the examined period.

Overall, approximately 3,572 number of COVID-19 cases detected in the examined cities are probabilistically related to polluted breathable air. This suggests that decreased air pollution would reduce the number of COVID-19 cases in these cities and beyond.

Tables 6–11 contain results obtained via computing target levels of admixture contents in ambient air for each city.

Target concentrations differ by city due to the differential estimated total health impact of city-specific air pollutants. However, almost everywhere, the target air pollutant levels that ensure a growth in COVID-19 incidence not exceeding 3 %, and in a better case 1 %, are lower than the average daily maximum permissible concentration (MPC) of an individual

Table 4

Parameters of relationships between a value $\partial\tilde{y}_t$ and average daily concentrations of chemicals in ambient air as per combined data collected in all 6 cities

Chemical	a_i	b_i	n	R^2
Hydrogen chloride (as per HCl molecule)	8.96	–0.0202	464	0.0369
di-Aluminum trioxide *	7.34	–0.0213	591	0.0105
Nickel oxide (recalculated as per Ni)*	39.8	–0.0298	619	0.0126
Ozone	1.76	–0.0223	382	0.0188
Carbon black (soot)*	1.39	–0.0135	1469	0.0077
Formaldehyde	2.09	–0.0146	1372	0.0036

Note: * occurs in ambient air as fine-dispersed solid particles.

Table 5

Computed numbers of associated disease cases as per cities, cases/year

City	COVID-19 incidence over the examined period				Share of cases associated with air pollution
	Actually detected		Including that associated with air pollution		
	Cases, total	cases / 1,000	Cases, total	cases / 1,000	
Bratsk	3,280	14.50	1,380	6.10	0.334
Krasnoyarsk	14,929	14.83	232	0.23	0.016
Norilsk	2,171	11.94	207	1.14	0.089
Omsk	33,238	28.79	723	0.63	0.022
Cherepovets	5,634	18.05	501	1.61	0.089
Lipetsk	15,326	30.46	529	1.05	0.035

Table 6

Target average daily concentrations of chemicals in ambient air in Bratsk that ensure a relative growth in COVID-19 incidence not exceeding 1–3 % (here and further on given a persistent set of pollutants typical for a specific city)

Chemical	Target concentration, mg/m^3, that ensures growth in incidence not exceeding		MPC*, mg/m^3	
	1 %	3 %	av.daily	av.annual
Benzopyrene	2.40E-07	7.2E-07	–	1.00E-06
Particulate matter PM_{10}	0.0003	0.0010	0.15	0.075
Particulate matter $PM_{2.5}$	0.0005	0.0014	0.035	0.025
Hydrogenchloride (as per HCl molecule)	0.0011	0.003	0.1	0.02
Dimethylbenzene (a mix of isomers)	0.0044	0.013	0.04	–
Methylbenzene	0.0027	0.008	–	–
Carbon (soot)	0.0045	0.013	0.05	0.025
Ethylbenzene	0.0006	0.002	–	0.04

Note: *MPC means maximum permissible concentration.

Table 7

Target average daily concentrations of chemicals in ambient air in Cherepovets that ensure a relative growth in COVID-19 incidence not exceeding 1–3 %

Chemical	Target concentration, mg/m^3, that ensures growth in incidence not exceeding		MPC av.daily, mg/m^3
	1 %	3 %	
Particulate matter PM_{10}	0.0017	0.051	0.06
Particulate matter $PM_{2.5}$	0.0019	0.006	0.035
Nickel oxide	0.0002	0.001	0.001

Table 8

Target average daily concentrations of chemicals in ambient air in Lipetsk that ensure a relative growth in COVID-19 incidence not exceeding 1–3 %

Chemical	Target concentration, mg/m^3, that ensures growth in incidence not exceeding		MPC av.daily, mg/m^3
	1 %	3 %	
Nitrogen oxide	0.017	0.051	0.06
Ammonia	0.004	0.012	0.035
Hydrogen sulphide	0.00008	0.001	0.001
Manganese and its compounds	0.00008	0.0003	0.002*
Ozone	0.006	0.018	0.1
Prop-2-en-1-al (acrolein)	0.0002	0.0006	0.01

Note: * average annual concentration is given in case there is no determine average daily one.

Table 9

Target average daily concentrations of chemicals in ambient air in Norilsk that ensure a relative growth in COVID-19 incidence not exceeding 1–3 %

Chemical	Target concentration, mg/m^3, that ensures growth in incidence not exceeding		MPC av.daily, mg/m^3
	1 %	3 %	
Nitrogen dioxide	0.0046	0.42	0.06
Hydrogen sulphide	0.0005	0.0004	0.002*

Note: * average annual concentration is given in case there is no determine average daily one.

Table 10

Target average daily concentrations of chemicals in ambient air in Krasnoyarsk that ensure a relative growth in COVID-19 incidence not exceeding 1–3 %

Chemical	Target concentration, mg/m^3, that ensures growth in incidence not exceeding		MPC av.daily, mg/m^3
	1 %	3 %	
Benzene	0.1136	0.42	0.06
Hydrogen sulphide	0.00012	0.0004	0.002*

Note: * average annual concentration is given in case there is no determine average daily one.

Table 11

Target average daily concentrations of chemicals in ambient air in Omsk that ensure a relative growth in COVID-19 incidence not exceeding 1–3 %

Chemical	Target concentration, mg/m^3, that ensures growth in incidence not exceeding		MPC av.daily, mg/m^3
	1 %	3 %	
Ethylbenzene	0.0037	0.011	0.04*
Nickel oxide	0.00004	0.0001	0.001

Note: * average annual concentration is given in case there is no determine average daily one.

pollutant and/or average annual MPC. A target level may be lower than hygienic standards by 1.5–3 times (for example, target concentrations of nitrogen dioxide and ammonia in Lipetsk; benzopyrene and xylene in Bratsk, or particulate matter PM_{10} in Cherepovets) or even by 10–20 times (target concentrations of manganese compounds and acrolein in Lipetsk or hydrogen sulphide in Krasnoyarsk). While target concentrations are determined by specific sets of pollutants in this or that city and actual existing concentrations of specific admixtures, the data overall predict that COVID-19 cases would drop with substantial improvement in ambient air quality.

Discussion. The present results support the hypothesis that variations in COVID-19 incidence is correlated with urban air pollution, and that these relationships are demonstrable regardless of the type and concentration of pollutants in breathable air. In brief, air pollution appears to increase the incidence of COVID-19. This relationship holds not only for particulate matter (as reported in multiple foreign publications) but also for a set of other chemicals that produce airway irritability among other physiologic effects. These chemicals include: sulfuric acid vapors, hydrogen chloride, formaldehyde, hydrogen sulphide, among others.

The apparent contribution of chemical pollution to an increased COVID-19 incidence in five of the six cities (i.e., Krasnoyarsk, Norilsk, Omsk, Cherepovets, and Lipetsk) varied within close ranges and amounted to approximately 5.0 ± 2.6 % over the examined period. A detected correlation between daily fluctuations in COVID-19 incidence and air pollution in Bratsk was substantially more intense and

amounted to approximately 33 %; additional research is required either to confirm or correct these results. Overall, these results assume that pre-existing disease and other factors that affect COVID-19 rates are distributed equally among the populations studied here. The findings also rely on the assumption that COVID-19 incidence is comparably reported across the cities studied. While COVID-19 mortality rates are not included, such data would be a valuable addition to the present analysis.

These results are relevant to economic entities that contribute to air pollution and to the governmental authorities that regulate the emission of pollutants from factories and other entities. Standards for permissible emissions can and should be the first obligatory step in making the living environment truly safe, but there should also be further measures designed to minimize air pollution and associated population health risks. Such steps are especially important when people are simultaneously exposed to a contagious respiratory virus (SARS-CoV-2) and air pollutants that are known to compromise airway and/or immune function. The latter should always be taken into account when a system for environmental standardization is being updated, including methodology for emissions quoting. Indeed, the management of pollutant emissions in cities should become a system for health risk management, especially in the case of specific sanitary-epidemiologic situations. These recommendations are highlighted by the present results showing that COVID-19 has uncovered underlying major health risks among urban populations in Russia and beyond that are continually exposed to airborne pollutants.

The present research is considered to be preliminary and aimed at testing methodological approaches. Primary analysis of all collected data revealed some uncertainties in initial data that produced significant effects on computation results and should be eliminated. Uncertainties are associated with some delay in entering data on morbidity into information bases and the lack of measurements for some days, which required “restoration” of data by interpolation methods.

Similar studies using more complete data collected over a longer period of time are recommended. Additionally, it will be important to examine how air pollution affects COVID-19 incidence and mortality by sex, age, occupation and preexisting medical conditions. Results obtained from such studies will allow the development of recommendations designed to optimize the quality of breathable urban air.

Studies on the efficiency of vaccination against SARS-CoV-2 and post-vaccination immunity persistence among people living under different external exposure conditions can be and should be another significant trend in future research. Such studies should be accomplished as soon as possible since they, among other things, are vital for organizing proper and efficient prevention of repetitive waves of COVID-19. Without doubt, there are grounds for such studies: first of all, there is evidence of declining post-vaccination immunity to other communicable diseases such as measles or diphtheria under exposure to environmental pollution [37–39]. Research [40] has shown that laboratory blood tests of children living in environments with permanent high chemical aerotechnogenic exposure have weakened immune systems and decreased immune responses to vaccine antigens.

Given the existence of a relevant database, wide-scale research that focuses on assessing the influence of environmental pollution on preserving immunity against COVID-19 could be launched without delay. Ambient air quality is constantly monitored within the “Ecology” National project in residential areas in cities where air pollution is high. Data on each COVID-19 case are registered in a unified database that contains information collected across Russia. There is also systemic national serologic monitoring [41] that provides uninterrupted objective assessment of specific post-vaccination immunity against agents of communicable diseases that can be managed via specific prevention; this monitoring is performed in “indicator” population groups and risk groups.

There should be permanent control over the existing situation, and wide and compre-

hensive discussion of the results obtained via this control; discussion and communications should involve not only scientists and practical experts in public health but also people who make decisions on ecology, medicine, public agents at all levels, and people representing business and civil society as well. All the above-listed steps are needed not only to control and surmount the current pandemic but also to provide critical information to help solve similar problems in future. These approaches are in line with opinions and attitudes expressed by many researchers [42–44].

Conclusions. An authentic correlation between COVID-19 incidence and the concentration of chemical pollutants in ambient air has been detected in all six examined cities; these are considered priority cities in the Russian Federation for air pollution and are included in the "Clean air" Federal project. Our data are in line with those obtained by foreign researchers and suggest a common trend of higher COVID-19 incidence among people living in urbanized territories of Russia and beyond. In sum, they suggest that continuous exposure to certain air pollutants with established adverse health impacts can increase the risk of COVID-19 and spread of SARS-CoV-2.

In five cities (Krasnoyarsk, Norilsk, Omsk, Cherepovets, and Lipetsk), the contributed excess risk varied within close ranges and amounted to approximately 5.0 ± 2.6 % over the test period. A detected correlation between daily fluctuations in the growth in COVID-19 incidence and air pollution in Bratsk was substantially more intense and amounted to approximately 33 %. This unexpected large result requires confirmation based on a more detailed analysis of modeled data.

Combined multi-component exposure to chemicals results in an authentic growth in medical and demographic losses even if concentrations of specific chemicals conform to hygienic standards. Standards for permissible emissions should be treated as the first obligatory stage in making the living environment truly safe, but in future there should be further actions aimed at minimizing pollution and population health risks.

The present research allowed testing some methodological approaches to detect fluctuations in COVID-19 incidence associated with air pollution. It seems advisable to accomplish similar studies using more complete data collected over a longer period of time, including examinations focusing on mortality due to COVID-19, especially among urban populations in Russia and beyond.

Studies on the efficiency of vaccination against SARS-CoV-2 and post-vaccination immunity persistence among people living under different environmental conditions should become another significant trend in future research.

The combination of epidemiologic trends and biomedical examinations that concentrate on the efficiency of SARS-CoV-2 vaccination can provide a scientific information base for the optimal management of environmental quality and population health risks; among other things, it helps prevent and control negative outcomes during complicated sanitary epidemiologic situations.

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Research article

RADON RISK COMMUNICATION ISSUES: RESULTS OF THE ALL-RUSSIAN PUBLIC OPINION SURVEY

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1,500 respondents took part in an Internet survey conducted in autumn 2020 in the Russian Federation. The survey was a part of the Radon Cross-Cultural Multilingual Public Opinion Survey (STEAM project) in the framework of the IAEA technical cooperation project RER9153. The survey was representative for the population of the country as per sex, age, and a region of residence. Random sampling error didn't exceed 2.5 % for 95 % confidence interval. All respondents were given some information on radon that was as similar as possible in questionnaires published in languages spoken in all 22 countries that took part in the STEAM project; it was done in order to provide an opportunity to make further cross-country comparison of the survey results. The objective of the survey was to investigate what attitudes people had towards their health and towards radon as a possible health risk factor.

The survey revealed that in the Russian Federation people were rather poorly aware about radon. Only 31.7 % respondents stated that they were to a greater or lesser extent informed about radon. The level of knowledge about radon as a health risk factor was at a comparable level. For the majority of respondents, information about radon received from Rospotrebnadzor and its subordinate research institute formed the perception of radon as a risk factor that requires actions to mitigate its impact on health. Medical specialists turned out to be the most trustworthy source of information about health risks, first of all, family doctors and physicians in polyclinics; people also trusted medical prevention centers, Rospotrebnadzor, regional and local public health care authorities.

Results of the presented survey that was the first social survey focusing on the radon problem and conducted throughout the country can be used as a basis for planning communication strategies within the framework of both national and regional radon programs.

Key words: *radon, natural exposure, risk communication, radiation risk, radiation protection, social survey, risk awareness, National radon program, risk perception.*

Radon is a natural radioactive gas, colorless, tasteless, and odorless. It is not only the most significant natural source of public exposure worldwide [1–3], but also a major contributor to the individual annual radiation dose due to all sources of ionizing radiation (accounting for more than 50 % of the total dose to the population of the Russian Federation [4–6]). At the same time, radon often remains in the "information shadow" of other sources, primarily nuclear power facilities and various medical equipment, which actually make a much smaller contribution to the collective dose. It is a proven fact that radon and its progeny are the second leading cause of lung cancer after tobacco smoking for smokers and the main one for non-smokers [7, 8].

International organizations, such as the International Atomic Energy Agency (IAEA), the World Health Organization (WHO), the International Commission on Radiological Protection (ICRP), emphasize the importance of developing national radon programs (or radon action plans), conducting national and re-

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gional radon surveys in dwellings, public buildings with high occupancy factors, and workplaces, assessment of health risks due to radon , establishment of reference levels for indoor radon concentration and standards for the activity concentration of natural radionuclides in building materials [1–3].

The strategy of communication with stakeholders, including the population, is supposed to be an integral part of national radon programs. The problem of low public awareness of radon and the health risk it poses is still typical for many countries [9–16], including the Russian Federation. This leads to the fact that there is no market for radon protection and remediation of buildings in our country yet [17].

Conducting any information campaigns on radiation safety issues requires preliminary social research due to the complexity of these issues, the presence of persistent myths about radiation and the possibility of increasing radiophobia among the population [18].

It should be noted that some critics claim that public opinion polls put a person in a model situation and are aimed at finding out something that a particular individual may not have, namely, opinion on a specific issue [19, 20]. It turns out that polls in that way do not reflect public opinion, but rather form it themselves [21]. This paradox can also manifest itself in the case of such a little-known issue for the population as radon because it is relevant for the radiation safety experts and, to some extent, public health care authorities, but not for the general population, since it rarely appears in the focus of the media. In case there is low public awareness of some issue, it is reasonable to use the survey methodology with the enrichment of public opinion, i.e. providing information on the issue with subsequent investigation of the reaction to it.

This methodology was chosen for the international radon cross-cultural multilingual public opinion survey “STEAM project”, coordinated by the Institute of Public Health (Bucharest, Romania). The survey was initiated within the framework of the IAEA Technical Cooperation Project RER9153 “Enhancing the Regional Capacity to Control Long Term Risks to the Public due to Radon in Dwellings and Workplaces”. St. Petersburg Research Institute of Radiation Hygiene after Professor P.V. Ramzaev conducted this survey in the Russian Federation.

It should be noted that any change of a person’s protective behavior related to health, from complete ignorance that protective behavior is possible to specific active actions (ordering measurements of indoor radon concentration, remediation in houses after initial measurements if necessary), goes through several stages:

1) Ignorance of protective behavior;

2) Awareness, but not engagement;

3) Engagement and decision-making on specific actions;

4) Making a decision not to take protective actions (termination of the ascending sequence to protective behavior);

5) Making a decision to take specific actions, but not taking them in fact;

6) Action (one-time, periodic);

7) Consolidation of a new protective practice in the field of health [22, 23].

At different stages, various risk communication strategies are effective, stimulating the population to move to the next stage. The present survey was focused on respondents who were in the first stages, with a cross-sectional type of research being best suited for them, as opposed to a longitudinal one used in case of transition from the stage of ignorance to the stage of decision-making on specific actions, when one group of respondents is interviewed several times over a certain period of time.

The purpose and objectives of the survey were set in accordance with the type of survey and the supposed low public awareness of radon and its health impact.

The purpose was to study the attitudes of Russians towards their health and radon as a risk factor for health.

Objectives:

– study the attitude of the population towards their health;

– measure the level of public awareness of radon;

– inform respondents of radon;

– determine a degree to which information on radon was assimilated by respondents and identify possible failures in risk communication;

– assess respondents' readiness to measure indoor radon concentration in their homes and take protective actions if necessary;

– identify the sources of information on health risk factors, including radon, that are most used by respondents and deserve their trust.

Materials and methods. An online survey was conducted with 1,500 respondents in the fall of 2020. The questionnaire was preliminarily translated from English into Russian and adapted. In addition, we carried out a pilot study to refine the wording of the questions. The survey was representative for the population of the country as per sex, age, and a region of residence. Random sampling error didn't exceed 2.5 % for 95 % confidence interval. Participation in the survey was voluntary. Quotas for the survey were calculated on the basis of the Federal State Statistics Service data[1]. At the beginning, a link to the online questionnaire was distributed in popular social media in Russia (Vkontakte, Odnoklassniki, Facebook), and then respondents were additionally selected with the help of service Anketolog.ru, which was the platform for the online survey.

Microsoft Excel was used to accumulate, correct, and systematize initial results and to visualize the results of the analysis. IBM SPSS was used to analyze initial results of the survey with parametric and nonparametric statistical methods. Nominal variables were described with absolute values and percentages.

The nominal data were compared using Pearson's χ^2 test to assess the significance of differences between the actual number of outcomes or qualitative characteristics of the sample falling into each category and the theoretical number that could be expected in the groups under study if the null hypothesis was true.

First, we calculated the expected number of observations in each cell of the contingency table under the condition that the null hypothesis of no relationship was true. For this purpose we multiplied the sums of series and columns (marginal totals) and then divided the obtained product by the total number of observations. Then we calculated the value of the χ^2 test according to the formula:

$$\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}},$$

where i is the number of row (from 1 to r), j is the number of column (from 1 to c), O_{ij} is the actual number of observations in cell ij, E_{ij} is the expected number of observations in cell ij.

The value of the χ^2 test was compared with the critical values for the $(r\text{-}1)\cdot(c\text{-}1)$ number of degrees of freedom. If the obtained value of χ^2 exceeded the critical value, it was concluded that there was a statistical relationship between the risk factor under study and the outcome at an appropriate level of significance.

All respondents were given the following key information on radon that was as similar as possible in questionnaires published in languages spoken in all 22 countries that took part in the STEAM project; it was done in order to provide an opportunity to make further cross-country comparison of the survey results.

«Radon is a naturally occurring radioactive gas that can be released from soil, rocks and building materials. Radon is colorless, tasteless and odorless. You can find out whether it is present in the air or not only with the help of special devices. Outdoors the concentration of radon is very low because it is rapidly diluted with atmospheric air. However, in closed rooms it can accumulate.

Radon enters the human lungs with inhaled air. There its radioactive radiation can damage cells, which further leads to the increased probability of developing lung cancer. Radon is the second leading cause of lung

[1] Federal State Statistics Service. Available at: https://rosstat.gov.ru (20.08.2020).

cancer after smoking. Moreover, the probability of developing lung cancer as a result of exposure to radon in smokers is 25 times higher than in non-smokers. In the Russian Federation, up to 3,000 people die every year from lung cancer caused by radon.

In the Russian Federation, a limit for indoor radon concentration is established and protective measures must be taken if this limit is exceeded. Specialized accredited laboratories in every region can measure radon concentration in your house or apartment and determine whether the limit is exceeded or not. If necessary, specialized project organization will be able to design protective and mitigation measures».

Results and discussion. Most of the respondents (82.4 %) assessed their health as "satisfactory" and "good". On average, respondents tended to evaluate their health better than the health of other Russian citizens (Table 1). The comparison of these questions made it possible to split the dataset into three parts: those who assessed their health worse than that of others (5.6 %), those who assessed their health better than that of others (50.8 %), and those who assessed their health similarly to others (36.3 %). Respondents over 55 years old prevailed among the first group and young people prevailed among the third group.

The majority of the respondents (70.8 %) claimed to be aware of health risk factors and only 16.1 % noted that they were not aware of them. The distributions of answers to the questions about awareness of risk factors for personal health and for the health of the Russian population were almost equal (Table 2).

According to the results of categorization of respondents' answers to the question "*What risk factors, in your opinion, can most strongly affect your health*?" with the freedom to choose the answer (without predefined options), the following groups of risk factors were identified: environmental, i.e. related to the state of the environment (air pollution,

Table 1

Respondents' self-assessment of their health and assessment of the health of other Russians, %

Answer options	Questions	
	You would say that your health in general is	You would say that the health of Russians in general is
Poor	4.4	20.8
Satisfactory	45.3	60.4
Good	37.1	9.6
Very good	6.9	0.4
Excellent	4.5	0.3
Don't know	1.0	7.5
I would prefer not to answer	0.8	1.0

Table 2

Respondents' assessment of their awareness of risk factors for themselves and for residents of Russia as a whole, %

Answer options	Questions	
	Regarding the risks factors for your own health, you would say you are	Regarding the risk factors for the health of Russians in general, you would say you are
Not at all informed	1.9	3.3
Not informed	14.2	16.7
Informed	48.0	51.1
Well informed	16.9	11.3
Very well informed	5.9	3.8
I don't know	12.6	13.1
I would prefer not to answer	0.6	0.6

Table 3

Respondents' agreement with statements related to their health control, %

Answer options	Statements			
	I protect myself from any risk factors that could affect my health	I have control over all risk factors for my health	The indoor air quality from my home is very important for my health	Decisions about health risks should be left to experts
Strongly disagree	2.5	3.4	0.4	4.7
Disagree	16.9	27.6	1.5	22.4
Neither agree nor disagree	47.1	43.8	5.7	25.0
Agree	25.6	19.3	62.7	34.8
Strongly agree	4.4	2.7	27.6	7.8
I don't know	2.9	2.4	1.4	4.2
I would prefer not to answer	0.6	0.7	0.7	0.9

Table 4

Respondents' awareness of radon in general and radon as a health risk factor, %

Answer options	Questions	
	How much would you say you know about radon?	How much do you say you know about the health risk due to radon exposure?
Nothing	38.5	43.5
Only a little	29.4	26.7
Something	23.2	20.1
Quite a bit	6.0	6.5
A lot	2.5	2.4
I would prefer not to answer	0.4	0.7

climate change, etc.), bad habits (alcohol, smoking, overeating or improper diet, drugs), various diseases (cardiovascular, including hypertension, heart attacks, diabetes, visual disturbances, etc.), lifestyle (work, sleep disturbances, sedentary lifestyle), stress, COVID-19, poor-quality food and drinking water.

The following population groups can be distinguished based on the respondents' assessment of the control over health risk factors (Table 3):

– People who take their health seriously and agree with the authority of experts – 7.0 %;

– People who take their health seriously and disagree with the authority of experts – 8.3 %;

– The rest of people who agree with the authority of experts – 35.6 %;

– The rest of people who disagree with the authority of experts – 49.1 %.

The category "people who take their health seriously" (15.3 %) was calculated as the sum of those who "agree" or "strongly agree" with the first three statements in Table 3.

The majority of respondents (90.3 %) claimed that air quality was very important to them, 30.0 % claimed that they protected themselves from any risk factors that might affect their health, 22.1 % believed that they controlled all risk factors for their health.

Among those who assessed their health as "good", "very good", or "excellent" (Table 1), the proportion of those who claimed to protect themselves from any health risk factors was higher (38.7 % vs. 21.8 %), and they were more confident that they controlled all risk factors for their health (29.5 % vs. 15.1 %). In general, self-assessment of health is positively associated with a serious attitude towards one's health.

The level of knowledge about radon in general turned out to be quite low. Only 23.2 % of respondents heard "something" about it, and another 8.5 % said they knew "quite a bit" or "a lot" about it (Table 4). The level of knowledge about radon as a health risk was at a comparable level, but two points need

to be noted. Firstly, incompetent individuals in any field tend to overestimate the level of their knowledge (Dunning – Kruger effect) [24]. Secondly, during the online survey, the respondents had the opportunity to get some information on radon before filling out the questionnaire, which could also affect the distribution of answers to the question. Thus, we can reasonably assume that the real awareness is even lower than that presented in the Table 4.

The proportion of those who assessed their knowledge of radon as confident was higher among people whose professional activity was related to ionizing radiation (15.0 % of respondents answered "quite a lot" and another 15.0 % answered "a lot"). However, awareness of radon remains very low even among this group.

No statistically significant differences in awareness of radon were found between those who were seriously concerned about their health (see Table 3) and those who were not. The relationship between the level of awareness and how respondents assessed their health was also not found. Based on this, we can conclude that information on radon does not reach the audience. It is not available even to people who take serious care of their health.

The first two groups of questions were followed by a part with some key information on radon (see Materials and Methods section) so that the respondents could broaden their knowledge. The next series of statements were offered to assess the perception of the given information (Table 5).

A quarter of respondents (24.1 %) agreed that measuring radon concentration in their house was a "priority". 13.1 % of respondents had the impression that measuring would be a simple task. A fifth of respondents (19.3 %) believed that they would have the financial means to take measures to reduce the radon level in their house. Two thirds (68.8 %) claimed that the decision to take actions to reduce the radon level in the house was not entirely up to them.

Despite the fact that the majority of respondents (84.2 %) claimed that their homes were always well ventilated, only a third of respondents (33.4 %) noted that there was no use to measure radon concentration for this reason (Table 6). Hence, we can conclude that ventilation of rooms is perceived by the majority of respondents as insufficient protective measure and the information given to respondents earlier caused them concern. It can be also noted that there were slightly more respondents who cared about ventilation among those who assessed their health positively (86.5 % vs. 81.7 %, calculated as the sum of "agree" and "absolutely agree" answers).

Table 5

Respondents' agreement with statements about reasons preventing them from taking protective measures, %

Answer options	Statements				
	Testing for radon is easy	Testing the house I live in for radon is a priority	There is no use to test the home I live in for radon. I always maintain good ventilation	I could not afford to take measures to reduce the radon level in the house I live, in five years time	The decision to take actions to reduce the radon level in the house I live is not entirely up to me
Strongly disagree	8.3	7.3	3.3	3.5	2.5
Disagree	24.7	22.6	19.1	15.8	11.6
Neither agree nor disagree	24.7	27.4	31.3	22.4	10.1
Agree	11.0	21.4	29.2	27.7	54.7
Strongly agree	2.1	2.7	4.2	7.5	14.1
I don't know	28.3	16.5	12.1	21.6	5.7
I would prefer not to answer	0.9	2.2	0.9	1.5	1.2

Table 6

Respondents' agreement with the statements about the need to measure radon concentration in a house with good ventilation, %

Answer options	Statements	
	There is no use to test the home I live in for radon. I always maintain good ventilation	Regardless of the weather, I make sure that my home is well ventilated
Strongly disagree	3.3	0.7
Disagree	19.1	3.3
Neither agree nor disagree	31.3	10.5
Agree	29.2	57.3
Strongly agree	4.2	26.9
I don't know	12.1	0.7
I would prefer not to answer	0.9	0.6

Table 7

Respondents' potential willingness to take actions to reduce radon levels in their homes, %

Answer options	Statements	
	I would take immediate actions to reduce the radon levels in the house I live	It would be a priority over other risks to take actions to reduce the radon level in the house I live
Strongly disagree	0.9	1.7
Disagree	1.7	5.8
Neither agree nor disagree	9.3	22.3
Agree	58.4	50.9
Strongly agree	24.8	11.9
I don't know	4.1	6.5
I would prefer not to answer	0.8	0.9

To determine the proportion of respondents who were convinced that radon was dangerous and the need to take protective measures in the absence of the above obstacles (Table 5), some questions about the potential readiness to take measures to reduce the radon levels in homes were asked (Table 7).

83.2 % of respondents claimed that they would take immediate actions, and it would be a priority over other risks to take such actions for 62.8 % of respondents. When analyzing these distributions, it is necessary to keep in mind the "illusion of focus" and the principle of WYSIATI ("What You See Is All There Is") which says that the issue being discussed or more relevant issue at a particular moment becomes more important in comparison with others [25].

Since the survey focuses specifically on radon, some respondents are more likely to assess the radon issue as significant and requiring action. However, we cannot be sure that in the future respondents will use their limited resources specifically to measure and / or reduce radon levels in their homes if other issues emerge.

A person answering questions about his or her life does not go into a detailed analysis and is prone to momentary mood. However, it can be noted at least that information on radon received from Rospotrebnadzor (Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing) and its scientific research institute forms attitudes towards the perception of radon as a risk factor for the majority of respondents requiring actions to mitigate its impact on health.

Among those who take their health seriously (Table 3), the proportion of those who are willing to take immediate actions to reduce radon level in their homes is higher (89.1 % vs. 81.8 % among the rest). This category also includes a higher proportion of those who say that taking actions to reduce radon levels in their homes would be a priority (74.8 % vs. 60.4 % among the rest).

Another significant factor influencing the willingness to take measures and give them higher priority is the trust in experts. For example, among those willing to delegate decisions on their health to experts, 87.0 % would immediately take radon protective measures (vs. 80.1 % among the rest) and for 67.8 % they would be a priority (vs. 58.7 % among the rest).

The statements presented in Table 8 also test the comprehension and persuasiveness of information on radon which was given earlier. After reading the box with some key information on radon, 83.0 % of respondents agreed with the statement that radon was a radioactive gas. To compare significance of risk of indoor exposure to radon compared to other risks turned out to be a difficult task for the respondents, thus most estimates were in the middle of the scale: "disagree" (25.8 %), "neither agree nor disagree" (26.1 %), "agree" (19.0 %). The reason for this lies in the fact that respondents were not provided with information on other risks due to the limitations, goals and objectives of the survey. Meanwhile, it is known that without reference points for comparison, it is difficult for a person to make a judgment [26]. This fact should be taken into account while communicating radon risk. Some experts emphasize that the population needs both practical guides to action and theoretical information [23, 27].

The question about radon as the cause of diseases revealed the potential for the emergence of myths about radon in the case of unwary and unprepared risk communication. 79.3 % of the respondents correctly claimed that radon could be the cause of lung cancer (Table 9). At the same time, despite the information provided, some respondents indicated that radon could also cause other diseases (50.6 % for asthma, 38.3 % for allergies, 35.2 % for skin diseases, and 48.4 % for other respiratory diseases).

Table 8

Respondents' agreement with statements testing the comprehension of information on radon, %

Answer options	Statements	
	Radon is a radioactive gas	Compared to other risks, exposure to indoor radon does not pose a significant risk for my health
Strongly disagree	1.1	7.9
Disagree	2.6	25.8
Neither agree nor disagree	6.3	26.1
Agree	59.6	19.0
Strongly agree	23.4	3.3
I don't know	6.3	17.1
I would prefer not to answer	0.8	0.8

Table 9

Respondents' agreement with the statement "Radon exposure in high concentrations increases the risk of developing the following diseases", %

Disease	Answer options			
	Strongly disagree or disagree	Neither agree nor disagree	Strongly agree or agree	I don't know or I would prefer not to answer
Asthma	8.2	18.7	50.6	22.6
Allergies	13.0	24.9	38.3	23.7
Lung cancer	2.3	7.0	79.3	11.4
Skin diseases	11.7	27.9	35.2	25.0
Other types of cancers	4.1	19.7	52.7	23.5
Other respiratory conditions	7.4	20.4	48.4	23.8

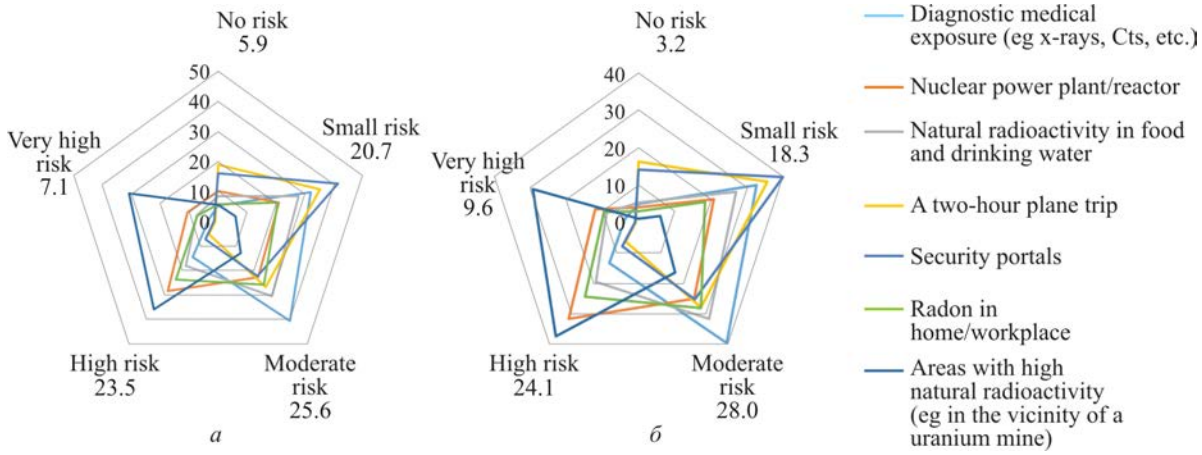

Figure 1. Comparison of respondents' perception of radon as a risk with other radiation risks: a) for themselves personally, and b) for Russians in general, %[2]

The published results of risk perception studies revealed a difference in the perception of personal and societal risks [28]. For example, the results of studies in the USA among those in whose homes radon measurements have already been carried out suggest that people tend to underestimate, rather than overestimate, the issue of radon in relation to their home [27].

No differences between the perception of radon at the personal and societal levels were found in our survey. This may be due to insufficient information provided to the respondents; this issue requires further research.

Figure 1 presents comparative data on the perception of radon among other radiation risks at the personal and societal levels. Given the "illusion of focus", the issue of radon is perceived by respondents after receiving key information as the third most dangerous among other radiation risks, for example, after areas with high natural radioactivity and nuclear power plants. It should be noted that for a half of the respondents (52.2 % in personal risk, 49.5 % in societal risk) the risk from radon is "moderate", "low" or "absent", and only for one-third of the respondents (30.6 % in personal risk, 33.7 % in societal risk) it is "high" or "very high".

Figures 2 and 3 show a comparison of respondents' use of various sources of information and trust in them. The most popular sources of information are the Internet, social media, and television (Figure 2). Medical specialists turned out to be the most trustworthy source of information on health risks, first of all, family doctors and physicians in polyclinics; people also trusted medical prevention centers, Rospotrebnadzor, regional and local public health care authorities (Figure 3).

Conclusions. According to the Requirement 50 of the IAEA General Safety Requirements Part 3, "The government shall provide information on levels of radon indoors and the associated health risks and, if appropriate, shall establish and implement an action plan for controlling public exposure due to radon indoors". At the same time, the strategy of communication with stakeholders, including the population, is considered as an integral part of the action plan (national radon program) [1]. Despite the fact that since the end of the Federal Target Program "Ensuring Nuclear and Radiation Safety for 2008 and for the period up to 2015", no radon-related activities have been carried out at the federal level in the Russian Federation, a possibility of establishing a national radon program in the future cannot be ruled out. In the Decree of the President of the Russian Federation No. 585 dated October 13, 2018 "On approval of the Fundamentals of State Policy in the field of ensuring

[2] The answer options "Do not know" and "I prefer not to answer" are not presented, so the sum does not reach 100 %

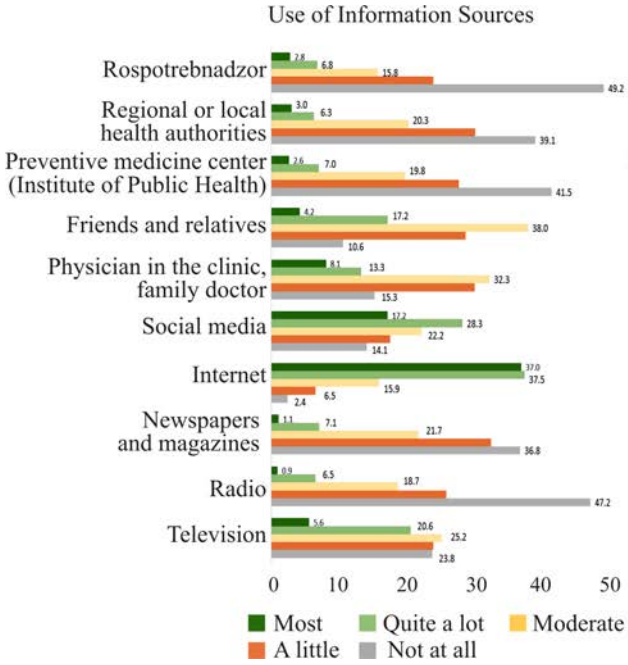

Figure 2. Respondents' use of various sources of information

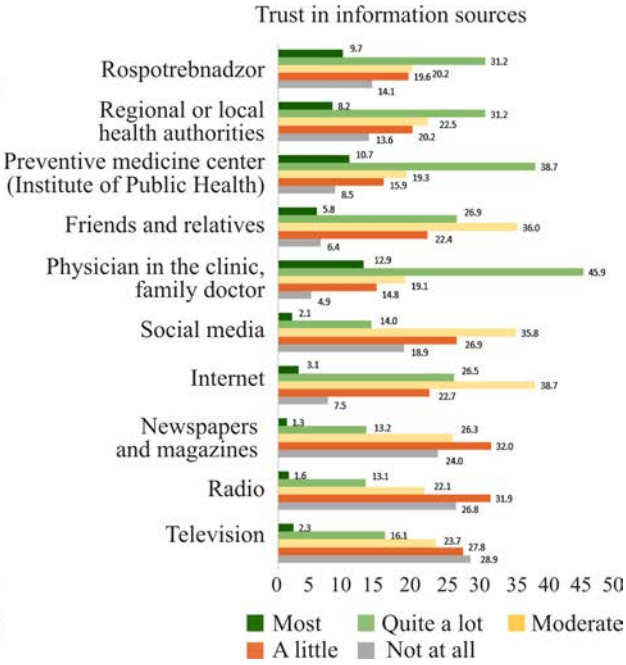

Figure 3. Respondents' trust in various sources of information

nuclear and radiation safety of the Russian Federation for the period up to 2025 and beyond", one of the main areas of work is designated as "improvement of state control (supervision) over the impact on human health of natural sources of ionizing radiation, including radon and its progeny, in dwellings, children's institutions, public and industrial buildings". In this regard, the results of the present survey, which was the first social survey focused on the problem of radon and conducted nationwide, can be used as a basis for planning communication strategies within both national and regional radon programs.

Since a very low level of knowledge about radon was revealed among the respondents, it becomes obvious that there is an urgent need to raise public awareness of this problem through a planned information campaign, especially for the population in radon-prone areas. The purpose of such a campaign may be, among other things, to refute existing myths about radon and prevent the appearance of new ones. It is extremely important to change the perception of the risk factor itself so that radon turns from a "natural radioactive gas" into a "harmful substance in the indoor air" for the population. This may prevent the appearance of an attitude that a person is unable to control and influence this risk factor, so it is useless to take any protective action. As part of the information campaign, the population should be informed of the diseases that radon can cause and cannot cause. It's very important to inform people that in everyday life radon is a much more potentially dangerous source of exposure than other sources of ionizing radiation, but at the same time it is possible to take protective actions against it or to find out after taking measurements that this risk factor is irrelevant for a particular dwelling. It should be emphasized that measuring the indoor radon concentration is the only reliable way to identify the presence or absence of this risk factor, but at the same time, measurements are not a significantly financial and time-consuming procedure.

The results of health risk assessment such as the annual number of radon-induced lung cancer deaths that could be prevented if the indoor radon concentrations in many dwellings became much lower [5, 29], may be used as an additional argument in favor of radon protective and mitigation actions. Respondents in the survey noted that the main obstacle to the implementation of protective measures is the fact

that the decision to take actions to reduce the radon level in the house is not entirely up to them. In this situation, risk communications can involve not only the residents of apartment buildings, but also the management companies or homeowners' associations in order to convince them and the residents of the houses they manage of the need for measurements and, if necessary, radon protective or mitigation actions. In this case, we can suggest using such means of risk communications as development of brochures, manuals and other guidance documents, informational and training videos, publications and interviews of experts in local media, including social media.

The results of the survey show that the population (note that the sample consisted of Internet users) prefers to get information on health risk factors from the Web, while representatives of the public healthcare authorities and supervisory agencies appear to be the most trustworthy. For this reason, it seems appropriate to use the interest of Russians in general and journalists in particular in health issues and the increased credibility of Rospotrebnadzor during the COVID-19 pandemic to conduct information campaigns to increase the public perception of such a risk factor as radon. Moreover, it seems logical to integrate radon issues into other health-related campaigns, for example, aimed at protecting human health from the effects of secondhand smoke and direct tobacco smoking or at reducing the mortality from malignant neoplasms through, among other things, early and mass diagnostics. In this case, we can suggest using the accounts of Rospotrebnadzor and health care authorities in various social media, where information is most often presented not only in text form, but also in graphic or audiovisual, which is much easier to perceive, as well as organizing interviews of experts on television, radio and in online media. It should be noted that companies on the market of services related to radon measurements, as well as the development and implementation of radon protective and mitigation measures, should in this case be ready for a potential increase in the number of applications from interested citizens and organizations.

This highlights the main feature of the development and implementation of radon programs: they can be successfully implemented only in case there is close and coordinated interaction between a large number of stakeholders. Their work should be based on the desire to improve the quality of life of citizens of the country, preserve their health and increase life expectancy.

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Review article

PREVENTIVE MEASURES TO REDUCE HARMFUL EFFECTS PRODUCED BY ELECTROMAGNETIC RADIATION ON HEALTH

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Man-made electromagnetic waves are the most widely and rapidly expanding exposure in today's world, including exposure in several frequency groups: extremely low frequencies (ELF) from electricity lines, hybrid car batteries and high power lines (>3 Hz–3 kHz), radiofrequency (RF) and microwave frequencies including millimeter waves (3 kHz–300 GHz) from mobile phones, towers, base stations and wireless devices, and intermediate frequencies "Dirty Electricity" emitted from power lines.

While such organizations as ICNIRP (the International Commission on Non-Ionizing Radiation Protection) still continue to claim that electromagnetic radiation can cause "only thermal effects", clinging to theory that does not match facts and upholding obsolete thermal safety standards, extensive scientific evidence has clearly demonstrated that non-thermal health effects produced by electromagnetic radiation do exist, are important to health, and should be taken into consideration when safety standards are set. This review aims to highlight some evidence of biologic effects in various body systems, and to suggest preventive measures to reduce such effects on health.

Exposure to electromagnetic radiation at intensities lower than thermal safety standards has been associated with non-thermal biological effects including damage and changes to cells and DNA.

This review presents evidence of such effects demonstrated in: the hematologic system, the nervous system, the immune system, the reproductive system, the skin and muscles, the cardiovascular system, glucose metabolism, and electrohypersensitivity ("Microwave sickness"). Protective measures are then suggested to reduce these effects.

Key words: *electromagnetic radiation, non-thermal exposure, health, electrohypersensitivity, protective measures, safety standards.*

Exposure to electromagnetic radiation at intensities lower than thermal safety standards has been associated with non-thermal biological effects [1–3] including damage to cells and DNA and changes in them. This review focuses on providing evidence of such effects demonstrated in the hematologic, nervous, immune and reproductive systems, skin and muscles, cardiovascular system, glucose metabolism, and electrohypersensitivity ("microwave sickness"). Protective measures are then suggested to reduce these effects.

DNA effects. Lai and Singh [4] demonstrated an increase in DNA single and double-strand breaks in brain cells of rats after two hours of exposure to 60 Hz ELF, and effect that could be blocked with melatonin and N-tert-butyl-a-phenylnitrone (PBN). Udroiu et al. [5] found significant increases in micronuclei in liver and peripheral blood samples from newborn mice that had been exposed in-utero to ELF, 50 Hz, 650 mT magnetic field. Zothansiama et al. [6] found significantly ($p < 0.0001$) higher frequency of micronuclei in the peripheral blood lymphocytes of people living within 80 meters from mobile base stations, compared to those living 300 meters away from the RF radiation source.

Hematologic system. Workers occupationally exposed to microwave radiation had hematological changes in peripheral blood in correlation with time of exposure [7]. Signifi-

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cant changes were seen in concentration and/or activity of glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) and rise in lipid peroxidation (LOO) in peripheral blood lymphocytes of people living near cell-phone base stations [6]. Lai [8] reviewed and summarized multiple studies that showed changes in levels of free radical activities such as oxygen (ROS) / nitrogen (RNS) species and endogenous antioxidant enzymes following exposure to EMF.

Nervous system. Kim et al. [9] discussed many effects seen in the nervous system, including neuronal cell apoptosis, changes in nerve myelin and in ion channels. Sheppard et al. [10] discussed the calcium-efflux effect in chick brain tissue, in specific frequency and amplitude windows of exposure to EMF. Eberhardt et al. [11] demonstrated effect produced by RF 900 MHz on rat blood brain barrier permeability and neuronal damage. Carruba et al. [12] demonstrated experimentally that low-frequency pulse by mobile phones triggered EEG evoked potentials in human volunteers. Effects produced by ELF on neurodegenerative diseases were shown by Benassi et al. [13] regarding Parkinson's disease, with significantly impaired redox homeostasis and thiol content in SH-SY5Y cells, and an increase in protein carbonylation; and by Bobkova et al. [14] with changes in spatial memory and brain amyloid-β in two animal models of Alzheimer's disease. A review by Terzi et al. [15] summarizes additional neurodegenerative effects. In children, ADHD symptoms were associated with higher use of cell phone [16]. Many health risks associated with exposure to Wi-Fi [17] as well as neuropsychiatric effects produced by EMF exposure are summarized by Pall [18].

Immune system. Szmigielski [19] conducted a literature review and concluded that "short-term exposure to weak RF radiation may temporarily stimulate certain humoral or cellular immune functions, while prolonged irradiation inhibits the same functions". El-Gohary & Said [20] reported effects produced by mobile phone RF EMF on immunoglobulin levels (IgA, IgE, IgM, and IgG); and on total leukocyte, lymphocyte, eosinophil, basophil, neutrophil and monocyte counts. Electrosmog effects on Vitamin-D receptor (VDR) and 1,25-dihydroxyvitamin-D (1,25-D), which are associated with many chronic inflammatory and autoimmune diseases are discussed by Marshall and Heil [21]. Lushnikov et al. [22] showed that under multiple repeated exposures, low-intensity extremely-high-frequency electromagnetic radiation (frequency of 42.0 GHz and energy flux density of 0.15 mW/cm^2 for 20 minutes daily) affected immunogenesis in mice. Belpomme & Irigaray [23] many of the patients had low-grade inflammation and an autoimmune response involving autoantibodies against O-myelin, and in 80 % of the patients with Electrohypersensitivity, several oxidative stress biomarkers were detected in peripheral blood.

Reproduction. Saygin et al. [24] reported pathophysiological changes in the testes of rats exposed to wireless frequencies (2.45 GHz) for 3 hours per day. Schauer & Mohamad Al-Ali [25] demonstrated that men who regularly carried mobile phones in trouser pockets showed higher percentage of pathologic morphology of sperm and lower levels of luteinizing hormone.

Skin. Johansson [26, 27] was one of the first to publish research papers about "screen dermatitis", an effect produced by EMF exposure on the skin reporting an increase in mast cells in facial skin samples of electrohypersensitive persons under long-term exposure. Cardona-Hernández et al. [28] reviewed some of the effects. Short-term skin exposure induces only a transient alteration of epidermal homoeostasis, but it may still alter the protective capacity of the skin [29]. Esen & Esen [30] found lengthened latency of facial & head skin resistance response by approximately 200 ms following cell phone exposure. Belpomme & Irigaray [23] demonstrated cutaneous lesions, mostly on the hands patients, and particularly on the hand which held a mobile phone. Feldman et al. [31] demonstrated that coiled sweat glands acted

similarly to arrays of helical antennas, with a resonating frequency in the THz range that influenced RF-energy absorption of millimeter and submillimeter waves into human skin.

Muscle. Blank [32] discussed changes in biosynthetic patterns in muscle cells exposed to EMF stimuli that were similar to changes caused by other known stresses, such as heat shock. McCarty et al. [33] demonstrated muscle twitches in respond to changes (on/off) in EMF exposure.

Cardiovascular system. Vangelova et al. [34] assessed long-term effects of occupational EMF exposure on the cardiovascular system and found that the radiofrequency EMR exposure was associated with greater risk of hypertension and dyslipidemia. Another occupational study by Wilen et al. [35] compared RF operators with unexposed workers and showed that time integrated exposure parameters were of some importance regarding symptoms such as fatigue, headaches, and warmth sensations in the hands, and that RF operators had lower heart rate and more episodes of Bradycardia.

Rats exposed to high-power microwave RF had lower heart rate than non- exposed (30 mW/cm^2 for 15 min – this is a very high exposure but the point is to demonstrate pathological changes in the myocardium). Exposed rats demonstrated histological and ultrastructural changes in the myocardium, with swollen and irregularly arranged myocytes, and some chromatin condensation with dark staining in nuclei [36].

My colleagues and I demonstrated severe and unique vascular calcification in the aortas of rats exposed to intermediate frequencies of 150–155 kHz in an animal model of chronic kidney disease. We thought that an irradiating device could remove calcium from cardiac valves, but the result was the opposite [37]. We repeated this study with ELF exposure to 50 Hz using cell phone chargers and found the same results but the data were not published. Two long-term RF exposure studies performed at the Ramazzini Institute and by the US Department of Health and Human Services National Toxicology Program (NTP) found clear evidence of an association between long-term low-intensity RF EMF exposure and tumors in the hearts (and brains) of male rats [38, 39].

Glucose & metabolism. Meo & Al Rubeaan [40] compared groups of rats exposed to mobile phone radiation. The rats that were exposed for longer than 15 min/day for a 3 months had higher fasting blood glucose ($p < 0.015$) and serum insulin ($p < 0.01$) compared to unexposed and their insulin resistance was significantly increased ($p < 0.003$) compared to control rats. Ben Salah et al. [41] exposed rats to Wi-Fi (2.4 GHz) and noted an alternation in blood glucose level between hypo- and hyperglycaemia during 21 days that the rats were exposed to Wi-Fi, compared to the unexposed group. The exposed rats had an increase in plasma total proteins, triglycerides, creatinine, ALAT, ASAT and iron levels and decrease in plasma uric acid.

Electrohypersensitivity (EHS), formerly known as "Microwave sickness". Most of the symptoms described by electrohypersensitive patients are a consequence of neural damage and over-sensitized neural responses [42]. Belpomme & Irigaray [43] showed an overlap between Multiple Chemical Sensitivity and Electrohypersensitivity in about 30 % of their large database on over than 2,000 patients. The patients' typical symptoms were: "headache, tinnitus, hyperacusis, dizziness, balance disorder, superficial and/or deep sensibility abnormalities, fibromyalgia, vegetative nerve dysfunction, and reduced cognitive capability, including immediate memory loss, attention–concentration deficiency, and eventually tempospatial confusion. These symptoms were associated with chronic insomnia, fatigue, and depressive tendency, in addition to emotional lability and sometimes irritability".

In an extensive study performed on one electrohypersensitive patient, McCarty et al. [33] demonstrated that within 100 seconds after initiation of EMF exposure ($p < 0.05$) the patient developed somatic responses to the exposure, including neural symptoms: temporal pain, headache; muscular symptoms: muscle twitching; and cardiovascular symptoms: skipped

heartbeats. The symptoms appeared following field transitions (off–on, on–off) rather than induced by the mere presence of the field. The patient was not able to consciously identify when the electromagnetic field was turned on or off.

Mechanistic studies. Many mechanistic studies have described the non-thermal interactions between electromagnetic fields and biological tissues [43]. Blackman et al. [44] presented the concept of multiple power-density windows. Liboff [45] discussed resonance effects. Panagopoulos et al. [46] suggested that an external oscillating field vibrating freed ions on the surface of a cell plasma membrane and irregularly gated electrosensitive channels on the plasma membrane disrupting the cell's electrochemical balance and function. Friedman et al. [47] proposed a mechanism of short-term ERK activation by RF frequencies. Giuliani et al. [48] researched the "Zhadin current", coherent excitations in mesoscopic regions of ions and biomolecules, that gave rise to electric currents and consequent magnetic fields in a cell, and Zhadin [49] analyzed Quantum mechanisms to further explain these effects. Blank and Goodman [50] demonstrated that DNA expressed two structural characteristics of a fractal antenna in an electromagnetic field, electronic conduction and self-symmetry.

Results. Based on the presented evidence presented that health effects from exposure to chronic exposure, low levels of EMF exist, the following Preventive & Protective Measures are adapted from Recommendations published by international scientist groups; medical organizations; environmental protection and self-help activist groups. Many of these groups have called for lowering allowed EMF exposure levels and halting plans to expand exposure with 5G networks [51–63].

An increase in distance and use reduction are important factors to reduce exposure and consequent health risks. Proximity is the most important factor in exposure. Radiation levels fall dramatically with distance from the source.

A. The public realm. Figure 1 presents a comparison between allowed exposure limits to RF radiation (including from cell towers) in various countries. Reduction of exposure from cell phone towers / antennas is technically possible and is carried out in some countries better than in others. Keeping long distance from kindergartens and schools is extremely important, since children are a more susceptible population to any environmental exposure; and distancing antennas from bedrooms, to avoid unnecessary and harmful exposure during sleep.

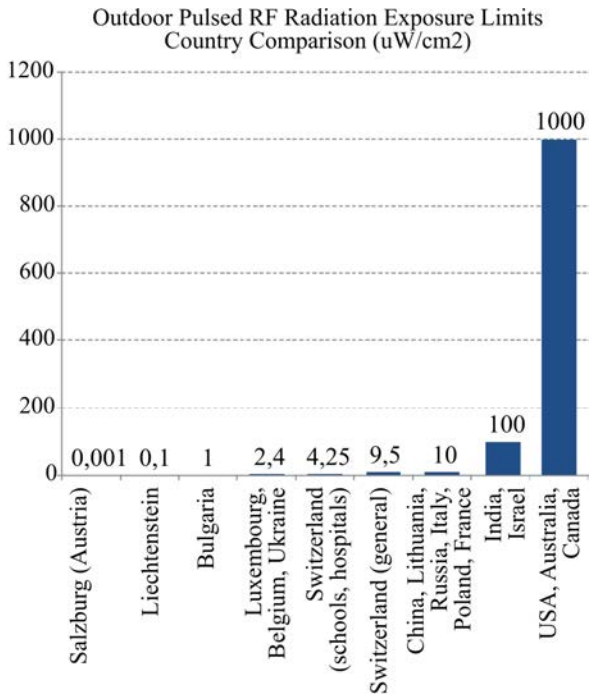

Figure 1. Country comparison. Outdoor Pulsed RF EMF exposure limits, uW/cm^2 [1]

Wireless utility meters and wireless devices in the public realm, while perhaps emitting low mean intensity levels of EMF, emit biologically important pulsed exposure that is more harmful, even at low measured intensities. Furthermore, EMF intensity measurements are often incorrect if performed using

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Figure 2. Ways to reduce Cellphone radiation from wireless devices[1]

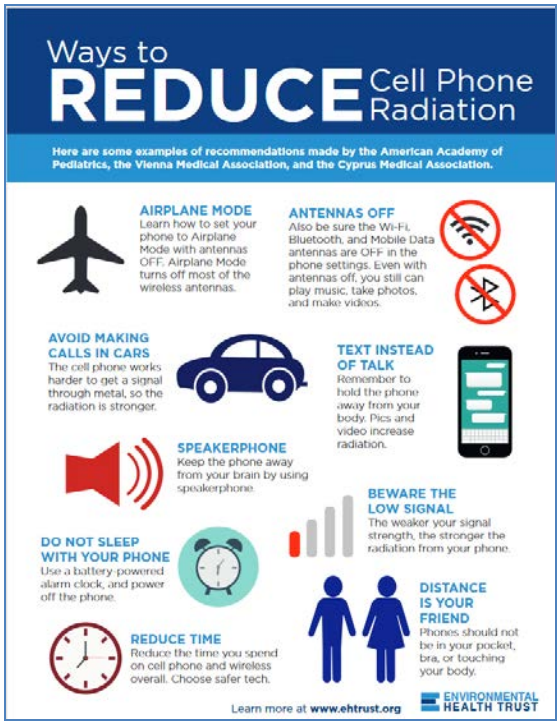

Figure 3. Ways to reduce exposure[1]

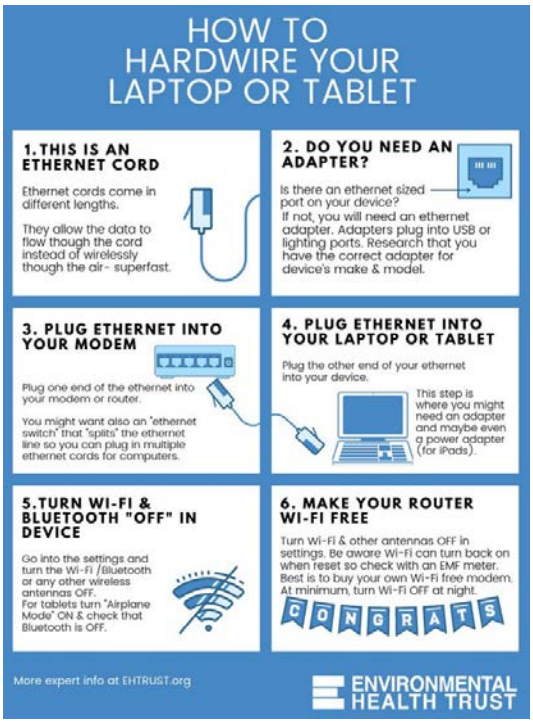

Figure 4. How to hardwire your mobile device[1]

Figure 5. Reduce computer EMF exposure[1]

a measurement device that does not have the adequate measurement speed [64].

Avoid use of Wi-Fi at school – always prefer safer wired technology that does not emit RF EMF.

B. Exposure from personal devices. The most extensive and problematic exposure comes from use of personal devices since they are closer to a body. Devices that emit pulsed waves are especially harmful even if the exposure intensity is not high. Figures 2–5 present exposure reduction strategies developed by the Environmental Health Trust and presented here with permission.

Recommendations on preventing health symptoms from EMF exposure. Distance wireless devices from your body:

- Avoid carrying cell phone on your body at all times. Reproductive organs, especially in pregnancy; the nervous system; and cardiovascular system are sensitive.
- When talking or texting, position the cell phone away from your body as far as possible, use speaker or an "air tube" wired headset (not Bluetooth).
- Avoid placing cordless home phones near you, especially when sleeping.
- Do not place a laptop or tablet device on your lap since these devices emit Radio Frequency radiation (RF EMF) like cell phones; it is especially important during pregnancy.
- Do not place a powered cell phone near you while you sleep.
- Do not charge a cell phone near your body.

Reduce use of emitting wireless devices in your home:

- Prefer use of corded home phones that do not emit RF radiation for most calls.
- Connect the internet router via Ethernet cables and turn off Wi-Fi.
- Use wired keyboard, mouse, etc.
- Turn Wi-Fi off when not in use (router and devices), especially at night.
- Turn off printers' Wi-Fi feature since printers continuously emit very high levels of RFR.

Minimize exposure:

- Minimize time of phone use. Prefer texting to long phone conversations.
- Reduce use of streamed media on the cell phone; whenever possible download the content and then access it on "airplane mode", with Wi-Fi and other modes (Bluetooth etc.) switched off, to reduce your exposure while handling the device.
- Children should only use cell phones for emergencies since cell phone radiation penetrates deeper into their brains.
- Avoid using cell phone inside spaces that are surrounded by metal, when the signal is weak, or when moving at high speed, for example in vehicles or elevators. The cell phone emits higher radiation because in such places it makes a greater effort to connect to the antenna, or to connect to changing distant antennas (when moving).
- Avoid charging laptops while in use. When charging, use a 3-pronged grounded cable [65].

Importance of this review. Health hazards caused by EMF exposure have not been adequately addressed by national and international organizations such as the World Health Organization [66–71]. It is necessary to reduce exposures to all frequencies of EMFs, according to the Precautionary Principle. Cellphone networks should be planned to emit reduced exposures – but global plans are only to expand exposure, on land and in space [70]. A recent study analyzed the expected exposure effects of planned G5 antenna density [72]. Calculating from known power consumption modeling, Ben Ishai [73] suggests that G5 networks would emit a 6-fold increased ambient radiation compared to current networks, and would require a thousand times as much electricity to power the networks.

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REGULARITIES IN DISEASES OF THE MUSCULOSKELETAL SYSTEM DEVELOPING IN SCHOOLCHILDREN UNDER COMPLEX EXPOSURE TO ENVIRONMENTAL FACTORS AND FACTORS RELATED TO LIFESTYLE

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The present research is vital due to diseases of the musculoskeletal system being widely spread and having great social significance as well as a variety of etiological factors that cause this pathology among schoolchildren. Overall, 339 schoolchildren were examined; they attended secondary schools located on territories with different sanitary-hygienic conditions regarding ambient air. Hygienic, clinical-laboratory and instrumental procedures as well as mathematical data processing were applied to fulfill the research tasks. Average daily consumption of food products was estimated as per "Dairies of a weekly schoolchildren's ration"; specific factors related to lifestyle were estimated via questioning performed with a specifically designed authors' questionnaire. Children who attended a secondary school in a city where metallic construction materials were manufactured had manganese concentrations in their blood that were by 8–9 % higher than among their counterparts from the reference group; lead concentrations in their blood were by 1.9–2.2 times higher than in the reference group and by up to 1.7 times higher than background levels. Priority factors that produce such negative effects on health as diseases of the musculoskeletal system include the following: school subjects with the same complexity do not interchange with simpler ones in schedules; breaks between classes do not conform to hygienic regulations; food rations are imbalanced as per milk products and eggs; physical training and doing sports are irregular; homework takes longer period of times than before. Average ionized calcium contents in blood of children from the test group were by 9.0–14.0 % lower and hydrocortisone contents were by 1.3–1.5 times higher than in the reference group (p = 0.0001–0.01). Lower mineral density of bone tissues was by 1.6 times more frequent among children from the test group (p = 0.04). Diseases of the musculoskeletal system were 4.6–15 times more probable and scoliosis was 8–11 times more probable due to persistent exposure to manganese and lead, violated hygienic principles of education process organization and food rations, and low physical activity of schoolchildren. Changes in bone metabolism, activation of a stress-releasing system, and a decrease in bone mass are basic etiopathogenetic regularities in developing chronic pathology of the musculoskeletal system among schoolchildren associated with exposure to specific environmental factors and factors related to lifestyle.

Key words: *diseases of the musculoskeletal system, schoolchildren, environmental factors, education process, nutrition, lifestyle, cause-and-effect regularities.*

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Introduction. Children's and teenagers' health is an integral indicator and a criterion showing socioeconomic welfare of the society and a state in general [1]. An important trend in scientific research at the moment is revealing basic tendencies of health disorders among contemporary schoolchildren that occur due to influencing hygienic, biological, and epidemiological factors. Studies on structure of morbidity and regularities in development of morphofunctional disorders as well as chronic diseases help determine priority trends in prevention and health-preserving activities provided for children [2].

According to latest research data diseases of the musculoskeletal system and connective tissue still hold the leading rank place in the structure of morbidity among schoolchildren [1–4].

According to statistic data, in 2019 the first diagnosed pathogenesis of the musculoskeletal system amounted to 3,107.1 cases per 100,000 children aged 0–14 (in 2000, 2,904.0 cases); 5,603.0 cases per 100,000 teenagers aged 15–17 (in 2000, 3,636.3 cases)[1].

Results provided by clinic research indicate that progressing changes in the musculoskeletal system can exert negative impacts on development and functioning of the respiratory, cardiovascular, digestive, nervous, and reproductive system [4].

At present postural disorders and foot flattening are the most widely spread functional disorders of the musculoskeletal system in children and teenagers; scoliosis and flat feet are prevailing chronic diseases [3].

Bone tissue is a dynamic system where interconnected resorption and osteosynthesis cycles go on continuously. Remodeling is especially intense at a time when a child goes to school and it results in the bone system being highly sensitive to adverse effects produced by medical-biological and social-hygienic environmental factors [5, 6].

According to domestic researchers, a disease is idiopathic in 45–90 % registered cases of musculoskeletal pathology. Dayer R. and colleagues (2013) made an attempt to systematize the existing theories of etiopathogenesis regarding this nosologic category and ranked metabolic disorders in a body among leading causes for the pathology development together with genetic predisposition [7].

Besides, multiple studies established that the most frequent reasons for disorders developing in the musculoskeletal system included low physical activity among children and teenagers and imbalanced food rations influenced by conditions in school as well as hygienic and social ones [4].

Contamination of environmental objects with technogenic metals is the most significant hygienic factor that produces negative effects on bone tissue structure; when such metals penetrate a body in excessive quantities, their long-term cumulation and metabolism lead to disorders in the skeleton mineralization [8].

It is necessary to reveal risk factors that exert negative influence on formation of the musculoskeletal system in schoolchildren and to establish relevant cause-effect regularities since it provides timely diagnostics, prevention, and proper correction of disorders and diseases of the musculoskeletal system.

Our research goal was to establish etiopathogenetic regularities in diseases of the musculoskeletal system in schoolchildren associated with specific exposures to environmental risk factors and factors related to lifestyle.

Data and methods. Overall, 193 schoolchildren attending a secondary school (hereinafter School) were examined; the school was located in a city where there was a production facility manufacturing contraction metal ware. The reference group was made up of 146 schoolchildren who attended a gymnasium (hereinafter Gymnasium) located in an area without any industrial objects. All examined children were divided into several sub-groups as per their grades; the test sub-group 1 and the reference sub-group 1 included 1st grade children (School, 37; Gymnasium, 48); the test sub-group 2 and the reference sub-group 2, 4th grade children (School, 40: Gymnasium, 46); the test sub-group 3 and the

[1] The Russian statistical annual, 2020: Statistic data collection. *Rosstat*. Moscow, 2020, 700 p.

reference sub-group 3, middle-school children (School, 58; Gymnasium, 28); the test sub-group 4 and the reference sub-group 4, senior schoolchildren (School, 58; Gymnasium, 24). The subgroups from the same grades were comparable as per sex and age ($p > 0.05$).

Children to be included into the study didn't have any hereditary diseases of the musculoskeletal system or grave chronic somatic diseases; their parents or legal representatives were asked to give written consent on their participation, and an absence of such consent was a criterion for exclusion.

Air quality inside classrooms as well as ambient air quality on territories where the examined educational establishments were located was hygienically assessed based on data provided by measurements accomplished by experts of the Department for Chemical and Analytical Research Techniques at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies in March–September 2020. There were three points at which samples were taken: the first one was located outside for taking ambient air samples; the second one was located inside classes where junior schoolchildren studied (junior schoolchildren in each class usually spend all their school day in the same classroom); the third one was located in equipped classrooms and other school premises where middle-school and senior schoolchildren had their lessons. 4 air samples were taken at each point during a day; they were then analyzed, and an average daily concentration of an analyzed chemical was calculated. Manganese and lead were detected in air samples with mass spectrometry in accordance with Methodical guidelines MUK 4.1.3481-17 "Measuring mass concentrations of chemicals in ambient air with mass spectrometry with inductively coupled plasma"[2].

Manganese and lead were quantitatively determined in children's blood with mass spectrometry with inductively coupled plasma on *Agilent 7500cx* mass spectrometer ("Agilent Technologies Inc.", USA) in accordance with methodical guidelines MUK 4.1.3230-14[3] and MUK 4.1.3161-14[4].

A comparative assessment was performed to determine whether an education regime corresponded to requirements fixed in sanitary legislation[5]; it was done based on analyzing school schedules including lessons and breaks.

Data taken from "Dairies of a weekly schoolchildren's ration" were obtained by 24-hour recording of a child' food ration; these data were used to compare food rations consumed by primary, middle, and senior schoolchildren in School and Gymnasium with average daily food rations recommended by Sanitary Rules and Standards SanPiN 2.4.5.2409-08 for children attending secondary educational establishments[6].

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[2] MUK 4.1.3481-17. Izmerenie massovykh kontsentratsii khimicheskikh elementov v atmosfernom vozdukhe metodom mass-spektrometrii s induktivno svyazannoi plazmoi [MUK 4.1.3481-17. Measuring mass concentrations of chemicals in ambient air with mass spectrometry with inductively coupled plasma]. Available at: https://files.stroyinf.ru/Index2/1/4293735/4293735234.htm (June 23, 2021) (in Russian).

[3] MUK 4.1.3230-14. Izmerenie massovykh kontsentratsii khimicheskikh elementov v biosredakh (krov', mocha) metodom mass-spektrometrii s induktivno svyazannoi plazmoi [MUK 4.1.3230-14. Measuring mass concentrations of chemicals in biological media (blood and urine) with mass spectrometry with inductively coupled plasma]. *KODEKS: an electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/495856222 (June 23, 2021) (in Russian).

[4] MUK 4.1.3161-14 Izmerenie massovykh kontsentratsii svintsa, kadmiya, mysh'yaka v krovi metodom mass-spektrometrii s induktivno svyazannoi plazmoi [MUK 4.1.3161-14. Measuring mass concentrations of lead, cadmium, and arsenic in blood mass spectrometry with inductively coupled plasma]. *KODEKS: an electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200121438 (June 23, 2021) (in Russian).

[5] SanPiN 2.4.2.2821-10. Sanitarno-epidemiologicheskie trebovaniya k usloviyam i organizatsii obucheniya v obshcheobrazovatel'nykh uchrezhdeniyakh [Sanitary-epidemiological requirements to the conditions and organization of training in secondary educational establishments]. *KODEKS: an electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/902256369 (23.06.2021) (in Russain).

[6] SanPin 2.4.5.2409-08. Sanitarno-epidemiologicheskie trebovaniya k organizatsii pitaniya obuchayushchikhsya v obshcheobrazovatel'nykh uchrezhdeniyakh, uchrezhdeniyakh nachal'nogo i srednego professional'nogo obrazovaniya [SanPiN 2.4.5.2409-08. Sanitary-epidemiological requirements to organizing nutrition for students attending secondary educational establishments and establishments for primary and secondary vocational training]. *KODEKS*: *an electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902113767 (June 23, 2021) (in Russain).

A social survey was conducted among schoolchildren by asking them to fill in distributed questionnaires; it was done to assess certain factors related to their lifestyle (educational loads beyond basic educational programs, children's and teenagers' physical activity).

Clinical examinations were performed in conformity with the basic ethical standards stipulated in Helsinki Declaration (last edited in 2008). The research program was approved by the Ethical Committee of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (the meeting report No. 17 dated February 03, 2020). All the participants were informed about the research goal and techniques; after that they gave their written informed voluntary consent on medical intervention being performed and their personal data being processed.

Spinal column deformities and postural disorders were diagnosed in children and teenagers with computer optical topography (KOMOT) of a human body with "TODP optical no-touch computer topograph for determining spinal column deformities" (Novosibirsk) that provided an opportunity to determine a three-dimensional body shape in the system of absolute coordinates.

Quantitative ultrasound densitometry was applied to detect any disorders of bone strength; it was done with *Sunlight Omnisense 7000* device (Sunlight Medical Ltd., Israel). The examination was performed on the distal radius involving assessment of integral Z-score for speed of ultrasound wave (Speed of Sound or SOS, m/sec). Z-score SOS value ranging from -1 to -2 SD corresponded to the 10 %- percentile. A decrease in bone strength was diagnosed when Z-score SOS value was lower than -1 SD taking into account age and sex in accordance with the reference base stored by a manufacturer in the analyzer software. A fall in speed of sound below 3 %-percentile (Z-score was lower than -2 SD) corresponded to the apparent decrease in bone strength.

A biochemical examination was performed regarding calcium-phosphor metabolism. To get a picture of calcium metabolism, contents of its ionized form were determined with *EasyLyte Calcium* (Medica Corp., USA) blood electrolyte analyzer.

Adaptive hormone hydrocortisone was detected with *BioTek ELx808* (USA) ELISA analyzer.

Morbidity with diseases of the musculoskeletal system was comparatively analyzed based on data taken from "Medical case history of a child attending an educational establishment" (Form No. 026/y-2000) and results of clinical examinations performed by pediatrists and experts on physical therapy.

Data were statistically analyzed with SPSS 10.0 and 16.0 software packages for Windows using correlation and regression analysis. To quantitatively describe correlations between indicators, we calculated odds ratio (*OR*) and its confidence interval (*CI*).

Multiple models that showed "factor – response (nosology)" dependence were used to assess a probability of specific responses caused by exposure to factors exceeding hygienic standards. Parameters of a multiple model that reflected "factor – probable response" were estimated with building up a logistic regression model:

$$p = \frac{1}{1 + e^{-(b_0 + \sum_i b_i x_i)}},$$

where p is a probability that a response will deviate from physiological standard;

x_i is a level of exposure to a factor;

b_0, b_i are parameters of a mathematical model.

Differences in the results were considered statistically significant at $p \le 0.05$.

Results and discussion. Assessment of air quality inside the tested educational establishments revealed that average manganese contents were by 1.6 times higher than the hygienic standard in classrooms for junior schoolchildren in School; manganese and lead contents were by 5.2–8.1 times higher in classrooms School in comparison with Gymnasium ($p < 0.05$) (Table 1).

Contents of these two chemicals were by 1.25–5.25 times higher in air inside equipped classrooms and other school premises for middle-school children and senior

Table 1

Average chemical concentrations inside classrooms

Chemical	RfC, mg/m^3	MPC av.d., mg/m^3	Concentration, mg/m^3		p
			School	Gymnasium	
Classrooms for junior schoolchildren					
Manganese	0.00005	0.001	0.000078 ± 0.000016	0.000015 ± 0.000003	<0.05
Lead	0.0005	0.0003	0.000025 ± 0.000005	0.0000031 ± 0.0000007	<0.05
Equipped classrooms and other school premises (middle and senior school)					
Manganese	0.00005	0.001	0.000042 ± 0.000009	0.000008 ± 0.000002	<0.05
Lead	0.0005	0.0003	0.000007 ± 0.000001	0.0000056 ± 0.0000013	<0.05

Table 2

Average chemical concentrations in ambient air on the territories where School and Gymnasium were located

Chemical	RfC, mg/m^3	MPC av.d., mg/m^3	Concentration, mg/m^3		p
			School	Gymnasium	
Manganese	0.00005	0.001	0.000183 ± 0.000038	0.000039 ± 0.000008	<0.05
Lead	0.0005	0.0003	0.000020 ± 0.000004	0.0000065 ± 0.0000013	<0.05

schoolchildren in School in comparison with Gymnasium ($p < 0.05$).

Lead was detected in ambient air on the territory where School was located (0.000020 ± ± 0.000004 mg/m^3) and its concentrations was by 3.1 times higher than in ambient air on the territory where Gymnasium was located (the reference territory) ($p < 0.05$) (Table 2). Manganese contents in ambient air on the territory where School was located were by 3.7 times higher than the reference value and by 4.7 times higher than on the reference territory ($p < 0.05$).

Chemical and analytical examinations revealed that children from the 1st grade in School had 1.9 times higher lead contents in blood than their counterparts from Gymnasium (0.0213 ± 0.0036 against 0.0111 ± 0.0014 mg/dm^3, $p < 0.05$). A share of blood samples with elevated lead contents was by 5.5 times higher in the test sub-group 1 than the reference sub-group 1 (72.7 against 13.3 %, $p < 0.0001$; $OR = 15.77$; $CI = 5.75–52.14$; $p < 0.0001$).

Lead contents in blood of children from the 4th grade in School were by 2.2 times higher than the same parameter in blood of their counterparts in Gymnasium (0.0245 ± 0.0031 against 0.0113 ± 0.0026 mg/dm^3, $p < 0.05$) and by 1.7 times higher than the regional background level ($p < 0.05$). Lead contents in blood of children from the test sub-group 2 were elevated in 82.7 % cases against 11.1 % cases in the reference sub-group 2 (by 7.4 times higher) ($p < 0.0001$).

Average lead contents in blood of middle-school children in School were also by 1.9 times higher than the same parameter in Gymnasium (0.0226 ± 0.0056 against 0.0118 ± ± 0.0019 mg/dm^3, $p < 0.05$) and by 1.6 times higher than the regional background level ($p < 0.05$).

Children from the test sub-groups 1, 2 and 3 had manganese contents in their blood that were by 8–9 % higher than the same parameters in the reference sub-groups 1, 2, and 3 (0.012 ± 0.001 against 0.011 ± 0.001 mg/dm^3, 0.013 ± 0.002 against 0.012 ± 0.001 mg/dm^3 and 0.012 ± 0.001 against 0.011 ± 0.002 mg/dm^3 accordingly, $p \leq 0.0001–0.01$). A share of blood samples with elevated manganese contents was by 1.1–2.1 times higher in junior and middle classes in School than in Gymnasium (the 1st grades, 27.3 against 24.4 %; middle school, 28.6 against 21.4 %, $p = 0.48–0.76$), difference were statistically significant in 4th grades (33.3 % against 15.6 %, $p = 0.049$).

We established statistically significant relationships between manganese and lead contents in blood and individual doses inhaled with air inside classrooms based on cause-effect relations modeling ($R^2 = 0.13–0.66$; $47.32 \leq F \leq 603.60$; $p \leq 0.00001$) (Table 3).

Table 3

Model parameters for relationships "A chemical dose inhaled with air inside classrooms – a chemical concentration in blood"

Chemical	b_0	b_1	F	p	R^2
Manganese	0.01079	453.20881	47.3183	0.0000	0.13
Lead	0.01267	10107.92284	603.6043	0.0000	0.66

Research results regarding metal contents in blood of children and teenagers who live and study under aerogenic exposure to chemical environmental factors are in line with data provided by several authors who accomplished their research on territories where industrial and energy-producing enterprises were located [9–11].

Educational regimes in School and Gymnasium were assessed to determine whether they conformed to requirements fixed in sanitary legislation. The assessment revealed that the most frequent violation involved absence of interchanges between difficult and easy subjects during a day and a week (item 10.8)[5]. The principle was violated regarding interchanges between subjects that involved different activities; subjects with different ratio of a static and dynamic component; subjects with prevailing loads on the first or second signaling system; all these violations were established in schedules of schoolchildren from all the grades in School. Schedules drawn up for 5 days out of total 5 study days (100 %) in School had the most difficult subjects as the 1st lesson when schoolchildren were just warming up and/or as the 5th or 6th lesson when they were already tired instead of the 2nd or 4th lesson as it was stipulated by hygienic requirements. Similar violations were established for 4 days out of 6 study days in Gymnasium (66.7 %). Maximum educational loads for middle-school children were determined according to schedules and difficulty of subjects; in School the peak loads were determined on Tuesday (55 scores) and the easiest day was Wednesday (37 scores) instead of Thursday or Friday (item 10.11 violated) and it failed to provide preservation of optimal working capacity during the whole study week. The same item 10.11 and Appendix 3 in SanPiN 2.4.2.2821-10[5] were also violated in schedule of senior schoolchildren in School since Tuesday was the easiest day (31 scores) whereas the peak educational load was detected on Friday (56 scores). When a child has to face intense mental loads at a time his or her working capacity is declining, it leads to depleting energy potential of a body and chronic stress [12].

Emotional stress was proven to influence long-term contraction of neck muscles and shoulder-girdle muscles that resulted in weaker body balance which, in its turn, led to postural disorders [13].

Education process in School involved using *SMART Board* during IT classes for 45 minutes and this didn't conform to hygienic requirements fixed in item 10.8 of SanPiN 2.4.2.2821-10[5] where it was stipulated that use of such teaching aids for children in 3–4th grades and older should be limited to 30 minutes. *SMART Board SBD600 series* was used from 5 to 45 minutes during classes in Gymnasium (median value was equal to 10 minutes).

Besides, item 10.12 was violated in School regarding small breaks (5 minutes instead of stipulated 10) and long ones (15 minutes instead of obligatory 20). It failed to provide the 2nd phase in rest that was responsible for recovered "functional potentials" [14].

Violation of standards regarding breaks in education process leads to lower working capacity, growing fatigue, and stress. When educational process is organized without taking into account physiological principles of changes in schoolchildren's working capacities, it results in a necessity to remain in the same forced working posture for a long time and this posture creates significant loads on the musculoskeletal system [4].

Food rations consumed by schoolchildren in the analyzed educational establishments were compared with average daily rations recommended by the SanPiN 2.4.5.2409-08[6] for children attending secondary educational establishments. This comparison revealed a statistically significant ($p < 0.05$) lower consumption of wheat bread by all analyzed sub-groups (deficiency in School was 61–69 %; in Gymnasium, 66–88 %); rye bread (deficiency in School, 98–99 %; in Gymnasium, 95–100 %); potato (deficiency in School, 60–70 %; in Gymnasium, 62–76 %); fresh vegetables (deficiency in School, 85–88 %; in Gymnasium, 64–89 %); fish (deficiency in School, 84–91 %; in Gymnasium, 71–88 %);

milk (deficiency in School, 65–83 %; in Gymnasium, 64–95 %); sour milk products (deficiency in School, 54–75 %; in Gymnasium, 39–97 %); curds (deficiency in School, 58–74 %; in Gymnasium, 29–97 %); butter (deficiency in School, 79–88 %; in Gymnasium, 55–87 %); eggs (deficiency in School, 35–61 %, in Gymnasium, 35–99 %) (Table 4). Confectionary products were consumed in quantities on average being by 3.1–6.7 times higher than recommended (excess in School was equal to 293–934 %; in Gymnasium, 389–742 %, $p < 0.0001$).

Having compared average daily food rations consumed by schoolchildren who attended the two analyzed establishments, we reveled that children from School consumed vegetables and butter in smaller quantities (by 1.6–2.4 times and 1.4–2.2 times accordingly) than children from Gymnasium due to smaller amounts of these products in food rations consumed by junior and senior schoolchildren ($p = 0.0001$–0.0004). Butter was consumed in quantities that were lower than recommended by the standard, by 4.8–8.1 times lower in School and by 2.2–6.5 times in Gymnasium ($p < 0.0001$). Junior schoolchildren in School consumed curds in quantities that were by 2.4 times lower than recommended ($p < 0.0001$) and by 1.7 times lower than their counterparts in Gymnasium ($p = 0.004$). Schoolchildren consumed 15.8–26.2 g of eggs daily and it was by 1.5–2.5 times lower than recommended ($p < 0.0001$–0.03). Average meat consumption was lower than recommended in School (deficiency was equal to 46–61 %) and it was detected for children from all the analyzed grades ($p < 0.0001$) whereas in Gymnasium deviations from recommended rations (37–49 % deficiency) were statistically significant only in junior and middle school ($p < 0.0001$–0.02). Senior schoolchildren from School consumed poultry in quantities that were by 2.3 times lower than those consumed by their counterparts from Gymnasium (17.5 ± 22.6 against 39.9 ± 22.7 g, $p = 0.01$) and by 3 times lower than recommended ($p < 0.0001$). Actual quantities of macaroni in food rations consumed by junior schoolchildren from School amounted to

Table 4

Average daily food ration for junior, middle, and senior schoolchildren from School and Gymnasium, (g, ml)

Food products	Recommended quantity for children aged 7–10 (g, ml)	Junior school		Recommended quantity for children aged 11 and older (g, ml)	Middle school		Senior school	
		School	Gymnasium		School	Gymnasium	School	Gymnasium
Rye bread	80	1.6 ± 3.1	3.8 ± 9.3	120	1.2 ± 3.2	0.0 ± 0.0	1.6 ± 4.0	0.0 ± 0.0
Wheat bread	150	57.7 ± 28.4	51.3 ± 40.2	200	61.4 ± 53.3	50.0 ± 39.4	63.5 ± 83.7	23.8 ± 35.0
Cereals, beans	45	46.3 ± 21.0	54.0 ± 38.3	50	38.3 ± 26.3	45.4 ± 27.2	43.1 ± 33.5	58.9 ± 23.5
Macaroni	15	32.3 ± 20.4	20.2 ± 15.7	20	20.6 ± 11.6	35.7 ± 24.2	19.8 ± 18.7	12.6 ± 13.4
Potato	188	56.6 ± 41.3	71.4 ± 44.1	188	75.4 ± 53.1	63.1 ± 24.5	68.2 ± 57.3	44.9 ± 37.2
Fresh vegetables, greenery	280	41.6 ± 47.6	99.7 ± 67.9	320	37.1 ± 40.4	34.0 ± 40.3	47.8 ± 57.3	76.8 ± 59.7
Fresh fruits	185	3.0 ± 8.0	155.1 ± 97.7	185	145.9 ± 150.6	49.7 ± 54.9	107.2 ± 91.0	48.9 ± 52.9
Meat	70	37.6 ± 28.7	36.0 ± 22.5	78	39.45 ± 22.0	49.1 ± 27.7	30.2 ± 26.2	57.9 ± 62.9
Poultry	35	33.8 ± 32.4	45.8 ± 33.1	53	8.8 ± 8.6	8.6 ± 8.6	17.5 ± 22.6	39.9 ± 22.7
Fish	58	9.1 ± 10.8	16.8 ± 18.6	77	7.3 ± 10.3	9.1 ± 5.4	9.4 ± 16.6	11.6 ± 13.0
Sausages	14.7	43.6 ± 27.9	22.7 ± 24.0	19.6	33.7 ± 23.8	14.3 ± 11.3	40.1 ± 39.6	19.2 ± 25.0
Milk	300	106.1 ± 76.4	108.0 ± 81.7	300	90.8 ± 110.4	22.9 ± 51.1	51.5 ± 39.9	16.0 ± 23.8
Sour milk products	150	62.2 ± 70.7	91.3 ± 81.5	180	83.2 ± 68.8	5.6 ± 12.5	45.0 ± 55.1	60.0 ± 73.0
Curds	50	21.2 ± 28.1	35.4 ± 28.9	60	16.7 ± 21.9	1.7 ± 3.8	15.6 ± 21.8	12.4 ± 18.2
Cheese	9.8	13.2 ± 14.4	11.7 ± 10.8	11.8	16.7 ± 18.3	10.3 ± 4.0	10.7 ± 13.5	12.5 ± 12.6
Cream	10	2.2 ± 7.1	7.6 ± 8.7	10	3.9 ± 6.0	10.3 ± 16.2	2.5 ± 6.2	8.9 ± 19.6
Butter	30	6.3 ± 5.2	13.6 ± 7.4	35	6.1 ± 12.3	4.6 ± 4.4	4.3 ± 10.8	6.2 ± 5.3
Eggs	40	26.2 ± 33.9	25.9 ± 16.3	40	24.7 ± 23.6	0.34 ± 0.35	15.8 ± 21.5	13.3 ± 22.2
Confectionary products	10	103.4 ± 52.1	84.2 ± 48.3	15	62.0 ± 42.0	100.0 ± 53.4	58.9 ± 49.7	73.4 ± 82.1

32.3 ± 20.4 g a day and it was by 1.6–2.1 times higher than in Gymnasium and quantities recommended by the standard ($p < 0.0001$–0.01).

All examined children from School consumed sausages in excessive quantities, by 1.7–3.0 times higher than recommended, $p < 0.0001$–0.008). Junior and middle-school children from Gymnasium consumed these products in quantities that were by 1.9–2.4 times higher than recommended ($p = 0.003$–0.01).

Children from Gymnasium faced much greater deficiency of wheat bread due to a decrease in actual quantities of this product in rations consumed by senior schoolchildren (23.8 ± 35.0 against 63.5 ± 83.7 g in School, $p = 0.05$); deficiency of milk occurred due to its lower quantities in food rations consumed by middle and senior schoolchildren (22.9 ± 51.1 against 90.8 ± 110.4 ml in School and 16.0 ± ± 23.8 against 51.5 ± 39.9 ml in School accordingly, $p = 0.003$–0.05). We also detected that middle-school children from Gymnasium consumed sour milk products in extremely low quantities (5.6 ± 12.5 against recommended 180.0 ml, $p < 0.0001$) and it was by 14.9 times lower than in School ($p = 0.0001$).

All these data indicate there is an authentic 2.4–3.8-time decrease ($p < 0.0001$) in consumption of curds by schoolchildren although this product is a source of calcium that is the most easily assimilated by a body. Butter and egg yolk are basic sources of vitamin D that plays a significant role in bone calcification; yet, these products were also consumed in quantities by 4.8–8.1 and 1.5–2.5 times lower than physiological needs accordingly ($p < 0.0001$–0.03).

Questioning revealed that most schoolchildren (79.5 % in School and 98.9 % in Gymnasium) had some additional education beyond school studies; 75 % schoolchildren from School and 97.1 % schoolchildren from Gymnasium spent 15–60 minutes on doing additional homework ($p < 0.0001$). An amount of time spent on doing homework related to additional education grew by 2.0–8.0 times for 25 % schoolchildren from School (120 minutes and more) whereas a share of schoolchildren from Gymnasium who spent more than 2 hours a day doing their homework was only 2.9 % ($p < 0.0001$). An increase in amount of time spent on doing homework raises "physiological costs" of education.

Hypodynamia is another behavioral aspect that exerts negative influence on the musculoskeletal system. Physical activity is known to improve blood supply and to activate osteoblast functioning, protein synthesis, calcification, and bone tissue metabolism [15, 16].

60.5 % schoolchildren from Gymnasium were quite committed to doing sports since they did physical exercises or sport from 4 to 7 times a week whereas most schoolchildren from School (76.0 %) attended sports clubs or did physical exercises less than 3 times a week. Only 2.8 % schoolchildren in School had physical activity every day and it was by 4.7 times lower than in Gymnasium (13.2 %). Differences detected in this variable ("How regularly does a child do sport or physical exercises?") were statistically significant (Mann – Whitney test, $p < 0.0001$; Cramer's V correlation coefficient = 0.377, an average correlation, $p < 0.0001$).

We detected statistically significant differences in the variable "How many hours a week does a child spend on average on doing sports or exercises?" between School and Gymnasium (Mann – Whitney test, $p < 0.0001$; Cramer's V correlation coefficient = 0.306, an average correlation, $p = 0.002$). A number of schoolchildren from School who spent 9 hours a week on doing sports or exercises was by 4.6 times lower than in Gymnasium (2.9 % against 13.3 %). 10.1 % schoolchildren from School spent 6–8 hours on doing sport and it was by 2.6 times lower than in Gymnasium (26.7 %). One third of schoolchildren from School did sports for not more than 2 hours a week (31.9 % against 17.8 % in Gymnasium) and more than a half spent on it not more than 3–5 hours a week (55.1 % against 42.2 %) in Gymnasium.

Therefore, physical activities of most school children who participated in the research didn't conform to standards fixed by the World Health Organization that recommends children older than 5 to spend not less than 60 minutes on sport every day and to do power exercises not less than 3 times a week[7].

[7] Adolescent and young adult health. *WHO*, 2021. Available at: https://www.who.int/news-room/fact-sheets/detail/adolescents-health-risks-and-solutions (August 18, 2021).

We assessed topographic parameters that characterized a body posture in the frontal plane to reveal that in School only 2.7 % children in the 1st grade had scoliosis but the number grew by the end of the junior school (4th grade) and in senior school since 42.9 % and 41.7 % children accordingly had this pathology ($p < 0.0001$). There was a 27-time growth in probability of scoliosis among schoolchildren by end of the middle school ($OR = 27.05$; $CI = 3.37–217.22$; $p < 0.0001$), and almost a 26-time growth by the end of the senior school ($OR = 25.78$; $CI = 3.30–201.10$; $p < 0.0001$). In Gymnasium, a probability that scoliosis would occur grew by 8.5 times in senior school ($OR = 8.52$; $CI = 2.74–26.48$; $p < 0.01$). When analyzing postural disorders in the sagittal projection, we revealed that by the end of junior school "sway back" was detected only in children from School in 14.3 % cases (against 0.0 % in Gymnasium, $p = 0.008$).

Quantitative ultrasound examination of bone strength established that a share of schoolchildren from School who had lower bone mineral density (BMD) (Z-score was lower than -1 standard deviation (SD)) was by 1.6 times higher than the same value in Gymnasium (60.0 % against 36.4 %, $p = 0.04$).

A share of schoolchildren in 4th grade and in middle grades in School who had apparent decrease in bone strength (Z-score was lower than -2 SD) amounted to 21.4 % and 15.0 % accordingly and it was by 1.5–1.6 times higher than in Gymnasium (14.3 and 9.1 %) but differences were not statistically significant ($p = 0.4$). We should note that such a disorder requires additional profound examination with X-ray densitometry.

Laboratory tests aimed at examining mineral metabolism established that average contents of ionized calcium in blood of children from the test sub-group No. 1, No. 2, No. 3 and No. 4 were by 9.0–14.0 % lower than in blood of their counterparts from the reference sub-group No. 1, No. 2, No. 3 and No. 4 ($p = 0.0001–0.001$) (Table 5). It is well-known that even a trend towards a decrease in this microelement contents in a body results in calcium being released into blood flow from bones to make up for temporary deficiency [2].

Phosphor contents in blood of 4th grade children and middle-school children were within physiological range both in School and Gymnasium ($p > 0.05$) and there were no statistically significant differences between test and reference sub-groups (1.61 ± 0.07 in the test sub-group No. 2 against 1.6 ± 0.12 mmol/dm^3 in the reference sub-group No. 2, $p = 0.64$ and 1.55 ± 0.12 in the test sub-group No. 3 against 1.58 ± 0.08 mmol/dm^3 in the reference sub-group No. 3, $p = 0.23$). Contents of this microelement were also within physiological ranges in 1st grade children and senior schoolchildren but differences were multidirectional ($1.58 \pm \pm 0.06$ against 1.62 ± 0.07 mmol/dm^3 in the test and reference sub-groups No. 1 accordingly, $p = 0.007$; and 1.41 ± 0.08 against 1.30 ± 0.06 mmol/dm^3 in the test and reference sub-groups No. 4 accordingly, $p < 0.0001$).

Average hydrocortisone contents didn't exceed reference levels in blood of children from School but were higher than the same parameter in their counterparts form reference sub-groups: in 1st grade, by 1.4 times higher; 4th grade, by 1.3 times higher; in middle school, by 1.5 times higher ($p = 0.001–0.01$) (Table 5).

Table 5

Calcium and hydrocortisone contents in blood of children from the 1st grade, 4th grade, middle school and senior school in School and Gymnasium

Grade	Sub-group	Ionized calcium		Hydrocortisone	
		mmol/dm^3	p	nmol/cm^3	p
1st grade	Test sub-group No. 1	1.12 ± 0.02	0.001	295.22 ± 40.59	0.001
	Reference sub-group No. 1	1.26 ± 0.01		207.02 ± 22.24	
4th grade	Test sub-group No. 2	1.07 ± 0.01	0.001	272.45 ± 40.78	0.01
	Reference sub-group No. 2	1.22 ± 0.01		205.95 ± 22.98	
Middle school	Test sub-group No. 3	1.11 ± 0.011	0.001	301.49 ± 34.86	0.01
	Reference sub-group No. 3	1.22 ± 0.02		199.08 ± 21.85	
Senior school	Test sub-group No. 4	1.12 ± 0.01	0.0001	281.22 ± 32.59	0.01
	Reference sub-group No. 4	1.27 ± 0.01		367.74 ± 48.72	

Somatic state was comparatively assessed based on profound medical examinations and data taken from "Medical case history of a child attending an educational establishment" (Form No. 026/y-2000). The assessment revealed that diseases of the musculoskeletal system and connective tissue constantly held the 1st rank place in the structure of morbidity among 4th grade children as well as middle and senior school children. These results are in line with data provided by foreign and domestic researchers [2, 3, 16–21].

In School, prevalence of diseases of the musculoskeletal system and connective tissue was by 1.5 times higher among middle-school children and by 1.6 times higher among senior schoolchildren than among 1st grade children (86.9 against 58.8 %, $p = 0.003$ and 96.4 against 58.8 %, $p = 0.0001$). In Gymnasium the difference was 1.15–1.2 times (100.0 against 83.3 %, $p = 0.02$ and 95.8 against 83.3 %, $p = 0.13$). There was a 4.6-times growth in probability of diseases of the musculoskeletal system and connective tissue for middle-school children against 1st grade children in School ($OR = 4.60$; $CI = 1.61–13.12$; $p = 0.007$); this probability grew by 15 times for senior schoolchildren ($OR = 15.14$; $CI = 3.60–63.60$; $p < 0.001$).

We established an authentic correlation between elevated morbidity with diseases of *the musculoskeletal system and connective tissue* and elevated manganese and lead contents in blood ($0.14 \le R^2 \le 0.58$; $45.61 \le F \le 403.77$; $p \le 0.0001$), shorter small breaks between lessons ($R^2 = 0.84$; $F = 1{,}838.01$; $p \le 0.0001$), insufficient consumption of curds, eggs, and butter ($0.17 \le R^2 \le 0.86$; $25.84 \le F \le 765.14$; $p \le 0.0001$), more time spent on doing homework due to additional education, and less regular sports and physical exercises ($R^2 = 0.46$; $90.56 \le F \le 249.76$; $p \le 0.0001$).

Other deforming dorsopathies or postural disorders were diagnosed by 1.7 times authentically more frequently among senior schoolchildren in School than among their counterparts in Gymnasium (56.4 against 33.4 %, $p = 0.06$).

We established an authentic correlation between elevated morbidity with deforming dorsopathy and improper interchange between easy and difficult subjects during a day/week ($R^2 = 0.44$; $F = 269.96$; $p \le 0.0001$) and elevated manganese and lead contents in blood ($0.32 \le R^2 \le 0.64$; $70.03 \le F \le 555.97$; $p \le 0.0001$) since these two elements compete with calcium in bone tissue and disrupt biosynthesis and mineralization.

Morbidity was also analyzed in age dynamics; the analysis revealed that in School acquired deformities of limbs (flat foot [*pes-planus*] acquired) were by 1.4 times more frequently detected among senior schoolchildren than among 1st grade children (60 against 41.2 %, $p = 0.07$) whereas a share of school children with this pathology was stable in Gymnasium and amounted to 72.9–79.2 %.

There was also an authentic correlation between elevated morbidity with acquired deformities of limbs and elevated lead contents in blood ($R^2 = 0.42$; $F = 177.57$; $p \le 0.0001$), improper interchange between easy and difficult subjects during a day/week, a longer period of using an interactive whiteboard ($0.77 \le R^2 \le 0.86$; $1{,}112.63 \le F \le 2{,}144.36$; $p \le 0.0001$), and time spent on doing homework assigned due to additional training ($R^2 = 0.23$; $F = 24.20$; $p \le 0.0001$).

Osteoporosis without pathological fractures was not diagnosed in 1st grade children in School; still, it was detected in 10 % of 4th grade children ($p = 0.05$) and 18.9 % of middle-school children ($p = 0.01$). In Gymnasium a share of children who had osteoporosis went down from 6.3 % in junior school to 3.6 % in middle school ($p = 0.60$). Therefore, this pathology was diagnosed by 5.2 times more frequently in middle-school children in School against their counterparts in Gymnasium (18.9 against 3.6 %, $p = 0.05$).

Scoliosis, being one of the most widely spread chronic diseases of the musculoskeletal system, was by 5.1 times more frequently detected among 4th grade children in School than among 1st grade ones (15 against 2.9 %, $p = 0.07$). A share of middle-school children who had scoliosis was by 6.5 times higher than among 1st grade children and amounted to 18.9 % (against 2.9 %, $p = 0.03$). A number of senior schoolchildren in School who had scoliosis grew by 8.7 times against 1st grade children (25.5 against 2.9 %, $p = 0.01$). A probability of scoliosis grew by almost 8 times for middle-school children in School against 1st grade

children (OR = 7.67; CI = 0.94–62.99; p = 0.06) and by 11 times for senior schoolchildren (OR = 11.27; CI = 1.41–90.19; $p < 0.001$).

We detected an authentic correlation between morbidity with scoliosis and elevated manganese and lead contents in blood ($0.10 \le R^2 \le 0.62$; $33.92 \le F \le 261.42$; $p \le 0.0001$), improper interchange between easy and difficult subjects during a day/week and distribution of difficult subjects during a day, a longer period of using an interactive whiteboard, shorter small breaks between lessons ($0.19 \le R^2 \le 0.88$; $78.83 \le F \le 2{,}605.12$; $p \le 0.0001$), insufficient consumption of butter (R^2 = 0.23; F = 35.15; $p \le 0.0001$), more time spent on doing homework due to additional education (R^2 = 0.43; F = 43.91; $p \le 0.0001$), less regular sports and physical exercises (R^2 = 0.04; F = 12.90; p = 0.002).

Cause-effect regularities of scoliosis occurrence and relevant laboratory and instrument indicators were established based on analyzing research data, assessing cause-effect relations, and performing step-by-step modeling. These regularities and indicators characterize development of negative effects that occur due to exposure to adverse priority chemical factors in the environment, contemporary education process, eating habits and lifestyle.

Given long-term aerogenic exposure to technogenic chemicals, and adverse factors related to education process and lifestyle, ionized calcium contents in blood is a laboratory indicator that can be used to assess a probability of scoliosis (R^2 = 0.35; F = 42.30; $p \le 0.0001$). These contents are related to effects produced by lead (r = –0.41; $p \le 0.0001$) that competes with calcium in bone tissue thus leading to demineralization.

Correlation analysis revealed a correlation between insufficient consumption of curds, a significant source of calcium in food, and contents of this micronutrient in its ionized form in blood (r = 0.17; p = 0.04). Negative trends in eating habits of contemporary Russian schoolchildren, including insufficient milk and milk products consumption, are confirmed by multiple epidemiological researches accomplished by Rospotrebnadzor experts and experts in public healthcare [3].

There are different opinions by researchers on deviations in children's and teenagers' biochemical parameters and their relations with postural disorders. Our research results are in line with data provided by authors who point out negative calcium balance in case there is small orthopedic pathology [22].

There was a correlation between less regular sports and physical exercises (r = 0.22; p = 0.005) as well as shorter small breaks between lessons (r = –0.57; $p \le 0.0001$) and ionized calcium contents in blood. Our data confirm other researchers' opinions on calcium homeostasis being supported not only by sufficient quantities of calcium, phosphor, and vitamin D, but also proper mineralization of the skeleton that depends on how physically active a person is [15, 23].

Dependence between greater probability of scoliosis and growing hydrocortisone contents in blood (R^2 = 0.18; F = 66.72; $p \le 0.0001$) detected in our research confirms a hypothesis on impacts exerted by chronic stress on changes in bone tissue [6].

Elevated glucocorticoid contents produce negative effects on remodeling of bone tissue and its quality due to a decrease in calcium absorption from the gastrointestinal tract and resorption in kidney tubules [24]. Stress-releasing system activates due to negative factors related to contemporary education process and lifestyle such as shorter small breaks between lessons (r = 0.30; $p \le 0.0001$) and less regular sports and physical exercises (r = –0.13; p = 0.03). Research by P.N. Samikulin and colleagues revealed a role played by muscle work in reducing elevated hydrocortisone contents in young males with different levels of training [25].

Bone strength being lower than -1 SD as per Z-score SOS value is another regularity in scoliosis occurrence associated with negative factors related to lifestyle and eating habits (R^2 = 0.39; F = 56.95; $p \le 0.0001$) such as insufficient consumption of curds and (R^2 = 0.25; F = 35.96; $p \le 0.0001$) and low commitment to doing sports (R^2 = 0.24; F = 88.79; $p \le 0.0001$). A relation between mineral bone density and idiopathic scoliosis was first revealed by F.R. Burner and colleagues in 1982. As per data provided by V.T. Verkhoturova, changes in bone tissues in children and teenagers with scoliosis are caused by disorders in bone tissue micro-architectonics,

osteosynthetic cells being less active, and changes in the skeleton configuration [26].

The multiple model for "factor – probable response" dependence revealed the following contributions by negative into scoliosis occurrence: 25.8 %, factors related to education process; 30.9 %, factors related to lifestyle; 20.3 %, chemical environmental factors; 23.0 %, factors related to eating habits.

Conclusion. Therefore, a probability that diseases of the musculoskeletal system would develop grows by 4.6 times for middle-school children and by 15.0 times for senior school children from the test sub-groups and probability of scoliosis grows by 8.0 and 11.0 times accordingly due to several adverse factors. These factors include persistent exposure to metals resulting in lead concentrations in blood being 0.018–0.028 mg/dm^3 (by 1.9–2.2 times higher than in reference sub-groups and up to 1.6–1.7 times higher than regional background levels) as well as manganese contents being equal to 0.011–0.015 mg/dm^3 (by 8.0–9.0 % higher than in reference sub-groups); educational process not conforming to requirements fixed inn sanitary legislation; imbalanced food rations; low physical activity.

Cause-effect regularities of scoliosis associated with exposure to adverse environmental factors, contemporary education process, eating habits, and lifestyle include changes in cellular metabolism (ionized calcium) caused by effects produced by lead, less regular sports and physical exercises, shorter small breaks between lessons, and insufficient consumption of curds ($-0.57 \le r \le 0.22$; $0.0001 \le p \le 0.04$); activation of stress-releasing system (hydrocortisone) caused by shorter small breaks between lessons and low commitment to doing sports ($-0.13 \le r \le 0.30$; $0.0001 \le p \le 0.03$); osteopenic syndrome (Z-score) caused by less regular sports and physical exercises and insufficient consumption of curds ($-0.25 \le R^2 \le 0.24$; $88.79 \le F \le 35.96$; $p \le 0.0001$).

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Research article

ON ASSESSING RISK FACTORS THAT CAUSE MORTALITY DUE TO MALIGNANT NEOPLASMS AMONG MEN LIVING IN INDUSTRIAL MONOTOWNS

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The paper dwells on examining mortality among men due to malignant neoplasms (MNs) in Russia and in Norilsk and Monchegorsk, two monotowns located in the Arctic zone with the only industry there being nickel production. Nickel is a well-known carcinogen.

Given rather small population numbers in these two towns, the authors calculated mortality due to MNs that was averaged over 8 years (2010–2017) for 5-year age groups and standardized mortality ratios (SMR) for employable and post-employable ages as per this nosology in general and specific MNs localizations as well.

Mortality was comparatively analyzed in two male populations in the following pairs: Monchegorsk and Russia, Norilsk and Russia; the analysis was based on data on climatic peculiarities in the towns, working conditions at industrial enterprises, ecological situation, and socioeconomic features including an existing situation in public healthcare.

Socioeconomic welfare and public healthcare quality were close to average Russian ones in Monchegorsk, but SMR for employable population was higher than on average in the country: due to MNs in general, by 34.7 %; nickel-specific MNs such as MNs of the lip, mouth, and throat, by 2.2 times; MNs in the stomach, by 1.5 times.

In Norilsk working conditions were similar to those in Monchegorsk but the environmental conditions were worse; still, mortality among employable population was lower: due to MNs in general, by 15.4 %; MNs of the lip, mouth, and throat, by 14.0 %; due to MNs in the stomach, by 39.3 %. In comparison with Russia as a whole, mortality due to MNs was also lower at employable age but higher by 21.6 % at post-employable one.

A decrease in MNs-related mortality and carcinogenic effects becoming apparent at older ages were achieved due to organizing up-to-date oncologic aid in Norilsk including high-tech diagnostic, treatment, and rehabilitation procedures as well as due to higher living standards in the town.

Key words: *mortality, malignant neoplasms, nickel, monotowns, risk factors, high-tech medical aid.*

In Russia malignant neoplasms (MNs) occupy either the second or the third rank place in the structure of mortality among adult men falling behind diseases of the circulatory system and in some years external causes of death[1]. The most unfavorable oncologic situation is usually in industrially developed regions where chemical enterprises, ferrous and non-ferrous metallurgy are located and where most people are exposed to technogenic risk factors including carcinogens [1–5].

National cancer control programs issued by the WHO point out that cancer is quite preventable in one third of cases; it can be successfully treated in another one third provided early diagnostics; palliative help results in a significant improvement of life quality for such patients in the remaining one third of cases [6].

Copper and nickel production in Russia is listed among carcinogenic ones[2] and IARC

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[2] GN 1.1.029-98. Perechen' veshchestv, produktov, proizvodstvennykh protsessov, bytovykh i prirodnykh faktorov, kantserogennykh dlya cheloveka [HS 1.1.029-98. A list of substances, products, production processes, household and natural factors that are carcinogenic to humans]. *KODEKS: an electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200007321 (September 06, 2019) (in Russian).

assigns nickel and its compounds to Group 1, that is, these compounds are undoubtedly carcinogenic to humans [7].

It is well known that in Russia adverse, hard, and dangerous work is mostly accomplished by men. In 2018 in Russia a share of men who had to face adverse and hazardous working conditions at their workplaces varied from 40 to 60 % depending on an economic activity. The most hazardous conditions at workplaces are usually in mining and in processing industries and this share is even higher in industrially developed regions where it can reach 70–80 %. We should also note that in Russia mortality among people of employable age is typical exactly for men [8].

Sanitary-hygienic conditions are the most significant factor that influences mortality levels in industrial monotowns. This includes working conditions at an enterprise where a considerable part of a male population are employed (a city-forming enterprise) and environmental conditions that are influenced by an economic activity performed at this enterprise which is a dominating economic entity in a given monotown. But still, population mortality is influenced by a whole set of living conditions including climate, geographic conditions, socioeconomic and other features that can mitigate or, on the contrary, aggravate consequences of industrial activities.

Given all that, **our aim** was to assess contribution made by leading risk factors into mortality due to MNs among male population in industrial monotowns.

Materials and methods. We selected two industrial monotowns located in the Arctic zone in Russia where copper and nickel production was located. These monotowns were Monchegorsk (Murmansk region) located on the Kola Peninsula and Norilsk (Krasnoyarsk region) located on Taimyr.

We examined industrial, ecological, climatic and geographic and socioeconomic features of Monchegorsk and Norilsk and comparatively analyzed mortality due to oncologic diseases in these two monotowns and in Russia as a whole.

Occupational environment at enterprises located in the examined cities was analyzed based on data taken from state reports on sanitary-epidemiologic welfare of population in Murmansk region and Krasnoyarsk region; reports on sustainable development issued by "Norilsk Nickel" Group of Companies in 2010–2017; data obtained through studies performed by the North-Western Scientific Center for Hygiene and Public Health; etc. [9–13].

Ecologic situation in the towns was examined based on data provided by Murmansk regional office on hydrometeorology and monitoring over environmental pollution, the Committee on use of natural resources and ecology in Murmansk region, Taimyr Center on hydrometeorology and environmental monitoring and official annual statistical data bulletins issued by Rosgidromet in 2010–2017.

Socioeconomic features of Monchegorsk and Norilsk in 2010–2017 were examined based on official statistical annual reports and Rosstat databases as well as on data taken from reports available at official web-sites, *monchegorsk.gov-murman.ru* for Monchegorsk, and *norilsk-city.ru* for Norilsk. Additional data on public healthcare systems were taken from official web-sites of medical organizations in the examined towns.

Mortality among male population in the examined towns was analyzed based on the following statistical data:

– Sex and age population structure in 2010–2017;

– Number of death cases (Statistical Form C-51 "Distribution of deceased as per sex, age, and causes of death in 2010–2017).

Data on mortality among men of post-employable age provided by Murmansk statistical service were limited to an age group 70 and older and this was taken into account when statistical analysis was performed.

Since population number was relatively small in the towns and significant fluctuations in mortality levels were possible in some years, we calculated mortality rates due to MNs among adult males that were averaged over 8 years (2010–2017):

– for 5-year age groups (15–19, 20–24, ..., 85+) due to all MNs;

– mortality rates standardized as per age;

– for employable age (15–59);

– for post-employable age (Monchegorsk and Russia, 60–70+; Norilsk and Russia, 60–85+).

Standardized rates were calculated for MNs in general, MNs of respiratory organs and MNs of digestive organs as well as for leading MNs localizations that were specific for exposure to nickel according to literature data: MNs of lip, oral cavity, and pharynx; MNs of trachea, bronchus, and lung; MNs of stomach [7, 10, 12–18].

Standardization was performed with direct method. Age structure of male population in Russia determined in the Census-2010 was taken as a standard.

Results. Monchegorsk and Norilsk are located in the Polar Regions practically at the same latitude (67° n.l. for Monchegorsk and 69° n.l. for Norilsk); however, climatic conditions differ greatly in these two towns. Climate is milder in Monchegorsk due to close proximity to Gulfstream and average temperature in winter doesn't drop below –18 °C. Norilsk had more severe subarctic climatic conditions with temperature falling down to –53 °C in winter, higher wind speed and longer polar day and night.

Enterprises belonging to "Norilsk Nickel" Group of Companies are city-forming ones in both towns. In Monchegorsk a metallurgic plant is located where only finished products are manufactured. An enterprise located in Norilsk deals with the whole production cycle and includes several plants where ores are mined and refined as well as several powerful metallurgic plants.

Water-soluble nickel compounds in workplace air are a major source of carcinogenic hazards at metallurgic enterprises. Average nickel concentrations during a shift amounted to 6–37 MPC in Monchegorsk [9–11].

Workers employed at metallurgic plants in Norilsk are exposed to nickel compounds with average shift concentrations varying from 3.1 to 34 MPC and to arsenic oxide, up to 2.3 MPC [12–13].

Besides, workers employed at mining and refining enterprises where copper and nickels ores are processed are also exposed to carcinogenic factors including nickel dust in average shift concentration being from 3 to 8 times higher than MPC and benzopyrene, 1.3 MPC (cargo handling machinery operators) [12].

The most hazardous working conditions are usually at metallurgic enterprises. Nickel and its compounds are the most significant risk factors that can cause MNs among workers employed at such enterprises in both monotowns. Nickel penetrates the body predominantly through the airways, gastrointestinal tract and skin[3] [17].

Exposure to nickel didn't have any significant difference at different enterprises; hence, we can assume that occupational carcinogenic hazard was equal at all workplaces.

We analyzed ecological situation in Monchegorsk to reveal that emissions from stationary sources contained dust, sulfur dioxide, carbon oxide, nitrogen dioxide, as well as such carcinogens as benzopyrene and formaldehyde. Average annual concentrations were higher than MPC only for formaldehyde (up to 2.5 MPC)[4]. In 2018 45.7 tons overall were emitted into ambient air in Monchegorsk[1]. But at the same time cyclones that are typical for the Kola Peninsula make for pollutants dispersion. Complex atmosphere pollution index (IZA 5), all major pollutants taken into account, didn't ex-

[3] Vrednye veshchestva v promyshlennosti. T. 3. Neorganicheskie i elementorganicheskie soedineniya: spravochnik [Adverse chemicals in industry. Volume 3. Non-organic and elemental-organic compounds: reference book]. In: N.V. Lazarev, E.N. Levina eds. Leningrad, Khimiya, 1977, 608 p. (in Russian).

[4] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Murmanskoi oblasti: Gosudarstvennyi doklad [On sanitary-epidemiologic welfare of the population in Murmansk region: The State Report]. Murmansk, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing. Murmansk regional office Publ., 2010–2018 (in Russian).

ceed 5.2[3, 5] in 2010–2017 and pollution was estimated as "elevated"[6].

Ambient air in Norilsk is excessively polluted with adverse chemicals belonging to the 1st and 2nd hazard categories and they are detected in air in the town 350 days a year, 80 % of them in concentrations reaching 5 MPC[7]. Hygienic standards are violated as per contents of sulfur dioxide, phenol, nitrogen dioxide and oxide, copper, and cobalt that are detected in concentrations varying from 1.3 to 4.2 MPC. Average annual ambient air pollution with carcinogens was by 9.5 times higher than permissible levels for formaldehyde and by 7.5 times higher for nickel in residential areas in the town[8]. Overall, in 2018 1,798.5 thousand tons were emitted into ambient air in Norilsk. Adverse chemicals are accumulated in ambient air due to a peculiar relief since the town is located in a hollow surrounded by mountain ranges on the south-west and north-east with their height being 500–900 meters. IZA 5 varied from 4.19 to 31.4 and pollution was estimated either as "high" or "extremely high"[5].

Therefore, environmental pollution can be considered a risk factor of developing MNs in the examined towns. This pollution is significantly higher in Norilsk due to the peculiar relief and substantial production scales; the town is usually among three the most polluted urban settlements in Russia[6].

Having analyzed socioeconomic features we revealed that in 2018 average monthly wages in Russia amounted to 43,724 rubles; they were approximately by 30 % higher in Monchegorsk and amounted to 59,734 rubles; and they were almost two times higher in Norilsk and amounted to 93,129 rubles.[1]. Other socioeconomic features indicated that standard of living was slightly higher in Monchegorsk than in Russia on average according to most parameters whereas it was substantially higher in Norilsk. For example, a share of people with their incomes being lower than living wage amounted to 12.9 % in Russia; 10.8 % in Monchegorsk; and 6.6 % in Norilsk, almost two times lower than on average in the country. Unemployment rate was 4.8 % in Russia, 2.2 % in Monchegorsk, and 0.8 % in Norilsk; capital investments per capita amounted to 119.8 thousand rubles, 171.2 thousand rubles, and 524.9 thousand rubles accordingly[1].

We paid special attention to quality of medical aid and public healthcare systems in the examined cities when analyzing their socioeconomic features. According to official parameters related to public healthcare systems, there were only slight differences between Monchegorsk, Norilsk, and Russia as a whole: number of doctors per 10 thousand people amounted to 54.9, 50.9, 47.5 accordingly; nurses per 10 thousand people, 146.6, 189.5, 103.8; in-hospital beds, 84.2, 70.1, and 80.5 per 10 thousand people accoridngly[1]. However, when it comes down to population health preservation and mortality reduction, such factors as high-tech diagnostic and treatment techniques, professional skills and staffing are becoming more and more important. All this is necessary for efficient use of complicated unique equipment, especially regarding two nosology categories which are the leading causes of death, namely diseases of the circulatory system and MNs. Given that, we accomplished more profound analysis of oncologic aid provision in the examined monotowns.

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[5] Murmanskoe upravlenie po gidrometeorologii i monitoringu okruzhayushchei sredy [Murmansk office on hydrometeorology and environmental monitoring]. Available at: http://kolgimet.ru/ (June 13, 2019) (in Russian).

[6] RD 52.04.186-89. Rukovodstvo po kontrolyu zagryazneniya atmosfery (utv. Goskomgidrometom SSSR 01.06.1989, Glavnym gosudarstvennym sanitarnym vrachom SSSR 16.05.1989) [Guide 52.04.186-89. Guide on control over ambient air pollution (approved by the USSR Goskomgidromet on June 01, 1989, and the USSR Chief Sanitary Inspector on May 16, 1989)]. *KODEKS: an electronic fund for legal and reference documentation*. Available at: http://docs.cntd.ru/document/1200036406 (February 24, 2021) (in Russian).

[7] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Krasnoyarskom krae: Gosudarstvennyi doklad [On sanitary-epidemiologic welfare of the population in Krasnoyarsk region: The State Report]. Krasnoyarsk, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, Krasnoyarsk regional office Publ., 2010–2018 (in Russian).

[8] Taimyrskii tsentr po gidrometeorologii i monitoringu okruzhayushchei sredy [Taimyr center on hydrometeorology and environmental monitoring]. Available at: http://www.meteorf.ru/about/structure/cgms/3154/ (June 30, 2019) (in Russian).

In Monchegorsk there is only a primary oncologic aid office operating in the central town hospital; a diagnosis can be verified and any treatment can be provided only in Murmansk Regional Oncologic Dispensary which isn't either equipped with x-ray and endoscopic devices. Rospotrebnadzor, Gospozhnadzor, and Roszdravnadzor point out in their directions that the central town hospital doesn't have enough oncologists in the staff and its premises are unsatisfactory. The situation makes it next to impossible to detect MNs at early stages and can result in advanced oncologic diseases and, accordingly, higher lethality [19].

Medical aid is of different quality in Norilsk. Norilsk hospital is the only one in the Polar Regions which is located in a multistoried building and equipped with 1,000 beds. Its oncologic division has up-to-date high-tech equipment and there are highly qualified oncologists in the staff; all this provides complex and instant diagnostics of malignant neoplasms and sparing therapy for patients including low-invasive surgery etc. Medical and preventive programs are being implemented in the town including oncologic screening with its main focus on diseases that are typical for exposure to nickel: chronic and pre-cancer gastrointestinal disorders, neoplasms of lip, oral cavity and pharynx. There is a center for outpatient oncologic aid which is opened in Norilsk municipal polyclinic within the Regional project "Fighting against oncologic diseases". People who are older than 40 can have an annual medical check-up there [20].

Therefore, most parameters that describe socioeconomic welfare of population as well as quality of public healthcare were close to average country levels in Monchegorsk whereas they were substantially higher in Norilsk.

Demographic issues are known to be closely connected with economic and social development. There are the same demographic issues in Monchegorsk as in the country in general, namely declining population and young people migrating to larger cities, and this results in intense "population ageing"; the issues are further aggravated with low birth rates and high mortality levels, especially among males of employable age. In Russia, in 2018 overall mortality rate was 12.0 ‰ and higher than birth rate which was only 11.0 ‰. The difference between mortality and birth rates was even greater in Monchegorsk where they were 12.6 ‰ and 9.0 ‰ accordingly[1].

Demographic situation in Norilsk is quite different from that typical for the Arctic zone and Russia in general. The town has "young" population with a higher share of employable population, 68.7 %, and in 2018 it was higher than in Monchegorsk and in Russia by 10.6 % and 12.7 % accordingly.[1] The town is quite attractive for economically active population and a lot of people migrate there, predominantly young ones. But still, population increases mostly due to natural growth since in 2018 overall birth rate in Norilsk amounted to 13.1 ‰[1].

All these occupational and non-occupational factors can influence not only overall demographic parameters but also mortality due to oncologic diseases.

Given difference in age structure occurring due to both natural and mechanic population flows, we comparatively analyzed sex and age mortality rates for 5-year age groups as well as mortality rates standardized as per age separately for employable people (aged 15–59) and people of post-employable age (60 and older). Thus we eliminated impacts by differences in age structure of male population in the examined towns when identifying mortality levels.

To assess influence exerted by occupational activities on health, we compared mortality among male population in Monchegorsk and Russia since socioeconomic development and quality of public healthcare systems in this town were quite similar to those existing in Russia and a major difference was a carcinogenic copper and nickel production located there.

To assess influence exerted by socioeconomic and other non-occupational factors, we compared mortality in Norilsk and Monchegorsk where copper and nickel productions were located with comparable working condi-

tions at workplaces; but still, two towns were quite different regarding living standards and quality of public healthcare systems; more adverse climatic conditions in Norilsk were also taken into account.

Figure 1 shows mortality rates due to MNs average over 2010–2017 for specific age groups of employable male population (aged 15–59) in Monchegorsk, Russia, and Norilsk.

Analysis revealed that mortality rates were higher in Monchegorsk than in Russia or Norilsk practically in all age groups (Figure 1). On the contrary, mortality rates in most age groups in Norilsk were lower not only than in Monchegorsk but also than on average in Russia.

The highest mortality rates in two age groups of people older than 60 were also detected in Monchegorsk (Figure 2).

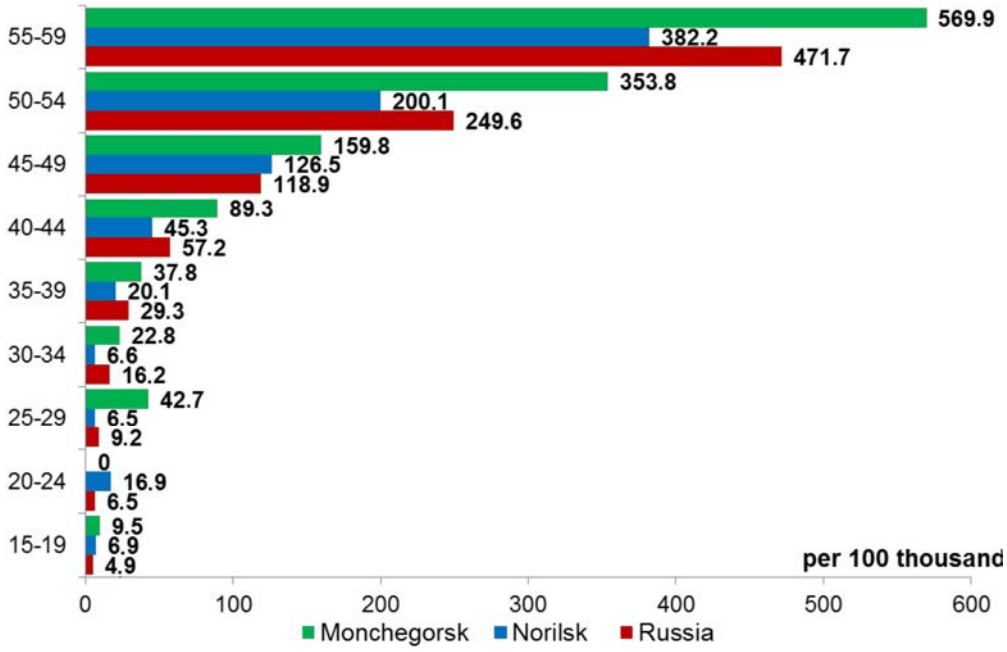

Figure 1. Mortality due to MNs among employable male population in Monchegorsk, Norilsk, and Russia as per 5-year age groups on average in 2010–2017 per 100 thousand people of a respective age

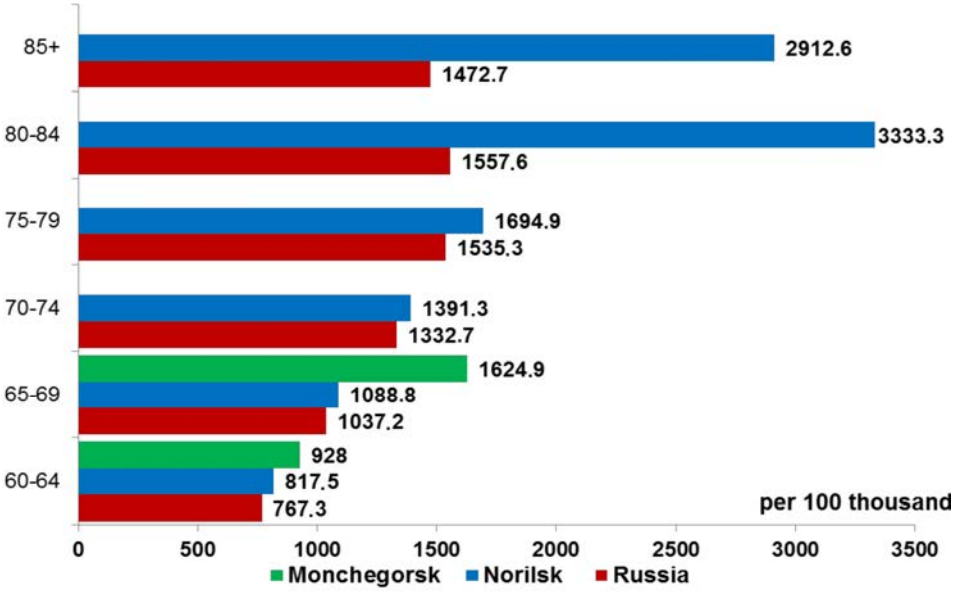

Figure 2. Mortality due to MNs among post-employable male population in Monchegorsk, Norilsk, and Russia as per 5-year age groups on average in 2010–2017 per 100 thousand people of a respective age

Table

Standardized mortality rates due to MNs among male population of employable and post-employable age in Monchegorsk, Norilsk, and Russia per 100 thousand people of a respective age

Territory	All MNs	MNs of respiratory organs	MNs of trachea, bronchus and lung	MNs of digestive organs	MNs of stomach	MNs of lip, oral cavity and pharynx
	Employable age (15–59)					
Monchegorsk	133.8	37.0	32.5	42.2	15.9	16.4
Norilsk	84.0	25.7	22.3	31.1	6.6	6.5
Russia	99.4	30.9	26.8	33.4	10.8	7.5
	Post-employable age (truncated data 60–70+)					
Monchegorsk	1204.6	317.3	298.9	441.3	156.3	73.6
Russia	1134.1	329.1	297.0	427.5	135.4	41.9
	Post-employable age (60–85+)					
Norilsk	1367.9	414.2	387.3	476.9	138.9	43.9
Russia	1124.7	331.6	299.1	422.5	134.2	42.4

The difference between mortality rates in Russia and Norilsk changed in groups of people of post-employable age. Mortality was higher in Norilsk than in Russia in all older age groups with maximum difference being reached for people older than 80.

Mortality rates among male population were standardized as per age separately for employable and post-employable age groups. The results are shown in Table.

Standardized mortality rate due to MNs among employable male population was by 34.7 % higher in Monchegorsk than in Russia (133.8 and 99.4 per 100 thousand men of employable age accordingly). Mortality due to MNs of respiratory organs was also by 19.7 % higher; MNs of trachea, bronchus and lung were by 21.5 % higher. Maximum difference was detected for malignant neoplasms typical for exposure to nickel: MNs of stomach, by 1.5 times higher than in Russia; MNs of lip, oral cavity and pharynx, by 2.2 times higher (Table).

Our analysis of mortality based on truncated data for post-employable age also revealed that mortality rates due to all MNs for male population were by 6.2 % higher in Monchegorsk than in Russia. The greatest difference was detected for MNs of target organs for exposure to nickel, MNs of stomach by 15.4 % and MNs of lip, oral cavity and pharynx by 1.7 times.

In Norilsk age mortality rates and standardized mortality rates for employable males were lower over the 8-year period, both in comparison with Russia and Monchegorsk.

Lower mortality rates were detected for specific MNs localization as well. Mortality rate among employable men due to MNs of respiratory organs, MNs of trachea, bronchus and lung included, was by one third lower in Norilsk than in Monchegorsk and almost by 17 % lower than in Russia. Mortality due to MNs of digestive organs was by 26.2 % and 6.9 % lower among employable population in Norilsk than in Monchegorsk and Russia accordingly; mortality due to MNs of stomach was by 2.4 and 1.6 times lower accordingly. Mortality due to MNs of lip, oral cavity and pharynx was by 2.5 times lower than in Monchegorsk and by 14.0 % lower than on average in Russia.

Results and discussion. Long-term examination of mortality due to MNs in Monchegorsk revealed higher mortality rates due to the nosology than on average in the country regardless of similar socioeconomic development and demographic features and similar quality of public healthcare in this town and in Russia in general.

Similar results are described by other foreign and domestic researchers indicating there are high risks of developing MNs both for workers employed at copper and nickel produc-

tion and for people living in settlements where such productions are located [15, 16, 21–23].

Elevated oncologic morbidity among workers employed at nickel production has been mentioned by V.P. Artyunina, G.P. Chashchin and others [21]. According to these authors when nickel concentration is by multiple times higher than MPC, oncologic incidence grows by more than 3 times among workers employed at such enterprises in comparison with population in general; the greatest difference is usually detected for lung cancer [21].

G.I. Tikhonova, T.Yu. Gorhakova and A.N. Churanova [23] analyzed mortality rates among employable male population in towns located in Murmansk region depending on an economic activity performed at city-forming enterprises. It was established that mortality rates among male employable population were higher in Monchegorsk than in Murmansk, a city without any large industrial enterprises. Mortality due to all MNs was by 17.1 % higher; due to MNs of respiratory organs, by 8.5 % higher; MNs of digestive organs, by 26.7 % higher; MNs of lip, oral cavity and pharynx, by 80.2 % higher [23].

There was a study on morbidity with malignant neoplasms among workers dealing with electrolysis at a nickel production plant in Port-Colborne (Ontario, Canada) over a period from 1930 to 1992; it revealed an elevated risk of MNs of nasal cavity and nasopharynx regardless of levels of exposure to nickel and its compounds [15].

M. Pavela, J. Uitti, and E. Pukkala [16] examined oncologic incidence among workers employed at nickel and oil processing productions in Harjavalta, Finland. They examined MNs incidence among 1,115 people who were exposed to nickel. A reference group was made up of 194 people who were not exposed to this metal at their workplaces. Overall examination period was 45 years, from 1967 to 2011. The authors revealed that exposure to nickel compounds was a basic reason for elevated risks of nasal cavity cancer and lung cancer among workers employed at nickel production. Overall number of cancer cases amounted to 251 among men (Standardized incidence rate (SIR) amounted to 1.05, 95 %) and to 12 among women (SIR 1.22, 95 %). 14 cases of lung cancer (SIR 2.01, 95 %) and 3 cases of nasal cavity cancer (SIR 26.7, 95 %) were detected at workplaces where exposure to nickel was the highest [16].

V. Ciannameo with colleagues [22] examined a correlation between exposure to nickel and mortality in a cohort made up of 2,991 Italian workers dealing with galvanizing. The research results revealed that exposure to nickel compounds could result in elevated risks of lung cancer even if this occupational exposure didn't exceed maximum permissible concentration. They also revealed a correlation between exposure to nickel and developing MNs of digestive organs and MNs of kidneys [22].

Therefore, we revealed higher mortality rates due to MNs among employable population in Monchegorsk who were predominantly employed at a city-forming nickel production enterprise and these results are well in line with those obtained by other Russian and foreign researchers. We can conclude that elevated mortality due to cancer among adult male population as a negative health outcome results from occupational and ecological risks caused by economic activities performed at a city-forming enterprise.

We could expect even higher mortality rates due to MNs in Norilsk since working conditions at city-forming enterprises also involved risks of developing MNs and environmental pollution there was much higher than in Monchegorsk with higher contents of carcinogens and could be further aggravated by more adverse climatic conditions. This assumption was also confirmed by literature data on high oncologic incidence rates in Norilsk available in works by O.A. Ananina with colleagues [24], D.V. Goryaev and I.V. Tikhnova [25], V.V. Karasyov with colleagues [26], B.A. Revich [4].

But our analysis of socioeconomic development and quality of medical and preventive aid provided to population allows concluding that lower mortality due to MNs among male employable population in Norilsk in compari-

son with Monchegorsk is to a greater extent due to high efficiency of public healthcare organizations in the town. Revealing a disease at an early stage contributes greatly to reducing mortality due to neoplasms and the process is quite efficient in Norilsk due to oncologic screening programs aimed at making wider population groups have medical check-ups and due to greater attention paid by medical experts to MNs localizations typical for exposure to nickel. It is confirmed by extremely low mortality rates due to MNs of stomach and MNs of lip, oral cavity and pharynx among employable population; mortality rates among population of post-employable age due to these MNs localizations are similar to those on average in the country (mortality due to MNs of stomach is only by 3.5 % and due to MNs of lip, oral cavity and pharynx, by 3.6 % higher in Norilsk than on average in Russia). It is very important since mortality due to all MNs among population of post-employable age was by 21.6 % higher in Norilsk than on average in Russia.

Several works by Russian and foreign authors have also stressed it is important to improve oncologic aid in order to achieve higher quality of life and longer life expectancy for patients with malignant neoplasms [27–29].

Analysis of all the obtained results indicates that there is high risk of developing MNs both in Norilsk and Monchegorsk and, given all the examined hygienic characteristics, it can be higher than on average in the country but mortality due to this nosology usually occurs in older age groups. This detected regularity can be estimated as postponed realization of carcinogenic risks caused by technogenic factors but adjusted by more efficient public healthcare systems including up-to-date oncologic aid as well as by higher living standards.

So, our study on mortality due to oncologic diseases in Norilsk revealed that a medical component, that is, early diagnostics, timely and efficient treatment and following rehabilitation had the most significant influence on mortality due to MNs in an industrial monotown with carcinogenic productions, especially when it comes down to employable population.

Therefore, economic activities performed at a city-forming enterprise can produce negative effects on health of workers and town population but the same enterprise brings all social and economic welfare to population being a life source for a given monotown [23]. And occupational and ecological risks can be mitigated significantly in case business is socially responsible. This can give grounds for social-demographic and corporate policies aimed at health preservation and reducing mortality among workers and town population in industrial monotowns.

Conclusions:

1. Our social-hygienic study on mortality among adult male population in two industrial monotowns in Russia allowed differentiated estimation of consequences caused by exposure to factors related to economic activities performed by copper and nickel production enterprises. It was done through comparative analysis of mortality in Monchegorsk and Russia and non-occupational factors in comparing mortality in Norilsk and Monchegorsk.

2. Socioeconomic features and quality of public healthcare in Monchegorsk were comparable to average country parameters; but still, there were higher mortality rates due to all MNs and specific localizations typical for exposure to nickel revealed there among male population. It allows concluding that the detected differences correlate with exposure to adverse occupational factors and environmental pollution.

3. We established that mortality rates among male population were lower in all 5-year age groups in Norilsk in comparison with Monchegorsk despite comparable occupational factors at city-forming enterprises in these two towns and more adverse climatic conditions and more polluted environment in Norilsk. These lower mortality rates were due to higher quality of life in Norilsk including better availability of medical and preventive aid, high-tech diagnostics, treatment and rehabilitation provided for patients with malignant neoplasms.

4. Higher mortality rates due to MNs were detected in Norilsk in comparison with Russia for people of post-employable age as opposed to lower standardized mortality rates among men younger than 60; that is, carcinogenic health risks persisted though they turned into actual diseases at older ages. This indicates that hygienic activities aimed at improvement of working conditions and ecological situations should be considered a top priority.

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Research article

ELECTROMAGNETIC ENVIRONMENT CREATED BY MOBILE COMMUNICATION AS A RISK FACTOR CAUSING HIGHER PREVALENCE OF CIRCULATORY DISEASES

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Our research goal was to examine energy and time parameters of electromagnetic environment created by mobile communication terminals in Crimea and to detect a relationship between morbidity with diseases of the circulatory system (DCS) among people living in the republic due to electromagnetic environment.

Electromagnetic environment was determined as per energy flux density (EFD) at terminals, average daily electromagnetic exposure (ADEE) and individual electromagnetic burden (IEB). Correlation analysis of all the obtained data was performed to detect possible correlations with diseases of the circulatory system.

Measurements of electromagnetic environment created by terminals in the region during a period from March 2019 to February 2020 (4,204 measurements at points where people used mobile communications intensely) yielded the following results: average EFD value amounted to 1.43 ± 0.04 μWt/cm^2; IEB, 117.80 ± 6.55 (μWt/cm^2)·min; ADEE, 60.56 ± 1.15 min.

Electromagnetic environment parameters and prevalence of the most common DCS in Crimea were statistically processed and the following authentic correlations were revealed: between overall morbidity with circulatory diseases and energy (EFD (Tau=0.399; p < 0.01)) and integral (IEB (Tau=0.437; p < 0.01)) properties of electromagnetic radiation; between overall morbidity with diseases that involved elevated blood pressure and IEB (Tau=0.377, p=0.01); between primary morbidity with diseases that involved elevated blood pressure and all electromagnetic radiation properties: EFD (Tau=0.304, p=0.04), IEB (Tau=0.342, p=0.02), and ADEE (Tau=0.299, p=0.04); between primary morbidity with cerebrovascular diseases (CVD) and ADEE (Tau=0.411, p < 0.01) as time property of the examined factor.

Energy and time parameters of electromagnetic environment created by mobile communication terminals that have been measured in Crimea are authentically correlated with distribution of morbidity with circulatory diseases and exert their influence on a risk of probable growth in morbidity with these nosologies among people living in Crimea.

Key words: *electromagnetic radiation, electromagnetic burden, mobile phone, primary diseases, pathology of the circulatory system.*

Diseases of the circulatory system have significant influence on labor and life potential of the contemporary world society as well as demographic safety of any state; they are a vital issue for contemporary medicine since such diseases are among primary causes of population mortality [1, 2]. However, morbidity with diseases of the circulatory system and its outcomes for population are different in different countries. Thus, an authors' team conducted a research work based on analyzing trends in morbidity over 2001–2016 in Great Britain and as a result it was established that life expectancy grew approximately by 3 years per a decade. The primary factor here was a decrease in frequency of hospitalizations, first of all, due to diseases of the circulatory system [2].

Statistical data collected in Belarus were analyzed; as a result, the authors detected a decrease in primary morbidity with diseases from this nosologic category by 16 % from

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2011 to 2015 [3]. In Russia in 2018 a number of registered cardiovascular diseases grew by 12.68 % against 2010[1]. When analyzing specific nosologies within the category, for example, hypertensive diseases, experts detected that morbidity grew by 28.12 % including first diagnosed cases that grew by 78.69 %[1]. Foreign researchers pay close attention to conventional factors that influence risks of cardiovascular diseases (imbalanced nutrition, low physical activity, overweight, etc.) and their scientifically grounded correction [4]. Some research works focus on patients' age and there are conclusions in them on the necessity to implement efficient preventive activities for middle-aged and elderly people [1]. Domestic researchers have revealed impacts exerted by climatic-geographic and ecologic factors on deviations in population health in a given region from the country trends [5].

At the same time there are data on effects produced on non-carcinogenic health risks by electromagnetic radiation (EMR) created in radio frequency (RF) spectrum [6, 7]. New procedures are being developed to estimate intensify of electromagnetic background and population exposure [8] and there are studies focusing on possible health risks caused by environmental pollution with electromagnetic fields generated by mobile phone base stations [7]. Levels of electromagnetic fields (EMF) are being examined, among other things, within a context of assessing population exposure [9].

It is quite promising to examine epidemiologic situation regarding cardiovascular diseases in regions with different climatic and geographic conditions [5]. In this respect Crimea seems an interesting region, especially given mass reconstruction of mobile communications that is taking place in the republic at the moment. It allowed us to formulate our research goal that was to examine energy and time parameters of electromagnetic environment created by mobile communication terminals in Crimea and to detect a relationship between electromagnetic environment and morbidity with diseases of the circulatory system among population in the republic.

Data and methods. We took data from statistical reports issued over 2015–2019 to analyze primary and total morbidity with diseases of the circulatory system among population in Crimea; the data were provided by the Crimean Medical and Information Analytical Center. We analyzed total and first detected morbidity rates calculated as per 100 thousand people living in Crimea; rates were analyzed for the following classes of diseases according to the International classification of diseases (ICD-10) (the latest edition as of 2019): hypertensive diseases, ishaemic heart diseases, and cerebrovascular diseases.

We applied several parameters to estimate electromagnetic environment: energy flux density (EFD) as an energy parameter of EMR produced by mobile communication terminals; average daily electromagnetic exposure (ADEE) as a time parameter of the examined factor; and individual electromagnetic burden (IEB) as an integral parameter that depended both on energy flux density and duration of exposure.

Electromagnetic environment was estimated in 22 administrative districts in Crimea through mobile monitoring over energy flux density in radio frequency spectrum of radiation created by mobile communication terminals. To do that, we used *PZ-34* measuring device (NTM "Zashchita", Moscow, RF) with *AP 3-34 VNF* antenna. The antenna was fixed on a support device together with two *Samsung A30 mobile phones* 0.37 meters away from them and was located in a box on a car's trunk. *MS2712E* portative spectrum analyzer (Anritsu, USA) with *Aaronia AG* antenna (Aaronia, Germany) was used at each measuring point to analyze the background and to remove side EMF sources in radio frequency

[1] Zdravookhranenie v Rossii. 2019: stat. sb. [Public healthcare in Russia. 2019: statistical data collection]. Moscow, Federal State Statistic Service Publ., 2019, pp. 70–78 (in Russian).

spectrum. Then, we determined energy flux density three times during 1 minute at 1.7 meters high, both background level and those created by each mobile phone during a phone conversation with a remote interlocutor (provided that background level was lower than 0.5 μW/cm^2). We determined access burst (AB) for each call, a time period necessary to establish a connection during which a mobile phone creates maximum electromagnetic radiation; duration of this period is linearly dependent on overall loads on a mobile network in a given region [8]. To determine an average duration of a phone conversation, we applied crowdsourcing that involved collecting data on time spent on using mobile phones provided by volunteers. They collected data on average daily time spent on using mobile phones from detail reports on phone calls provided by Mobile TeleSystems (MTS). We calculated individual electromagnetic burden (IEB) using data on EFD, duration of a phone conversation and correction index CI (normalizaiton of a current value to a minimum one detected in a given region). To estimate duration of people's exposure to the examined factor, we took average daily electromagnetic exposure (ADEE) [8] that was calculated taking into account duration of a phone call, CI, and average daily number of phone conversations determined according to detail reports on phone calls. Experimental data were tested to determine whether distribution was normal according to Kolmogorov – Smirnov procedure. In case a data series was distributed normally, we calculated simple mean and error of mean; if distribution was not normal, we calculated median (*Me*) and upper and lower quartiles (*Q1*; *Q3*). Data series with normal distribution were tested with Pearson's linear correlation analysis to detect any correlations; data series with distribution that was not normal, with Kendall rank correlation coefficient.

Results and discussion. Having analyzed data on morbidity with diseases of the circulatory system among population in Crimea over 2015–2019, we were able to calculate medians (*Me* (*Q1*; *Q2*)) of total morbidity that amounted to 47,923.3 (43,230.1; 51,877.8) cases per 100 thousand people for diseases of the circulatory system; 18,266.6 (6,877.7; 19,747.5), for hypertensive diseases; 19,543.9 (18,742.1; 20,740.2), for ishaemic heart diseases; and 6,050.7 (5,557.8; 6,676.2), for cerebrovascular diseases. Therefore, we can note that diseases of the circulatory system are mostly ishaemic heart diseases and hypertensive diseases. As for primary morbidity with diseases of the circulatory system among population, medians calculated for primary morbidity amounted to 2,911.8 (2,780.2; 4,355.7) for diseases of the circulatory system; 697.2 (688.2; 1,187.0), for hypertensive diseases; 906.6 (808.9; 1,538.4), for ishaemic heart diseases; and 811.7 (774.2; 951.8), for cerebrovascular diseases. Given that, it seems interesting that a share of cerebrovascular diseases has grown considerably in the structure of primary morbidity with diseases of the circulatory system in comparison with the same parameter in total morbidity with the same diseases.

Experimental studies on electromagnetic environment created by mobile communications in the region from March 2019 to February 2020 (4,204 measurements accomplished at points where people used mobile communications quite actively) yielded the results given in Table.

Calculated average EFD values varied from 0.94 ± 0.03 μW/cm^2 (Belogorskiy district) to 2.04 ± 0.06 μW/cm^2 (Simferopol); average value in Crimea was 1.43 ± 0.04 μW/cm^2, and simultaneously measured access burst (AB) varied from 6.35 ± 0.19 sec (Dzhankoyskiy district) to 11.31 ± 0.41 sec (Simferopol), average value in Crimea was 9.05 ± 0.41 sec. Average daily duration of phone conversations varied from 44.5 ± 2.76 min (Pervomaiskiy district) to 67.51 ± 3.07 min (Yalta), and average value in Crimea amounted to 56.94 ± 2.77 min. Average calculated IEB in the region amounted to 117.80 ± 6.55 (μW/cm^2)·min. Having analyzed the parameter in greater detail, we spotted out cities (Simferopol (254.11 ± 11.51 (μW/cm^2)·min),

Table

Parameters of electromegnatic environment created by mobile communications on administrative districts in Crimea in 2019–2020

Adminitrsative district	EFD[1] (μW/cm^2)	Access burst (sec)	Duration of phone calls a day (min)	IEB[2] (μW/cm^2·min)	Average daily exposure (min)
Crimea	1.43 ± 0.04	9.05 ± 0.41	56.94 ± 2.77	117.80 ± 6.55	60.56 ± 1.15
Simferopol	2.04 ± 0.06	11.31 ± 0.23	67.32 ± 1.94	254.11 ± 11.51	72.96 ± 1.34
Yevpatoria	1.30 ± 0.05	9.39 ± 0.68	58.11 ± 2.45	108.09 ± 5.11	62.79 ± 1.07
Feodosyia	1.22 ± 0.05	9.39 ± 0.71	55.62 ± 1.87	102.75 ± 4.89	60.30 ± 1.75
Kerch	1.11 ± 0.04	8.08 ± 0.43	62.41 ± 3.44	79.60 ± 3.94	60.44 ± 0.89
Yalta	1.45 ± 0.05	9.99 ± 0.51	67.51 ± 3.07	156.44 ± 6.17	72.49 ± 1.11
Alushta	1.52 ± 0.06	11.13 ± 0.44	47.10 ± 2.75	124.76 ± 4.34	52.67 ± 2.97
Armyansk	1.09 ± 0.04	7.94 ± 0.17	45.28 ± 3.10	64.11 ± 2.84	49.17 ± 1.91
Sudak	1.34 ± 0.05	9.14 ± 0.22	62.84 ± 3.23	120.16 ± 5.34	67.37 ± 2.77
Krasnoperekopsk	1.74 ± 0.06	8.89 ± 0.40	58.55 ± 2.18	136.16 ± 4.71	62.95 ± 2.51
Bakhchysaray district	1.23 ± 0.05	9.71 ± 0.34	51.91 ± 2.19	91.26 ± 3.58	56.76 ± 2.75
Belogorskiy district	0.94 ± 0.03	7.57 ± 0.29	46.46 ± 1.88	54.13 ± 1.87	50.19 ± 1.77
Dzhankoyskiy district	1.24 ± 0.04	6.35 ± 0.19	64.86 ± 2.54	80.35 ± 2.90	67.85 ± 2.54
Kirovskiy district	1.24 ± 0.05	8.62 ± 0.28	58.21 ± 2.32	104.58 ± 2.15	62.62 ± 2.82
Krasnogvardeiskiy district	1.76 ± 0.06	9.44 ± 0.32	65.44 ± 2.95	152.17 ± 5.52	70.16 ± 3.72
Lenisnkiy district	1.48 ± 0.05	9.36 ± 0.26	61.6 ± 2.78	140.04 ± 4.88	66.29 ± 2.09
Nizhnegorskiy district	1.37 ± 0.06	8.22 ± 0.39	45.2 ± 2.11	83.34 ± 4.51	49.31 ± 1.94
Pervomaiskiy district	1.88 ± 0.05	9.43 ± 0.39	44.5 ± 2.76	130.63 ± 6.02	49.28 ± 2.11
Razdol'nenskiy ditsrict	1.32 ± 0.04	8.22 ± 0.32	62.8 ± 3.07	114.70 ± 4.31	67.02 ± 1.52
Sakskiy district	1.54 ± 0.06	11.05 ± 0.27	59.7 ± 2.95	162.47 ± 6.53	65.22 ± 2.92
Simferoplskiy district	1.27 ± 0.03	8.65 ± 0.41	53.1 ± 1.85	91.56 ± 3.70	57.50 ± 1.87
Sovetskiy district	1.35 ± 0.05	6.52 ± 0.32	56.5 ± 2.78	80.40 ± 2.98	59.72 ± 2.14
Chernomorskiy district	1.98 ± 0.07	10.38 ± 0.41	48.1 ± 1.95	159.41 ± 4.74	53.29 ± 1.84

N o t e : [1] means energy flux density; [2] means individual electromagnetic burden.

Yalta (156.44 ± 6.17 (μW/cm^2)·min)) and districts (Chernomorskiy (159.41 ± 4.74 (μW/cm^2)·min) and Sakskiy (162.47 ± 6.53 (μW/cm^2)·min)) where individual electromagnetic burden was higher than on average in Crimea. IEB was next to minimal in some cities (Armyansk (64.11 ± 2.84 (μW/cm^2)·min), Kerch (79.60 ± 3.94 (μW/cm^2)·min)) and districts (Belogorskiy (54.13 ± 1.87 (μW/cm^2)·min), Dzhankoyskiy (80.35 ± 2.90 (μW/cm^2)·min). Calculated average daily electromagnetic exposure (ADEE) varied from 49.17 ± 1.91 min (Armyansk) to 72.96 ± 1.34 min (Simferopol) and amounted to 60.56 ± 1.15 min on average in Crimea.

Next, we applied Kendall correlation procedure to statistically calculate correlations between EFD, IEB, ADEE, and basic parameters of morbidity with diseases of the circulatory system in Crimea. We managed to detect authentic correlations between average annual EFD and total morbidity with diseases of the circulatory system ($Tau = 0.399$; $p < 0.01$) and primary morbidity with hypertensive diseases ($Tau = 0.304$; $p = 0.04$); we also detected authentic correlations between IEB and total morbidity with diseases of the circulatory system ($Tau = 0.437$; $p < 0.01$), total morbidity with hypertensive diseases ($Tau = 0.377$; $p = 0.01$), and primary morbidity with hypertensive diseases ($Tau = 0.342$; $p = 0.02$). Having analyzed overall annual data, we detected authentic correlations between ADEE and primary morbidity with hypertensive diseases ($Tau = 0.299$; $p = 0.04$) and primary morbidity with cerebrovascular diseases ($Tau = 0.411$; $p < 0.01$).

At present there are different opinions on impacts exerted by exposure to EMR created by mobile phones on the cardiovascular system. Thus, for example, authors of a review [10] based on results obtained in several ex-

perimental studies came to a conclusion that mobile phones didn't produce any effects on hemodynamic parameters (heart rate or blood pressure) or on electrical activity of the heart. However, authors of a similar review [11], though having mentioned absence of any effects on the heart by EMR created by mobile phone frequencies, still stressed there were certain compensatory mechanisms that would weaken over time together with growing adverse potential of probable cardiovascular effects. If we keep in mind that a disease of the circulatory system occurs after a necessary time period, such potential growth of cardiovascular effects makes our detected correlation between total morbidity with diseases of the circulatory system and EFD and IEB look quite regular. In its turn, results obtained by colleagues from India who revealed changes in blood pressure, heart rhythm, and heart rate under exposure to GSM standard electromagnetic radiation provide another illustration for our detected correlation between total morbidity with hypertensive diseases and individual energy burden [12, 13].

Duration of phones calls using a mobile device also has its impacts on the cardiovascular system. Szyjkowska A. and colleagues (2019) proved in their experimental study that if a person communicated over mobile phone for more than 60 minutes a day, it had significant influence on his or her blood pressure; it was authentically (p = 0.04) different from blood pressure of people who spent less than 1 hour a say on communicating over a mobile phone [14]. Our detected correlations between primary morbidity with hypertensive diseases and ADEE confirm these data.

Another interesting finding is a correlation between primary morbidity with cerebrovascular diseases and ADEE; it is in line with literature data on detected ischemia of the brain vessels under exposure to electromagnetic radiation created by mobile phones [15]. It is also indirectly illustrated with research works that focused on influence exerted by different EMF frequencies on hormones and enzymatic activity of the brain [16]; the authors examined mechanisms that underlay effects produced by EMF at tissue and cellular levels and described how calcium channels in membranes activated as a response to exposure to EMF. Besides, in our research, we detected a difference between a place that belonged to cerebrovascular diseases in total and primary morbidity with diseases of the circulatory system as well as considerable growth in a share belonging to such diseases in primary morbidity with diseases of the circulatory system. So, we can assume that the overall structure of morbidity with diseases of the circulatory system might change in future. Given that, it seems vital to accomplish further experimental epidemiologic research focusing on adverse effects produced by exposure to EMF created by mobile phones.

Conclusion. We examined electromagnetic environment created by mobile phones in Crimea from March 2019 to February 2020; as a result, the following average parameters were determined: energy flux density (EFD), 1.43 ± 0.04 μW/cm^2; individual energy burden (IEB), 117.80 ± 6.55 (μW/cm^2)·min; average daily electromagnetic exposure (ADEE), 60.56 ± 1.15 min. Having analyzed correlations between prevalence of circulatory diseases and parameters of electromagnetic environment, we revealed the following authentic correlations: between total morbidity with diseases of the circulatory system and the energy parameter of electromagnetic radiation or EFD (Tau = 0.399; $p < 0.01$) and its integral parameter or IEB (Tau = 0.437; $p < 0.01$); between total morbidity with hypertensive diseases and the integral parameter or IEB (Tau = 0.377, p = 0.01); between primary morbidity with hypertensive diseases and all three parameters, or EFD (Tau = 0.304, p = 0.04), IEB (Tau = 0.342, p = 0.02), and ADEE (Tau = 0.299, p = 0.04); between primary morbidity with cerebrovascular diseases and the time parameter of the examined factor or ADEE (Tau = 0.411, $p < 0.01$). Therefore, energy and time parameters of electromagnetic environment created by mobile phones that have been measured in Crimea are

authentically correlated with distribution of morbidity with circulatory diseases, in particular, hypertensive diseases and cerebrovascular diseases, and have their influence on growth in morbidity with diseases of the circulatory system among people living in the republic.

A research work of such type doesn't require any ethical approval.

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Research article

DEOXINIVALENOL AS A RISK FACTOR OF FOOD GRAIN CONTAMINATION: MONITORING RESULTS OF 1989–2018 YEARS HARVESTS IN RUSSIAN FEDERATION

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The paper dwells on the results obtained via long-term monitoring over food grain (wheat, barley, corn, oats, and rye) contamination with mycotoxin deoxynivalenol (DON). From 1989 to 2018 6,800 grain samples from Central, Southern, Volga, Ural, Siberian, North-Caucasian, Far Eastern, and Northwestern Federal Districts (FD) of the RF were analyzed. Depending on a year harvest, DON occurrence varied from 0 to 42 % and maximum toxin content reached 6.65 mg/kg. Over the whole examined period 10 % samples turned out to be contaminated and one forth of them contained the toxin in quantities exceeding maximum permissible levels (MPL). DON occurrence amounted to 24–42 % in years of mass epiphytoties (1989, 1992 and 1993) as well as in crops gathered in 2014 and 2017; DON was detected in quantities exceeding MPL in 9–27 % of examined samples in those years. 78 % contaminated samples came from Southern and North-Caucasian FD and another 10 % were from Far Eastern FD. A significant correlation between DON occurrence and a number of rainy and sunny days in May was established on the example of wheat samples from Krasnodar region. Analysis of contamination dynamics has revealed that over the last years there has been an ascending trend in frequency of DON detection in wheat that came not only from regions where Fusarium head blight was widely spread but also from regions in North-western, Siberian and Volga FD. Health risks related to DON intake with wheat grains processing products were assessed; the assessment revealed that DON intake higher than tolerable daily intake (TDI) for the residents of Southern and North-Caucasian FD in 1992, 1993, 2014 and 2017.

Average occurrence of DON was 4.2; 11.9; 3.0 and 0.6 % for barley, corn, rye, and oats samples and its maximum contents amounted to 8.95; 0.95; 0.96 and 0.44 mg/kg accordingly. Just as it was the case with wheat, the most of contaminated samples came from Southern, North-Caucasian and Far Eastern FD. Contamination tended to grow for all the examined grains and it calls for relevant measures aimed at controlling food grains safety

Key words: *monitoring, mycotoxins, food grain, wheat, barley, oats, corn, rye, Fusarium head blight, occurrence, deoxynivalenol, health risk assessment, tolerable daily intake, weather, correlation analysis.*

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Grain and the processed products are the traditional diet basis of most people in Russia; therefore, grain quality and safety is the most significant element of food safety in the Russian Federation. Phytopathogens, including toxicogenic micromycetes are an integral part of any agricultural biocenosis. Improper crop rotations, technological breakdowns in grain cultivation and low quality of seed grains together with unfavorable weather make for "field" fungi development, micromycetes from *Fusarium* genus being among the most widely spread ones. Toxic secondary metabolites produced by microfungi, or mycotoxins, pose a health risk. The greatest health risk is associated with chronic intake of mycotoxins in small amounts with food [1–3].

Deoxynivalenol (DON) is the most widely spread fusariotoxin in the world. It was extracted in 1972 in Japan and the USA and later it was confirmed to be a constant contaminant in grain cultures in most regions all over the world. DON is the most frequently detected in wheat, a bit less frequently in corn, barley, rye, and oats, as well as in their processed grain products [4–12]. Its major producers, causing *Fusarium* head blight, are fungi from several *Fusarium* species including *F. graminearum, F. culmorum, F. nivale* [3, 6, 10, 11, 13]. Toxin accumulation depends on toxigenic properties of producing fungi strain, climatic and weather conditions, techniques applied to grow and protect plants, and storage conditions [14–16].

High humidity during and after flowering stage of the crop creates auspicious conditions for *Fusarium* head blight to appear. DON contents in contaminated grain grow starting from flowering and up to milky-wax ripeness and then they fall dramatically during wax and full ripeness [15, 17]. Moderate climate that is typical for North America, China, and Europe is optimal for *Fusairum* head blight (FHB) spreading in wheat [2, 6, 11, 16, 18]. In the Russian Federation grain grown in the North Caucasian, the Southern, and the Far Eastern Federal Districts (FDs) is the most susceptible to the disease [2, 13, 15, 19].

Acute DON poisoning resulted in vomiting in animals studies (DON is also known as vomitoxin due to this fact). DON was established to cause alimentary toxicosis not only among farm animals [11, 16, 20] but among people as well [2, 20]. Studies revealed that it bonded to ribosome at molecular level and inhibited protein synthesis thus disrupting proper cell functioning [2]. DON in low doses is able to inhibit immunity. However, exposure to the toxin in its lethal dose can result in leukocytosis, bleeding, diarrhea, and endotoxicosis [21]. The Joint FAO – WHO Expert Committee on Food Additives (JECFA) established provisional maximum tolerable daily intake (PMTDI) and acute reference dose of DON and acetylated metabolites (3-acetyl DON and 15-acetyl DON) for people at 1 and 8 μg/kg of body weight a day accordingly; the determined doses were results of toxicological studies [20]. The prevalence of the toxin and irrefutable evidence that it is truly hazardous for human health justified the introduction of hygienic standards for its contents in food in some countries. Codex Alimentarius Commission established international standards for DON contents in cereal grains (wheat, corn and barley) destined for further processing at the level of 2,000 μg/kg [22]. In the European Union countries maximum permissible levels (MPL) are regulated according to [23]: they should not exceed 1,750 μg/kg in unprocessed durum wheat, oats, and corn; 1,250 μg/kg in other unprocessed grains; 750 μg/kg in cereals and cereals products intended for direct human consumption. In the RF there are Technical Regulations of the Customs Union (TR CU) No. 021/2011 "On food safety"[1], and No. 015/2011 "Safety on grain"[2]; according to them DON MPLs in food wheat and barley as

[1] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: tekhnicheskii reglament Tamozhennogo soyuza [TR CU 021/2011. On food safety: The Technical Regulations of the Customs Union. Approved by the Decision by the Customs Union Commission on December 9, 2011 No. 880]. *KODEKS: the electronic fund for legal and reference documentation.* Available at: http: //www. eurasiancommission.org/ru/db/techreglam/Documents/TR % 20TS % 20bezopProd.pdf (March 12, 2021) (in Russian).

[2] TR TS 015/2011. O bezopasnosti zerna: tekhnicheskii reglament Tamozhennogo soyuza [TR CU 015/2011. Safety on grain (last edited on September 15, 2017): The Technical Regulations of the Customs Union. Approved by the Decision by the Customs Union Commission on December 9, 2011 No. 874]. *KODEKS: the electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902320395 (March 12, 2021) (in Russian).

well as their products are equal to 0.7 mg/kg and 1.0 mg/kg respectively.

Long-term mycotoxicological monitoring has been accomplished to examine food grain contamination with mycotoxins, both in areas where *Fusarium* head blight is widely spread and in other regions where grain is grown. This research work focuses on long-term 30-year monitoring results on DON contamination in food grain (wheat, barley, corn, rye, and oats) harvested in 1989–2018 in order to reveal basic health risks and identify activities for those risks to be reduced.

Materials and methods. Food grain samples (6,800 overall) were provided by Rospotrebnadzor regional offices from Central, Southern, Volga, Ural, Siberian, North Caucasian, Far Eastern, and Northwestern Federal Districts of the RF[3]. Experts examined in total 4,009 wheat samples, 1,293 barley samples, 1,020 rye samples, 278 corn samples and 200 oats samples harvested from 1989 to 2018.

Grain samples were taken from homogenous lots stored at grain reception and grain processing facilities in conformity with GOST (State Standard) R ISO 24333-2011[4]; DON contents in samples were determined with enzyme-linked immunosorbent assay (2009–2012), high performance liquid chromatography with diode-array and ultraviolet detection (HPLC-UV)[5] (1989–2018) and liquid chromatography with tandem mass spectrometry (HPLC-MS/MS)[6] (2018).

Data were statistically analyzed in IBM SPSS Statistics 23 (Statistical package for social sciences, USA) and Microsoft Office Excel 2007 (Microsoft Corp., USA). DON contents data in samples were presented in simple mean (*M*) and median (*Me*) values and 90-th percentile (90 %) (contamination levels below minimum detectable concentration for the selected procedures (0.05 mg/kg) were taken as 0). We accomplished two-factor dispersion and correlation analysis to study correlations between frequency of DON contamination in wheat harvested in 2006–2018 in Krasnodar region and temperature and humidity, duration of sunny weather days and precipitations from May to August. Data were analyzed within the Latin square 3x3 where lines were numbers of samples and columns were years of harvest, values of dependent variables for a selected wheat class were given in cells with Latin alphabet letters, and frequency of DON contamination was a dependent variable (the significance level was 0.1). Additionally, to establish influence of a parameter on the dependent variable in contamination analysis, we applied Mann – Whitney non-parametric test after performing class-interval grouping into two class-intervals for each factor, upper and lower levels, 1 and 2 accordingly. If after dispersion analysis there were more than two deviations in null hypothesis and levels for a fixed factor, we applied Tukey's range test to perform multiple comparisons of simple means with the significance level being 0.05.

Health risks caused by wheat grain being contaminated with DON were assessed on the basis of comparing total daily DON intake with wheat-based foods and PMTDI DON. Estimated daily DON intake with wheat-based foods was calculated as per the following equation (1):

$$N_{calc} = \frac{M \cdot P \cdot 1000}{w}, \qquad (1)$$

[3] The authors express their deep gratitude to personnel of the Federal Service for Surveillance over Consumer Rights protection and Human Well-being and its Regional Centers for Hygiene and Epidemiology for long-term cooperation, selection and provision of food grain samples to be examined by experts from the Federal Research Center of Nutrition, Biotechnology, and Food Safety.

[4] GOST R ISO 24333-2011. Zerno i produkty ego pererabotki. Otbor prob [GOST R ISO 24333-2011.Cereals and cereal products – Sampling]. *KODEKS: the electronic fund for legal and reference documentation.* Available at: https://docs.cntd.ru/document/1200092274 (March 11, 2021) (in Russian).

[5] MU 5177-90. Metodicheskie ukazaniya po obnaruzheniyu, identifikatsii i opredeleniyu soderzhaniya dezoksinivalenola (vomitoksina) i zearalenona v zerne i zernoproduktakh [Methodical Guidelines 5177-90. Methodical guidelines on detecting, identifying and determining contents of deoxynivalenol (vomitoxin) and zearalenone in grain and grain products]. Available at: http://www.gostrf.com/normadata/1/4293828/4293828870.pdf (March 13, 2021) (in Russian).

[6] MVI 410/4-2020. Metod mul'tidetektsii mikotoksinov v zerne i pervichnykh produktakh ego pererabotki [Measuring procedures 410/4-2020. A procedure for multidetection of mycotoxins in grains and primary produces of its processing]. Moscow, 2020 (in Russian).

where N_{calc} is estimated daily DON intake (μg/kg of body weight); *M* is the average DON contents in wheat grains (mg/kg); *P* is consumption of processed wheat products (kg/day); *w* is body weight (kg); average body weight of a person is taken as 60 kg; 1,000 is conversion factor to converse into μg.

Results and discussion. ***Wheat grain contamination harvested in 1989–2018 all over the RF.*** Frequency of detection and average DON contamination in wheat varied from 0 to 42 % and from 0 to 0.43 mg/kg accordingly depending on a crop year (Table 1). In total, 10.0 % out of 4,009 grain lots harvested from 1989 to 2018 were contaminated; contamination levels varied from 0.05 to 6.65 mg/kg (Table 1). DON contents were higher than MPL in 101 (2.5 %) grain samples.

26 % samples from 1989 harvest contained DON and its contents exceeded MPL in 9 %. Frequency of DON contamination in 1992 was maximum, 42 % with the exceeding of MPL in 27 % cases. The frequency of DON detection in wheat harvested in 1993 was also high, it reached up to 24 % of the examined samples, and a share of samples with DON contents being higher than MPL reached 15 %. DON content in contaminated samples varied from 0.05 to 6.65 mg/kg in 1998; from 0.05 to 5.63 mg/kg in 1992; and from 0.1 to 3.95 mg/kg in 1993.

Table 1

Frequency and levels of DON contamination in wheat harvested in 1989–2018

Year	Number of samples			DON contents in contaminated samples, mg/kg	DON content in total samples, mg/kg		
	total, items	Contaminated DON	containing DON -exceeding MPL		*M*	*Me*	90 %
		items (% of the total number)					
1989	57	15 (26)	5 (9)	0.05 – 6.65	0.23	0	0.44
1990/91	67	4 (6)	1 (1)	0.05 – 0.74	0.02	0	0
1992	139	59 (42)	37 (27)	0.05 – 5.63	0.43	0	1.06
1993	156	38 (24)	24 (15)	0.10 – 3.95	0.22	0	0.63
1994	254	16 (6)	6 (2)	0.17 –0.96	0.03	0	0
1995	169	11 (6)	0 (0)	0.07 – 0.70	0.03	0	0
1996	120	15 (13)	0 (0)	0.06 – 0.70	0.02	0	0.07
1997	137	15 (11)	1 (0.7)	0.05 – 1.14	0.02	0	0.05
1998	126	12 (10)	1 (0.8)	0.05 – 1.09	0.03	0	0.03
1999	132	0 (0)	0 (0)	< 0.05	0	0	0
2000	222	6 (3)	1 (0.5)	0.09 – 0.77	0.01	0	0
2001	252	12 (5)	0 (0)	0.05 – 0.62	0.01	0	0
2002	158	6 (4)	1 (0.6)	0.05 – 0.78	0.01	0	0
2003	375	5 (1)	0 (0)	0.05 – 0.07	0	0	0
2004	213	2 (1)	0 (0)	0.07; 0.08	0	0	0
2005	147	12 (8)	0 (0)	0.07 – 0.69	0.02	0	0
2006	85	11 (13)	0 (0)	0.05 – 0.34	0.02	0	0.06
2007	98	9 (9)	1 (1)	0.06 – 0.91	0.05	0	0
2008	73	4 (5)	1 (1.4)	0.06 – 1.03	0.02	0	0
2009	109	8 (7)	0 (0)	0.06 – 0.12	0.01	0	0
2010	122	10 (8)	2 (2)	0.06 – 1.26	0.03	0	0
2011	158	13 (8)	0	0.05 – 0.44	0.01	0	0
2012	34	0 (0)	0	< 0.05	0	0	0
2013	111	9 (8)	0	0.05 – 0.52	0.01	0	0
2014	57	21 (37)	6 (10)	0.07 – 5.85	0.29	0	0.66
2015	64	6 (9)	0	0.05 – 0.33	0.01	0	0
2016	154	29 (19)	2 (1.3)	0.05 – 1.43	0.05	0	0.54
2017	105	32 (31)	9 (9)	0.05 – 2.46	0.18	0	0.51
2018	115	18 (16)	3 (2.6)	0.10 – 1.27	0.06	0	0.20
TOTAL	4,009	399 (10.0)	101 (2.5)	0.05 – 6.65	0.05	0	0.05

N o t e : DON MPL, mg/kg, is not higher than 0.7 for wheat (TR CU 015/2011 “Safety of grain”).

Over the next 20 years from 1993 to 2013 frequency of DON contamination in wheat was relatively rare and varied from 0 to 9 %. DON contents higher than MPL were detected in very few cases, and maximum DON content in contaminated samples amounted to 1.26 mg/kg. A diagram that showed harvests distributed as per average DON contents revealed a peak at 0.01–0.03 mg/kg.

Starting from 2014 frequency of wheat grain contamination grew to 9–37 % together with increasing numbers of samples with DON contents being higher than MPL. The only exception was the crop of 2015 due to lack of samples contaminated with DON at levels higher than MPL.

Therefore, wheat harvested in 1999, 2003, 2004 and 2012 turned out to be the least contaminated with DON since a share of contaminated samples didn't exceed 1 % and contamination levels were quite low. Frequency of DON contamination varied from 3 to 13 % in wheat harvested in 1990–1991, 1994–1998, 2000–2002, 2005–2011, 2013 and 2015; average DON contents were from 0 to 0.05 mg/kg; and 90-th percentile, from 0 to 0.07 mg/kg. Contamination with the toxin that was higher than MPL was detected in isolated samples harvested in 1990–1991, 1997, 1998, 2000, 2002, 2007, 2008 and 2010. A peak in DON contamination was detected not only in mass epiphytoties years (1989, 1992 and 1993) but also in recent years of 2014, 2016, 2017 and 2018. In these years frequency of DON contamination varied from 16 to 37 %; average DON contents, from 0.06 to 0.29 mg/kg; 90-th percentile, from 0.20 to 0.66 mg/kg. Grain samples with DON contents being higher than MPL were the most frequent in wheat harvested in 1989, 1992, 1993, 2014 and 2017 (9–27 % of all the examined samples).

Wheat contamination harvested in 1999–2018 throughout regions. DON contents in wheat were different depending on the region of grain production (Figure 1). Analysis of distribution of contaminated wheat harvested in 1999–2018 showed that 78 % samples (159 out of 205 contaminated samples) were grown in the Southern FD (Republics of Adygei and Kalmykia, Crimea, Astrakhan region, Volgograd region, and Rostov region) and the North Caucasian FD (the Republics of Ingushetia, North Ossetia – Alania, Kabardino-Balkaria, Karachay-Cherkessia, and Stavropol region); all these regions are primary areas in Russia where *Fusarium* head blight is widely spread in grains. DON contents varied from 0.05 to 5.85 mg/kg in these samples. 10 % of contaminated samples were received from the Far Eastern FD and 5 % from the Central FD. Frequency of DON contamination was significantly lower in harvests gathered in the Siberian, the Volga, the Northwestern, and the Ural FDs and amounted to 1–2 %.

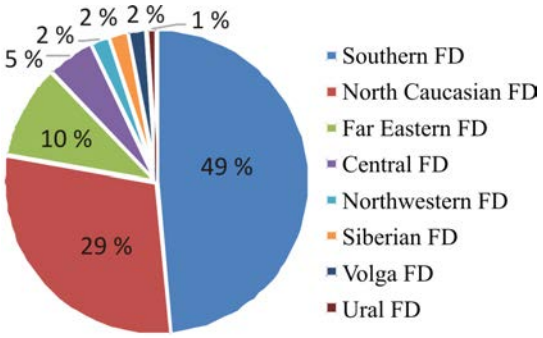

Figure 1. Region distribution of DON contaminated wheat harvested in 1999–2018 (% of the total contaminated samples)

According to Rosstat data, in 2018 the greatest contributions in total grain harvest belonged to Rostov region, 12.9 %; Krasnodar region, 12.4 %; and Stavropol region, 9.9 %. They were followed by Altai region and Volgograd region, 4.2 % each; Voronezh, Kursk, and Omsk region, from 3.1 to 3.8 %; Tatarstan, Lipetsk, Novosibirsk, Tambov, and Orel regionsб from 2 to 2.7 %; Orenburg, Penza, Kurgan, Tula, Chelyabinsk, Samara, and Krasnoyarsk regions – from 1.5 to 1.8 %[6]. As said above, grain produced in leading regions in wheat harvesting, namely Rostov, Krasnodar, and Stavropol regions, tended to be more heavily contaminated with DON than grain produced in other regions. Harvested wheat is further processed and consumed not only by people living in the Southern and the North Caucasian FDs but also by people living in other FDs; hence, DON intake due to consumption of contaminated wheat-based products can contribute significantly to DON in-

take with food products estimated for the country population as a whole.

Dynamics of grain contamination with DON was analyzed on the example of several wheat-producing regions in the RF; Table 2 contains the results.

From 1999 to 2018 wheat samples grown in ***the Southern FD*** mostly received from Krasnodar, Rostov, and Volgograd regions, and Adygei and Kalmyk Republics. DON grain contamination in these regions was significantly different depending on the area where grain was grown. For example, grain received from Kalmyk Republic and Volgograd region practically was not contaminated with DON or its content was very low. On the contrary, frequency of the toxin detection was the highest in grain received from Krasnodar region and Adygei Republic. Rostov region was somewhere in between as per this parameter within the Southern FD (Table 2).

Totally, 262 grain samples from Krasnodar region were analyzed; samples contaminated with DON were detected in 10 out of 17 harvests gathered in different years and frequency of detection varied from 0 to 79 % depending on a crop year. In some years a share of samples contained the toxin in concentration higher than MPL varied from 5.9 % (2016) to 42.9 % (2017) and maximum contamination levels reached 3.21 and 5.85 mg/kg.

65 grain samples from Adygei Republic were analyzed and DON was detected in grains from 8 out of 13 harvests gathered in different years in quantities varying from 0.08 to 0.78 mg/kg.

Table 2

Dynamics of wheat DON contamination harvested in 1999–2018 from different FDs in the RF

Region	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
The Southern Federal District (694 samples)																				
Krasnodar region (*n*** = 262)	3/0/0* (0)	4/0/0 (0)	**10/2/0 (20)**	1/0/0 (0)	5/0/0 (0)	3/0/0 (0)	1/0/0 (0)	**15/7/0 (47)**	**15/2/0 (13)**	–	–	**77/7/0 (9)**	8/0/0 (0)	–	**51/2/0 (4)**	**14/8/2 (57)**	**9/2/0 (22)**	**17/11/1 (65)**	**14/11/6 (79)**	**15/8/2 (53)**
Adygei Republic (n = 65)	–	**12/2/0 (17)**	**8/1/ 0 (13)**	**5/2/1 (40)**	**14/1/0 (7)**	–	–	–	–	1/0/0 (0)	–	–	**5/2/0 (40)**	–	1/0/0 (0)	**1/1/0 (100)**	**1/1/0 (100)**	1/0/0 (0)	1/0/0 (0)	**1/1/0 (100)**
Rostov region (*n* = 271)	25/0/0 (0)	**8/2/1 (25)**	**21/8/0 (38)**	**8/2/0 (25)**	11/0/0 (0)	**14/1/0 (7)**	**25/5/0 (20)**	**12/1/0 (8)**	7/0/0 (0)	5/0/0 (0)	**34/1/0 (3)**	4/0/0 (0)	–	–	25/0/0 (0)	8/0/0 (0)	**28/2/0 (7)**	**21/6/0 (29)**	7/0/0 (0)	8/0/0 (0)
The North Caucasian Federal District (333 samples)																				
Stavropol region (*n* = 215)	3/0/0 (0)	4/1/0 (0)	5/0/0 (0)	2/1/0 (0)	6/0/0 (0)	6/0/0 (0)	**4/2/0 (50)**	**2/1/0 (50)**	8/0/0 (0)	1/0/0 (0)	**16/1/0 (6)**	**9/1/0 (11)**	**17/2/0 (12)**	24/0/0 (0)	19/0/0 (0)	**17/7/3 (41)**	20/0/0 (0)	19/3/0 (0)	**15/9/1 (60)**	**18/2/1 (11)**
Kabardino-Balkaria, North Ossetia – Alania (*n* = 56)	–	–	–	–	–	–	–	–	**1/1/1 (100)**	–	**10/4/0 (40)**	–	**16/6/0 (38)**	–	**5/2/0 (40)**	**5/4/1 (80)**	**4/1/0 (25)**	1/0/0 (0)	**2/1/0 (50)**	3/0/0 (0)
Dagestan, Chechnya (*n* = 44)	–	2/0/0 (0)	–	–	–	–	**11/2/0 (18)**	–	**24/2/0 (8)**	2/0/0 (0)	–	–	2/0/0 (0)	–	–	5/0/0 (0)	–	–	–	–
The Far Eastern Federal District (56 samples)																				
Amur region (*n* = 12)	–	1/0/0 (0)	–	–	–	–	–	–	–	1/0/0 (0)	–	–	–	–	–	**1/1/0 (100)**	1/0/0 (0)	**2/2/1 (100)**	**3/3/1 (100)**	**3/2/0 (67)**
Primorye (*n* = 27)	–	1/0/0 (0)	–	1/0/0 (0)	–	–	**4/3/0 (75)**	**1/1/0 (100)**	**1/1/0 (100)**	**3/3/1 (100)**	**6/2/0 (33)**	**1/1/1 (100)**	–	–	–	–	–	–	**2/1/1 (50)**	–
The Central Federal District (693 samples)																				
Orel region (*n* = 150)	1/0/0 (0)	10/0/0 (0)	–	53/0/0 (0)	22/0/0 (0)	–	3/0/0 (0)	–	–	–	–	1/0/0 (0)	**20/1/0 (5)**	–	–	–	–	**6/1/0 (17)**	**5/2/0 (40)**	3/0/0 (0)
Voronezh region (*n* = 109)	7/0/0 (0)	10/0/0 (0)	–	7/0/0 (0)	11/0/0 (0)	14/0/0 (0)	15/0/0 (0)	5/0/0 (0)	**19/3/0 (16)**	–	–	1/0/0 (0)	–	–	–	–	–	–	3/0/0 (0)	3/0/0 (0)
Other regions (*n* = 434)	42/0/0 (0)	55/0/0 (0)	1/0/0 (0)	15/0/0 (0)	76/0/0 (0)	47/0/0 (0)	27/0/0 (0)	18/0/0 (0)	31/0/0 (0)	**28/1/0 (4)**	13/0/0 (0)	1/0/0 (0)	**17/1/0 (6)**	–	–	1/0/0 (0)	–	**17/1/0 (5)**	19/0/0 (0)	17/0/0 (0)
The Volga FD (331 samples)	1/0/0 (0)	29/0/0 (0)	4/0/0 (0)	69/0/0 (0)	23/0/0 (0)	65/0/0 (0)	5/0/0 (0)	5/0/0 (0)	10/0/0 (0)	13/0/0 (0)	5/0/0 (0)	3/0/0 (0)	42/0/0 (0)	10/0/0 (0)	–	–	–	18/0/0 (0)	**10/2/0 (20)**	**19/2/0 (11)**
The Siberian FD (266 samples)	2/0/0 (0)	48/0/0 (0)	55/0/0 (0)	19/0/0 (0)	33/0/0 (0)	25/0/0 (0)	9/0/0 (0)	–	4/0/0 (0)	3/0/0 (0)	8/0/0 (0)	10/0/0 (0)	**19/1/0 (5)**	–	–	3/0/0 (0)	–	5/0/0 (0)	**12/1/0 (8)**	**11/2/0 (18)**
The Ural FD (69 samples)	–	5/0/0 (0)	–	12/0/0 (0)	5/0/0 (0)	10/0/0 (0)	–	–	–	–	–	–	5/0/0 (0)	–	–	–	–	**21/2/0 (10)**	3/0/0 (0)	8/0/0 (0)
The North-western FD (35 samples)	–	16/0/0 (0)	–	1/0/0 (0)	1/0/0 (0)	7/0/0 (0)	–	–	–	–	1/0/0 (0)	–	1/0/0 (0)	–	–	–	–	**5/3/0 (60)**	**3/2/0 (67)**	–

N o t e : * means a number of examined samples, items / a number of samples contaminated with DON, items / a number of contaminated samples with DON contents being higher than MPL, items, (frequency of detection, %). ** is a number of samples received from a given region over the monitoring period from 1999 to 2018.

Grain samples (271 overall) from Rostov region were provided for monitoring purposes practically every year. DON was detected in grain from 9 out of 17 harvests gathered in different years in concentration varying from 0.05 to 0.77 mg/kg and frequency of its detection was lower than in Krasnodar region and Adygei Republic. DON contents were higher than MPL only in one out of 271 analyzed samples; it was a grain sample from the harvest gathered in 2000.

Grain samples from harvests gathered in 1999–2018 in ***the North Caucasian FD*** were mostly received from Stavropol region; samples from Kabardino-Balkaria, Dagestan, and North Ossetia–Alania were rarer (harvests gathered in 9 different years). In total 333 grain samples were received to be analyzed over the examined period; 215 out of them were from Stavropol region; 56 samples, from Kabardino-Balkaria and North Ossetia-Alania (Table 2). Frequency of DON detection in contaminated grain harvested in 1999–2018 in Stavropol region varied from 6 % (2009) and 60 % (2017) and maximum contents reached 1.56, 0.97 and 0.73 mg/kg in some grain samples in 2014, 2017 and 2018 crops accordingly. 9-year monitoring over wheat grain contamination in Kabardino-Balkaria and North Ossetia-Alania revealed the toxin contents in 7 out 9 examined grain harvests (Table 2). DON contents higher than MPL were detected in grains from harvests gathered in 2007, 2010 and 2014 and contamination levels reached 0.91, 0.71 and 0.83 mg/kg accordingly.

DON content was analyzed in 56 samples of wheat from harvests of 14 different years in ***the Far Eastern FD***. The toxin was detected in wheat from 9 out of 14 grain harvests gathered in Primorye and its contents exceeded MPL in some grain samples in 2008, 2010, 2016 and 2017 crops (Table 2). DON was detected in grains from 4 out of 7 harvests gathered in different years and its contamination levels were higher than MPL in grains in 2016 and 2017 crops.

DON was rarely detected in wheat from ***the Central FD.*** The toxin was detected in small quantities, from 0.05 to 0.16 mg/kg, in 1.4 % out of 693 examined samples of grains from harvests gathered in 20 different years.

Low (1.2 %) frequency of DON detection was revealed for grain samples received from ***the Volga FD***. The toxin was detected in quantities from 0.09 to 0.37 mg/kg in few samples out of 331 examined ones. It should be noted that samples contaminated with DON were from harvests gathered in 2017 and 2018.

Low (from 0.18 to 0.25 mg/kg) DON contents were detected in very few samples of grain from harvests gathered in 2011, 2017 and 2018 in ***the Siberian FD.***

DON contamination in grain received from ***the Ural*** and ***the Northwestern FDs*** was analyzed in few grain samples, 69 and 35 accordingly, from harvests gathered in 8 different years. Frequency of the toxin detection was 3 and 14 % accordingly. Contaminated samples were detected only in 2016 and 2017 crops. It should be noted that approximately 2/3 of samples received from the Northwestern FD, namely Kaliningrad region, were contaminated with DON in quantities varying from 0.14 to 0.46 mg/kg. High frequency of DON detection in Kaliningrad region is well in line with data collected in neighboring regions. Thus, DON was detected in 83 % out of 92 examined wheat samples received from Poland and harvested in 2016 in the amount from 0.01 to 1.27 mg/kg[2].

Therefore, analysis of dynamics of wheat grain contamination harvested in 1999–2018 in various RF regions revealed a growing trend for DON occurrence not only in the North Caucasian, the Southern, and the Far Eastern FDs where contamination was the highest during the survey but also in the North Western, the Siberian, and the Volga FDs recent years.

Analysis of environmental factors affecting the grain contamination with DON. Climatic and weather conditions are known to have their influence on DON accumulation by toxicogenic fungi of *Fusarium* genus [14, 15]. We estimated a correlation between grain contamination with DON (Figure 2) and several environmental factors on the example of grain samples from harvests gathered in 2016–2018 in Krasnodar region. The examined factors in-

cluded the following: average monthly humidity and temperature, a number of sunny hours (a period of time in each month when the sun was above the horizon in a given area and not hidden behind the clouds) and a number of days with precipitations exceeding 1 mm from May to August. Meteorological data were taken from "Aisori – remote access to DDL-archives", an online achieve that contained data on climatic research[7].

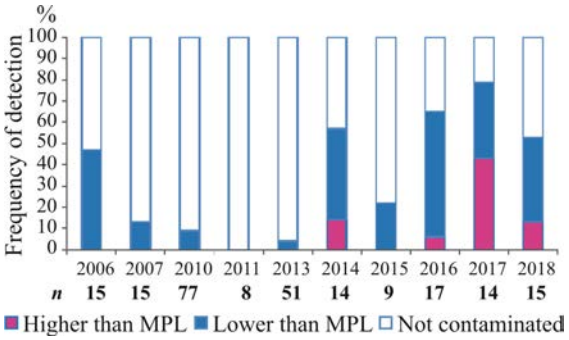

Figure 2. Dynamics of DON contamination in wheat harvested in 2006–2018 in Krasnodar region (*n* is a number of samples)

Correlation analysis confirmed there was an authentically significant correlation between frequency of the toxin detection and its average contents in contaminated grain samples harvested in 2006–2018: correlation coefficient was $r = 0.68$ and authenticity was $p = 0.02$ at the significance level $\alpha = 0.05$. Keeping in mind that variation coefficient for frequency of DON detection was significantly lower we took the former coefficient to use in further statistical analysis as a leading one. We didn't detect any correlation between frequency of DON detection and the examined weather factors from June to August. At the same time weather conditions in May, namely duration of sunny weather and a number of days with precipitations exceeding 1 mm authentically had the most significant influence on DON contamination, the significance level being 0.05 (Table 3). These data are well in line with the fact that wheat grain is the most susceptible to fungi infections during its milky ripeness and in Krasnodar region this period is from mid May to mid June. Clear and sunny weather and a decrease in a number of rainy days in May make for declining wheat contamination with DON and vice versa.

The detected correlation was confirmed with using Mann – Whitney non-parametric test. To apply it, we divided a parameter within a month into two class-intervals, upper and lower ones, and frequencies of DON detection were written down in a relevant class-interval. The analysis revealed that authenticity was higher than significance ($p > 0.05$) for average monthly air humidity and temperature in May; hence, they had only insignificant influence on contamination levels. But the significance was $p < 0.05$ for duration of sunny days and a number of rainy days with precipitation exceeding 1 mm and this indicates there is an authentic correlation between these two weather factors and grain contamination.

Table 3

Correlations between frequency of DON detection in wheat grain and examined weather conditions for harvests gathered in 2006–2018

Parameter	Correlation coefficient, *r* (authenticity, *p*)			
Month	May	June	July	August
Average monthly air humidity	0.49 (0.120)	–0.05 (0.868)	–0.22 (0.504)	–0.08 (0.811)
Average monthly air temperature	–0.46 (0.150)	–0.03 (0.916)	0.02 (0.946)	0.12 (0.718)
Duration of sunny hours per month	**–0.65 (0.029)**	–0.08 (0.805)	–0.07 (0.837)	–0.09 (0.782)
A number of days with precipitations exceeding 1 mm	**0.74 (0.008)**	–0.21 (0.525)	–0.11 (0.747)	–0.11 (0.756)

[7] Veselov V.M., Pribyl'skaya I.R., Mirzebasov O.A. Spetsializirovannye massivy dlya klimaticheskikh issledovanii. «Aisori – Udalennyi dostup k YaOD-arkhivam [Specialized data arrays for climatic research. "Aisori – remote access to DDL-archieves"]. VNIIGMI-MC Aisori D. Available at: http://aisori-m.meteo.ru/waisori/index0.xhtml (March 03, 2021) (in Russian).

Table 4

Two-factor dispersion analysis of a grain quality category, year of a harvest, and contamination

Dependent variable (DON content in a sample)					
Source	Sum of type III squares	Degree of freedom	Mean square	*F*-criterion	Significance
Adjusted model	3.422[a]	3	1.141	1.300	0.284
Year	0.503	1	0.503	0.573	0.452
Grain quality category	0.811	1	0.811	0.924	0.341
Year × category	2.034	1	2.034	2.319	0.134
Error	47.380	54	0.877		
Total	68.325	58			
Adjusted result	50.802	57			
[a] – R-square = 0.067 (Adjusted R-square = 0.016)					

Table 5

Frequency and levels of DON contamination in food barley, oats, rye, and corn harvested in 1989–2018

Grain	Number of samples			DON contents in contaminated samples, mg/kg	DON contents in total samples, mg/kg		
	Total, item	Containing DON	DON contents higher than MPL		*M*	*Me*	90 %
		items (% of total number)					
Barley	1,293	54 (4.2)	5 (0.4)	0.05 – 8.95	0.02	0	0
Corn	278	33 (11.9)	MPL not fixed	0.05 – 0.95	0.04	0	0.07
Oats	200	6 (3.0)	MPL not fixed	0.06 – 0.96	0.01	0	0
Rye	1,020	6 (0.6)	MPL not fixed	0.06 –0.44	0.00	0	0

N o t e : DON MPL, mg/kg, not exceeding 1.0 for barley (TR CU 015/2011 “Safety of grain”).

There was a growth in grain contamination observed from 2006 to 2018. Correlation analysis revealed a directly proportional correlation between a year of a harvest and frequency of DON detection: correlation coefficient was $r = 0.60$ and authenticity was $p = 0.049$ at the significance level $\alpha = 0.05$. Although it seems hardly possible to use this correlation to predict future situation due to $\alpha \approx p$, we still can’t ignore it.

We performed two-factor dispersion analysis of a grain quality category, a year of a harvest, and DON contents in a sample (Table 4). A year of a harvest was used as fixed factor A, and grain quality category, fixed factor B.

Analysis of barley, oats, rye and corn contamination harvested in 1989–2018. Aggregated data on DON contamination in barley, oats, rye, and corn harvested in 1989–2018 are given in Table 5. DON contamination ***in food barley*** was examined on the example 1,293 grain samples harvested in 1989–2018. Frequency of DON contamination in barley was lower than in wheat (10 %) and corn. DON was detected in 4 % samples (54 overall) in quantities varying from 0.05 to 8.95 mg/kg, and 5 samples contained DON in quantities higher than MPL. DON was detected most frequently in grains in 2017 and 2014 crops, 44 and 30 % accordingly, and this frequency was substantially lower in grains harvested in 2015 (22 %), 2016 (19 %), 1989 (16 %), 1992 and 2009 (14 % in both these years). In other years, frequency varied from 0 to 7 %. Notably, over the recent years, starting from 2014, DON contamination in barley has been growing.

Approximately 80 % of all barley production is located in the Central, the Volga, the Southern and the Siberian FDs [24]. The greatest number of samples was received from the Central and the Southern FDs. The toxin was detected in 11 % samples from the Southern FD in quantities varying from 0.05 to 1.7 mg/kg and its contents were higher than MPL in one sample of grain harvested in 2017. Barley received from Krasnodar region was the most contaminated with DON was, since 23 % out of 39 samples were contaminated

with DON in the amount from 0.05 to 1.7 mg/kg; the least contaminated barley was received from Rostov region: 7.5 % out of 40 samples were contaminated with DON in the amount from 0.05 to 0.11 mg/kg. According to our data the toxin was most frequently detected in barley received from the North Caucasian FD; DON was detected in 17 % of 64 examined samples, in particular, in low quantities in samples from Stavropol region (0.10 and 0.11 mg/kg). The range of DON contamination in grain from Kabardino-Balkaria was wider, from 0.07 to 8.95 mg/kg with exceeding MPL DON in grain harvested in 2017. DON in low quantities was detected in rare cases in grain received from Ingushetia and North Ossetia.

Although samples from the Far Eastern FD were sent for analysis irregularly and in relatively few numbers, approximately half of examined samples (10 out of 21) were contaminated with DON in quantities varying from 0.05 to 2.83 mg/kg with exceeding of MPL in one sample harvested in Amur region in 2018. Barley lots harvested in 2005–2009 in Primorye contained the toxin in low quantities, from 0.06 to 0.21 mg/kg.

Our research results revealed that, just as it was the case with wheat, samples received from the Far Eastern, the North Caucasian, and the Southern FDs were the most contaminated.

Corn grain was similar to wheat grain (11.98 % against 10 %) as per DON contaminations. DON contents in contaminated corn samples varied from 0.05 to 0.95 mg/kg (average contents amounted to 0.04 mg/kg). For reference, DON contents in contaminated wheat samples amounted to 0.05–6.65 mg/kg. Having analyzed dynamics of domestic corn contamination with DON, we revealed that DON occurred only in grains harvested in 2000, 2002, and 2012–2018, and the highest frequency of DON contamination was detected in recent years (2014, 2016–2018).

The Southern, the North Caucasian and the Central FDs are basic corn producers in Russia[8]. The greatest number of samples was sent for analysis from the Southern (217) and the North Caucasian FDs (47). The toxin was the most frequently detected in corn from the North Caucasian FD, namely, in 32 % out of 47 examined lots, in quantities varying from 0.05 to 0.68 mg/kg, 0.09 mg/kg on average. The toxin was detected less frequently, namely, in 6 % out of samples received from the Southern FD; its amount varied from 0.05 to 0.95 mg/kg, 0.02 mg/kg on average. DON was detected in rare corn lots received from the Central FD in quantities equal to 0.29 and 0.32 mg/kg. It should be noted that 4 out of 6 examined corn samples received from the Far Eastern FD was contaminated with DON in quantities varying from 0.13 to 0.55 mg/kg.

Oats contamination with DON was examined in 200 samples of grains harvested in 1999–2018. The toxin was detected in 3 % cases in quantities from 0.05 to 0.96 mg/kg, 0.008 mg/kg on average (Table 5). DON was detected in grains harvested in 2009, 2016 and 2017.

It should be noted that approximately 39 % of overall oats produced in the country is grown in Altai, Krasnoyarsk, Novosibirsk, Tyumen, Kemerovo, and Omsk regions and Bashkortostan [25]. The toxin was not detected in samples received from these regions. Contamination was detected only in samples from the Far Eastern (4 cases) and the North Caucasian FD (2 cases). Oats samples from Amur region and Primorye were the most contaminated with DON, its amounts varying from 0.09 to 0.96 mg/kg. The toxin was less frequently detected and in lower quantities in oats received from Ingushetia and Stavropol region (0.06; 0.09 mg/kg accordingly).

Generally the toxin was detected in ***rye*** samples were received from the North Caucasian FD during epiphytoties (harvests gathered in 1989 and 1992). The toxin was detected in rare cases in low quantities in grains harvested in 1996 and 2007, at the level of 0.22 and 0.06 mg/kg in the North Caucasian and the Central FDs accordingly.

[8] Sel'skoe khozyaistvo v Rossii. 2019: statisticheskii sbornik [Agriculture in Russia. 2019: statistical data collection]. *Rosstat*. Moscow, 2019, 91 p. (in Russian).

Approximately 74.4 % of the total grain harvest of rye in the country is grown in the Volga FD, Bashkortostan, Tatarstan, Orenburg and Saratov regions [24]. Rye samples received from these regions were not contaminated with DON.

According to monitoring results, it can be concluded that wheat and corn are the most frequently contaminated with DON among grain, and, barley to a lesser extent. The growth in DON occurrence in grains in recent years (2014, 2017 and 2018) could be explained not only by weather conditions auspicious for toxin production by *Fusarium* genus but also by changes in *F. graminearum* areas. According to Gagkaeva and others (2014), over the last few years these fungi spread into new territories located to the north from their usual habitat [13]. Starting from 2003, resistant populations of *F. graminearum* species occurred in Primorye and North Caucasus, spread into new areas, for example, into the north-western part of Russia, during local weather changes characterized with climatic conditions auspicious for microfungi [24]. The authors believe that the existing climate warming, especially in winter, makes for *F. graminearum* survival in new areas and the fungi get adapted to colder environment [13]. Agriculture intensification might be another reason for growing DON contamination.

Monitoring results confirmed that the Southern, the North Caucasian, and Far Eastern FDs remained the primary areas where *Fusarium* head blight was widely spread. At the same time in 2016–2018 frequency of DON detection grew in areas located to the north from the aforementioned regions.

Assessment of health risks caused by DON contaminations in food wheat grains. Wheat is a primary source of DON intake with food. When calculating probable DON intake into the human body, we used average mycotoxin contents in samples of grains harvested in the same year (*M*, Table 1). Toxin intake per person was calculated within a scenario when DON contents in food products made from processed wheat corresponded to its contents in grain; that is, processing didn't produce any significant effects on DON contents in a finished product. This approach corresponds to results obtained in studies on stability of the toxin during food processing and cooking: when grain is refined, the toxin contents usually remain the same or go down to 22–23 % [15, 27]; any thermal treatment doesn't influence DON contents [15, 27, 28].

Data on consumption of wheat products (wheat bread, grocery, flour confections, wheat flour, semolina, and macaroni) were taken from budget statistical reports issued by the Federal State Statistic Service and based on a sample survey over budgets spent by households[9]. Average consumption of food products made of processed wheat decreased over the last 25 years, from 267 g in 1993 to 209 g in 2018; it was taken into account when DON intake was calculated. Average person's weight was taken as equal to 60 kg.

Calculated total DON intake per person on average in Russia was significantly different from year to year. DON intake varied from 0.2 % (in 1999) to 140 % (in 1992) of DON PMTDI but remained lower than fixed DON PMTDI, 1 μg/kg of body weight, in the most cases except in 1992 (140 %) and 2014 (102 %) (Table 6) [20].

We should note that high average calculated DON intake with food products made of wheat harvested in 2014 for the country population didn't differ significantly from the intake calculated for the most risky regions; it was partly due to the fact that 88 % of all grain samples for analysis were received from the Southern and the North Caucasian FDs. More profound analysis of estimated daily DON intakes revealed that toxin intake was higher in the Southern and the North Caucasian FDs than on average in the country during the survey and varied from 3 % (in 2004 and 2009) to 410 % of PMTDI (in 1992). Calculated DON intake was also higher than PMTDI in the

[9] Potreblenie produktov pitaniya v domashnikh khozyaistvakh (byulleten') [Food products consumption in households (bulletin)]. *Rosstat*. Available at: https://rosstat.gov.ru/compendium/document/13292 (March 03, 2021) (in Russian).

Table 6

Calculated daily DON intake with food products made of wheat grain harvested in 1989–2018

A harvest year	Estimated daily intake ($N_{calc.}$), μg/kg of b.w. a day (% of PMTDI)	
	On average in Russia	The Southern and the North Caucasian FD
1989	0.96 (96.0)	–
1990–1991	0.07 (7.0)	–
1992	**1.40 (140.0)**	**4.10 (410.0)**
1993	0.89 (89.0)	**2.18 (218.0)**
1994	0.12 (12.0)	0.29 (29.0)
1995	0.12 (12.0)	0.68 (68.0)
1996	0.07 (7.0)	0.18 (18.0)
1997	0.08 (8.0)	0.20 (20.0)
1998	0.12 (12.0)	0.48 (48.0)
1999	0.002 (0.2)	–
2000	0.04 (0.4)	0.30 (30.0)
2001	0.036 (3.6)	0.22 (22.0)
2002	0.028 (2.8)	0.28 (28.0)
2003	0 (0)	0 (0)
2004	0.004 (0.4)	0.03 (3.0)
2005	0.084 (8.4)	0.36 (36.0)
2006	0.096 (9.6)	0.18 (18.0)
2007	0.066 (6.6)	0.24 (24.0)
2008	0.07 (7.0)	0.17 (17.0)
2009	0.025 (2.5)	0.03 (3.0)
2010	0.18 (18.0)	0.14 (14.0)
2011	0.04 (4.0)	0.12 (12.0)
2012	0 (0)	0 (0)
2013	0.04 (4.0)	0.04 (4.0)
2014	**1.02 (102.0)**	**1.12 (112.0)**
2015	0.05 (5.0)	0.05 (5.0)
2016	0.16 (16.0)	0.26 (26.0)
2017	0.63 (63.0)	**1.09 (109)**
2018	0.12 (12.0)	0.56 (56.0)

Southern and the North Caucasian FDs in 1993, 2014, and 2017 and accounted for 218, 112 and 109 % of PMTDI accordingly. The aforementioned ascending trend in wheat contamination calls for implementing necessary activities aimed at control over food grain safety.

Conclusions. We have analyzed the results obtained through long-term monitoring over food grain contamination with mycotoxins accomplished by the Federal Service for Surveillance over Consumer Rights Protection and Human Well-being with active participation by the Federal Research Center of Nutrition, Biotechnology, and Food Safety. The analysis confirmed that grains grown in the Southern, the North Caucasian, and the Far Eastern FDs were the most susceptible to DON contamination. At the same time, there is a growth in frequency of DON detection in grains grown in more northern regions and this indicates that *Fusarium* head blight is spreading over new areas and grain contamination with mycotoxins producers is becoming more probable. This area of contamination can increase when grain is transported from one region to another and this means it is necessary to control grain quality, primarily, when it is used as seed grain.

The highest frequency of DON detection and highest DON contents are typical for wheat and corn among all examined grains. It was shown that calculated daily DON intake with food wheat products was higher than PMTDI DON set by JECFA in some years over the examined period. DON intake was more likely to exceed PMTDI for people living in the Southern and the North Caucasian FDs.

To reduce health risks caused by food grain contamination with DON, it is vital to implement activities aimed at stricter control over spread of *Fusairum* head blight in the RF. Special attention should be paid to monitoring over safety of wheat and corn food grains, in particular, those grown in the Southern and the North Caucasian FDs.

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Research article

ASSESSMENT OF THE CONTRIBUTION MADE BY ONCOGENIC FACTORS TO THE RISK OF MALIGNANT NEOPLASMS DEVELOPMENT FOR THE URBAN POPULATION OF WORKING AGE

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High morbidity and mortality caused by malignant neoplasms in various age groups among urban population remains a pressing issue that public healthcare has to solve in contemporary megacities.

This research concentrated on assessing significance of oncogenic risk factors that could cause malignant neoplasms (MNs) occurrence among urban population aged 18–64 inclusively.

766 people living in an administrative and industrial center in Siberia took part in the research; MNs were diagnosed in 367 out of them (the test group). The research involved calculating individual carcinogenic risks. Oncogenic factors, both environmental and occupational ones, were examined for each patient; attention was also paid to peculiarities of medical and biological characteristics as well as tobacco smoking and alcohol intake. Impacts exerted by the examined factors on risks of MNs occurrence were determined via odds ratios with estimated confidence intervals.

First detected MNs frequency amounted to 638.9 ± 41.9 cases per 100 thousand people among women aged 18–64, and 532.6 ± 41.9 cases among men ($p > 0.05$). The following factors were determined as ones causing elevated risks of MNs occurrence: carcinogenic hazards at a workplace; hereditary predisposition; tobacco smoking; alcohol abuse; female sex; living under exposure to chemical carcinogens in the environment. Women tended to have certain diseases that are conventionally considered possible risk factors (viral hepatitis B and C and pancreatic diabetes) 1.3 and 2.4 times more frequently than men.

All the obtained data can be used for developing a targeted program aimed at malignant neoplasms prevention among urban population and they can also become a vital component in a system for managing risks of malignant neoplasms occurrence on a given territory.

Key words: *urban population of working age, malignant neoplasms, individual carcinogenic risks, oncogenic risk factors, tobacco smoking, sex, heredity.*

Malignant neoplasms (MNs) are among priority diseases as per their medical and social significance; they create negative demographic balance not only in the Russian Federation [1, 2] but also in many countries all over the world [3–5]. According to estimates by the World Health Organization, in 2016 MNs caused 9.0 million death cases accounting for 22 % of mortality due to non-communicable diseases [4]. Statistic data collected in the RF indicated that levels of oncologic morbidity taken as a ratio between male and female population were different in different age groups; these ratios amounted to 0.6 among people aged 15–29; 0.4, among people aged 30–39; 0.6, among people aged 40–49; 1.0, among people aged 50–59; 1.6, among people aged 60–69; 1.9, among people aged 70–79; 1.7, among people aged 80 and older [1].

Partial or total loss of health owing to MNs results in declining working capacities and, consequently, labor resources deficiency, the latter being a serious threat to the national security of the country. Substantial economic expenses on oncologic pathology treatment are a significant aspect related to the issue. In

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2016 integral economic losses caused by malignant neoplasms burden amounted to 241.3 billion rubles which was equal to 0.3 % of the country GDP. And there are hidden or unaccounted losses of working capacities by family members who have to take care of a patient with oncologic pathology and this leads to drastic changes in their working routines [6]. Therefore, issues related to MNs prevention are of great social and economic significance in the Russian Federation.

Primary prevention of malignant tumors occupies a most significant place in fight against cancer. Activities within such prevention should primarily be aimed at eliminating oncogenic environmental factors. At present there are multiple available research works that focus on detecting peculiarities of oncologic pathology occurrence among various population groups [7–12]. It was shown that a great contribution to adverse influence on health was made by chemical contamination of the environment, eating habits, working conditions, living conditions, education, and lifestyle. According to several researchers, environmental factors, primarily occupational ones, play the leading role (up to 75–80 %) in MNs occurrence and development [10, 11]. Ambient air pollution with chemicals is responsible for 41 % of respiratory diseases and more than 13 % of oncologic diseases among employable population [13]. Socioeconomic conditions are also thought to be a significant factor causing a risk of MNs occurrence [12, 14]. Influence exerted by this factor becomes possible due to multiple interrelated elements including material and social resources, physical and psychosocial stressors, and health-related behavior. Tobacco smoking is a basic risk factor of cardiovascular diseases and MNs and has negative social, ecologic, and economic consequences. In 2016 more than 1.1 billion people worldwide aged 15 or older were smokers (34 % of all men and 6 % of all women in this age group); in the RF, 58.3 % and 23.4 % accordingly [15]. Besides, socioeconomic status is tightly associated with smoking and alcohol abuse, the leading causes of malignant neoplasms in the airways and gastrointestinal tract [16]. In 2016 in the USA approximately 42.0 % of all MNs cases and 45.1 % death cases due to tumors were caused by risk factors [12]. According to estimates provided by Islami F. with colleagues, cigarette smoking caused the highest share of morbidity with MNs, 19.05 %, and mortality due to them, 28.87 %; overweight (7.8 % and 6.5 % accordingly) and alcohol intake (5.6 % and 4 % accordingly) were less significant [12]. It should be mentioned that complex effects produced by oncogenic factors result in higher risks [9, 17, 18]. MNs are considered to be more typical among elderly people; however, MNs of breast, ovary, lungs, thyroid gland, and the central nervous system become crucially frequent among people of working age [1, 8, 12] and this predetermines specific social significance of this nosology. It should be noted that knowing basic manageable factors that cause a significant share of morbidity and mortality due to malignant neoplasms gives grounds for primary and secondary prevention and provides relevant information for population and workers who are exposed to technogenic carcinogens. The most significant factors are revealed by using some statistical procedures; experience gained in their application as well as their advantages and drawbacks are described in several papers [19, 20].

Our research aim was to estimate significance of oncogenic risk factors for malignant neoplasms occurrence among urban population aged 18–64 inclusively.

Data and methods. The research was accomplished in an administrative and industrial center in Siberia with its population being equal to 430 thousand people (women accounted for 54 %); it included three stages. The first stage involved creating the test group (people with diagnosed MNs); to do that, we analyzed 8,206 individual case files on patients provided by a local oncologic dispensary with data collected in 2006–2016. Data were taken from the Form No. 030-6/u "Control case history of regular check-ups (onco)". Primarily diagnosed morbidity with MNs over years was given as an average an-

nual value with value error per 100 thousand people of relevant sex and age. The examined group was made up of 254 thousand people aged from 18 to 64 inclusively and this allowed creating representative samplings to assess risk factors. All patients permanently lived in Ulan-Ude; their age was within 18–64 inclusively.

At the second stage we calculated a necessary sampling to examine significance of risk factors. Given the gender ratio of population in their working age in the examined city, groups should include not fewer than 364 people according to [21]. 367 people were included into this study; they all applied to the local oncologic dispensary in 2014–2016, their diagnoses were confirmed, all medical documents were completed; age of all these people was within the selected range and they all lived in the examined city. The reference group was randomly created using data obtained through regular check-ups in 2016 and included 399 people without any diagnosed MNs.

This stage also involved individual questioning that was accomplished in person. Accounted risk factors included a place of residence, workplace, and bad habits; significant exposure levels were determined for tobacco smoking and alcohol intake and they were as follows: half a cigarette pack smoked daily for not less than 1 year and 1 standard drink 5 times a week [21]. The questioning provided necessary data on etiologically significant communicable diseases in a case history (viral hepatitis *B* and *C*, human papillomavirus); we also were able to find out whether a patient had had tuberculosis, HIV-infection and type II diabetes in the past or had one or several of these diseases at the moment of the research. Hereditary predisposition to MNs was taken into account in case they were diagnosed in patient's parents, children, or siblings.

Individual carcinogenic risks were calculated according to the procedure stipulated by the Guide R 2.1.10.1920-04[1] using data collected at monitoring posts of a hydrometeorology center, environmental monitoring system, production control, and results we obtained in our own examinations of qualitative and quantitative characteristics of workplace air and ambient air as well. Individual carcinogenic risk was assessed taking into account actual place of living for people who didn't work at productions with carcinogenic hazards; this individual carcinogenic risk reflected total aggregated ambient air pollution with substances that had oncogenic properties. We calculated individual carcinogenic risk that reflected total workplace air pollution (ICR_{wa}) for workers who were employed at major productions with carcinogenic hazards; the calculation procedure is described in details in our previous research [11, 22]. Frequency of a certain occurrence in examined groups was compared using Fischer's exact test, differences were considered statistically significant at $p < 0.05$.

Odds ratio (*OR*) and its confidence interval (*CI*) were calculated at the third stage to reveal significant risk factors of MNs occurrence in people aged 18–64. Factors with their bottom limit being $OR > 1$ were considered significant. We applied χ^2 test with Yates's correction to confirm that *OR* was statistically significant with critical significance being taken at $p < 0.05$.

MNs of trachea, bronchus, and lungs (ICD10 code is C34) were selected to estimate economic losses related to occurrence of MNs caused by exposure to adverse environmental factors. First of all, it was due to great frequency of this MNs localization (in 2014–2016 there were 70 cases among men and 9 cases among women in the examined age group) and substantial losses that, according to Kontsevaya and others [6], amounted to 73,501.2 million rubles in the RF in 2016. Total losses and ex-

[1] R 2.1.10.1920-04. Rukovodstvo po otsenke riska dlya zdorov'ya naseleniya pri vozdeistvii khimicheskikh veshchestv, zagryaznyayushchikh okruzhayushchuyu sredu [R 2.1.10.1920-04. Human Health Risk Assessment from Environmental Chemicals]. Moscow, The Federal Center for State Sanitary-Epidemiologic Surveillance of the RF Public Healthcare Ministry, 2004, 143 p. (in Russian).

penses borne by the examined settlement were calculated for men and women as per the following formula:

$$\text{ELE} = (N/3) \cdot \text{ELE (RF)}, \quad (1)$$

where $N/3$ is average annual number of MNs cases, ELE (RF) (economic losses and expenses) are average expenses per 1 case in the Russian Federation (1,202.675 thousand rubles [6]).

Population-attributed fractions (exposure levels) (*paf*) were calculated for specific risk factors as per the formula [23]:

$$paf = [E\,(OR - 1)] / [E\,(OR - 1) + 1], \quad (2)$$

where E is a population fraction exposed to a factor, OR is odds ratio for a disease developing in an exposed population group.

Bearing in mind that C34 nosology was caused by multiple risk factors, we applied the following formula to calculate combined *paf* in a multi-factor system:

$$\text{Combined } paf = 1 - [(1 - paf_1)(1 - paf_2)\ldots(1 - paf_n)], \quad (3)$$

where $paf_{1,2..,n}$ are exposure levels (population-attributed fractions) for specific factors (1.., n).

Results and discussion. We established that primarily diagnosed morbidity with MNs amounted to 638.9 ± 41.9 cases per 100 thousand people among women aged 18–64 living on the examined territory; 532.6 ± 41.9 cases among men per 100 thousand people of corresponding sex and age ($p > 0.05$). There were no statistically significant differences in frequency of most risk factors between men and women with MNs in the examined age group (18–64) (Table 1).

Frequency of active smoking was the only exception since each third male patient had this bad habit and it was 8 times more frequently than among women ($p = 0.000$). Certain diseases that the International Agency for Research on Cancer (IARC) considered to be probable carcinogenic risk factors were more frequent among women than among men: viral hepatitis *B, C*, by 1.3 times; pancreatic diabetes, by 2.4 times (however, these differences were not statistically significant). Since sex-dependent differences in frequency of risk factors were not significant, the second stage involved comparison between two groups.

We compared frequency of risk factors among patients who were treated at the local oncologic dispensary and among people who didn't have MNs (the reference group); Table 2 contains the comparison results. People with diagnosed MNs worked at productions with carcinogenic hazards and were occupationally exposed to carcinogenic risk factors 3.4 times more frequently than people from the reference group. The questioning revealed that several risk factors were more frequent among patients of the local oncologic dispensary than among people without diagnosed MNs; MNs were diagnosed in relatives 2.5 times more frequently; tobacco smoking, 2.2 times; alcohol abuse, 1.8 times; living on a territory where ICR_a was high, 1.1 times. Besides, it should be noted that women accounted for 62.3 % among all people of working age who had MNs and only for 53 % in the reference group.

We revealed the most significant risk factors by analyzing randomly selected individual primary medical documents of patients who were treated at the local oncologic dispensary and people who had regular medical check-ups and didn't have any diagnosed oncologic disease (Table 3).

Odds ratio was higher than 1 for all examined risk factors that caused MNs. However, an association between MNs and previous diseases or diseases a patient had at the moment of examination was not statistically significant (*OR* bottom limit was lower than 1). Risk factors were ranked as per their significance in the following way: occupational carcinogens > burdened heredity > tobacco smoking > alcohol abuse > sex > living on a territory with elevated ICR_i.

Economic losses were calculated for MNs of trachea, bronchus, and lungs that are

Table 1

Frequency of risk factors causing malignant neoplasms development among people aged 18–64 with diagnosed MNs (per 100 people)

Risk factors	Frequency (confidence interval)			*p
	men (n = 3,496)	women (n = 4,710)	both sexes (n = 8,206)	
Exposure to chemical carcinogens at workplace	14.2 (11.7–16.7)	8.9 (8.4–11.4)	12.1 (10.3–14.0)	0.15
Tobacco smoking	33.3 (29.9–36.7)	6.5 (4.4–8.6)	22.5 (20.2–24.8)	**0.00**
Burdened heredity	10.4 (8.3–12.5)	10.6 (7.9–13.3)	10.5 (8.9–12.1)	0.95
Viral hepatitis *B, C* in case history	3.8 (2.4–5.2)	4.9 (3.0–6.8)	4.2 (3.1–5.3)	0.64
Tuberculosis in case history	2.7 (1.5–3.9)	2.4 (1.0–3.8)	2.6 (1.7–3.5)	0.87
Pancreatic diabetes in case history	2.7 (1.5–3.9)	6.5 (4.4–6.6)	4.2 (3.1–5.3)	0.13

Note: * is statistical significance of differences between frequency among men and women.

Table 2

Frequency of risk factors causing malignant neoplasms development among urban population aged 18–64 (per 100 people)

Factors	Frequency (confidence interval)		*p
	People with oncologic disease (n = 367)	People without oncologic disease (n = 399)	
Exposure to chemical carcinogens at workplace	17.1 (15.2–19.0)	5 (3.9–6.1)	**0.000**
Tobacco smoking	18.8 (16.8–20.8)	8.7 (7.3–10.1)	**0.000**
Burdened heredity	12.5 (10.8–14.2)	5 (3.9–6.1)	**0.000**
Viral hepatitis *B, C* in case history	6.5 (5.2–7.8)	3.7 (2.8–5.5)	0.083
Tuberculosis in case history	2.6 (1.6–3.4)	1.7 (1.1–2.3)	0.417
Pancreatic diabetes in case history	4.2 (3.2–5.2)	2.3 (1.6–3.0)	0.118
Living under exposure to chemical carcinogens	76.4 (74.2–78.6)	70 (67.8–72.2)	**0.044**
Alcohol abuse	10.4 (8.8–12.0)	5.7 (4.6–6.8)	**0.019**
Female sex	62.3 (59.8–64.8)	53 (51.6–55.4)	**0.003**

Note: * is statistical significance of differences in frequency between the two groups, people with or without oncologic disease.

Table 3

Risk factors that cause malignant neoplasm development among urban population aged 18–64

Factors	*OR (CI)*	χ^2	*p*
Exposure to chemical carcinogens at workplace	3.97 (2.33–6.64)	28.0	0.000
Tobacco smoking	2.41 (1.16–3.92)	15.5	0.000
Burdened heredity	2.72 (1.57–4.69)	12.8	0.000
Viral hepatitis *B, C* in case history	1.79 (0.92–3.47)	2.5	0.114
Tuberculosis in case history	1.41 (0.52–3.82)	0.2	0.641
Pancreatic diabetes in case history	1.85 (0.79–4.27)	1.5	0.213
Living under exposure to chemical carcinogens	1.41 (1.02–1.94)	3.9	0.047
Alcohol abuse	1.89 (1.11–3.25)	4.9	0.027
Female sex	1.48 (1.11–1.97)	7.7	0.010

considered to be primarily caused by tobacco smoking and exposure to chemical carcinogens in workplace air and ambient air [9, 13, 15]. Contribution of oncogenic factors to economic losses due to malignant neoplasms of trachea, bronchus, and lungs among urban population aged 15–64 was ranked depending on gender [23]. Ranked *paf* for men were as follows: tobacco smoking (*paf* = 0.978), occupational exposure to carcinogens (0.97), exposure to carcinogens in ambient air (0.93); for women, occupational exposure to carcinogens (*paf* = 0.962), exposure to carcinogens in ambient air (0.93), tobacco smoking (0.9).

Growing MNs prevalence in various population groups predetermines not only medical significance of this pathology but also social and economic ones related to demographic issues becoming more and more serious (losses among overall and employable population), persistent loss of working capacities and disability, expenses borne by the society and a person on medical and social aid provided for oncologic patents. All this makes studies aimed at examining both clinical and hygienic and epidemiologic aspects of the issue more and more vital [8, 24].

Prevalence of certain risk factors varies depending on age and race / ethnic group. White M.C. with colleagues provided evidence that in the USA alcohol abuse was typical for each forth man and each eighth woman; tobacco smoking, each fifth and each seventh accordingly [25]. In our examined subpopulation frequency of the examined risk factors was rather different. Thus, one of 10 men and one of 20 women stated that they abused alcohol; each third examine man and one of 15 examined women smoked. It is probably due to a wider age range examined in our research (18–64 against 18–44 in the USA). Age-related factor can be quite significant since a share of people who abused alcohol was established to decline among people from older age groups [25]. Besides, data on frequency of smoking and alcohol abuse in the USA were obtained through a target survey whereas our estimates were based on questioning during a visit to a doctor and this indicates there are certain limitations in accomplished comparisons.

Most types of cancer are considered to be caused by a combination of factors with lifetime influence [26]. Cancer prevention is based on describing cancer burden, detecting reasons that cause it, assessing and implementing prevention activities. Approaches to cancer prevention should take into account non-genetic exposure that changes over time. Actions to reduce prevalence of adverse risk factors among young people can prevent or at least delay new cancer cases in future [27] and it is extremely important to assess contributions made by leading risk factors into morbidity for proper assessment of balance between expenses and benefits.

In the RF structure of morbidity with malignant neoplasms among men aged 30–59 is fundamentally different from the structure of morbidity among women of the same age. Neoplasms of trachea, bronchus and lungs (16.7 %), skin with melanoma (10.5 %), stomach (7.0 %), kidney (6.9 %) and lymphoid and hematopoietic tissue (6.7 %) prevail among men; neoplasms of breast (27.2 %), cervix uteri (10.6 %), skin with melanoma (10.2 %), corpus uteri (9.2 %), and ovary (6.1 %) prevail among women [1].

Our research revealed several most hazardous risk factors among the examined ones including occupational carcinogens and MNs occurrence in relatives (16.6 %), typical bad habits (smoking and alcohol), female sex, and carcinogens in ambient air. Overall, significance of factors available for our analysis doesn't contradict data provided by the WHO experts [9]. Environmental factors, first of all, occupational ones, make a significant contribution to morbidity with MNs and this is in line with research results described in [9, 24, 28]. We should note that our data on several factors are similar to the results obtained by Lezhnin and others; they assessed contributions made to MNs in lungs by various factors and detected that smoking and occupational hazards accounted for 22–23 %; biological agents, 17 %; chronic lung diseases in case history, 15 %; environmental pollution, 10 %; alcohol abuse, 9 %, stove heating, 4 % [29]. These results can be used to give grounds for programs aimed at primary prevention of malignant neoplasms among people of working age; they will help provide control over risk factors, implement sanitary-educational activities to create safety behavioral attitudes, and raise responsibility for personal and public health.

We should note that our research has certain limitations. Thus, we took into account only inhalation exposure to carcinogens although more and more attention is being paid to complex carcinogenic burden on the environment [7, 8, 17]. Vast majority of carcino-

gens can occur in water, air, soils, and food products and a person can contact them both at work and at home. And in most cases exposure to chemical carcinogens in small doses results in additive effects. Besides, uncertainty in estimates is related to genetic predisposition realized against a dominating role in etiology of malignant tumors belonging to environmental factors and lifestyle-related ones [13]. Our results can underestimate overall share of MNs caused by the examined factors since influence of all the established risk factors can't be determined quantitatively and many probable modifiable risk factors haven't been established as causal yet. Nevertheless, these results highlight that there is huge potential for reducing morbidity and mortality caused by MNs and, consequently, for minimizing economic losses and expenses borne by a person and the society as a whole through implementing well-grounded prevention activities

Conclusion. There were no statistically significant differences in frequency of most risk factors causing malignant neoplasms among men and women aged 18–64 with diagnosed MNs, excluding tobacco smoking that was much wider spread among men. The most significant oncogenic factors for urban population of working age included exposure to carcinogens at workplace; heredity burdened as per MNs; tobacco smoking; alcohol abuse; female sex; living under exposure to chemical carcinogens. Economic losses and expenses associated with malignant neoplasms of trachea, bronchus, and lungs were by 7.7 times higher among men than among women.

Research results can be used as a basis for making managerial decisions on malignant neoplasms prevention and development of a target program on providing sanitary-epidemiologic safety of the population. This program should be aimed at providing hygienic safety of the environment; protecting health of the most vulnerable population groups; informing public at large about prevention activities and healthy lifestyle; implementing secondary prevention activities.

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Research article

NEW APPROACHES TO ASSESSING AND FORECASTING MORBIDITY WITH ENTEROVIRUS (NON-POLIO) INFECTION IN THE RUSSIAN FEDERATION USING MATHEMATICAL MODELS

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At present it is impossible to develop epidemiologic surveillance and control over any infection regarding studies on dynamics of morbidity, seasonality and periodicity without using mathematical modeling techniques.

Our research goal was to study regularities in manifestations of epidemic process for enterovirus (non-polio) infection (EVnI) in the Russian Federation over 14 years (2006–2019) using mathematical models (linear, logarithmic, power, and exponential approximation).

An optimal mathematical model was selected using three statistical parameters, namely determination coefficient, Fischer's exact test, and standard error. Periodicity of rises and falls in morbidity was calculated with Fourier one-dimensional spectral analysis. Intra-year dynamics of morbidity with EVnI was estimated basing on monthly spread of the disease cases on the RF territory. Classic seasonal decomposition, Census I technique, was applied to analyze time series of monthly morbidity.

It was determined that EVnI epidemic process was unevenly spread over years in the RF in the examined period of time (2006–2019) and there were two opposite trends in it; the first one lasted from 2006 to 2010 when morbidity was declining and the second was from 2010 to 2019 when it was growing. Having analyzed manifestations of EVnI epidemiologic process in long-term dynamics given its uneven spread as per years, we established that it was advisable to use mathematical models approximated as per separate time periods. Average long-term morbidity with EVnI amounted to 8.09 $^0/_{0000}$ in the RF in 2010–2019 with growth rate being equal to 17.7 %. Maximum value was registered in 2017 (16.32 $^0/_{0000}$). An unfavorable prediction for further epidemic situation development was revealed for the examined period. The epidemic process was characterized with 4-year periodicity and summer-autumn seasonality with peaks usually occurring in August and September. Rates that characterized intensity of the trends in long-term morbidity dynamics and were calculated with mathematical models differed authentically from those obtained via conventional calculations of average values (χ = 11.08; d.f. = 1; p = 0.0009).

Key words: *enterovirus (non-polio) infection, non-poliomyelitic enteroviruses, epidemic process, epidemiologic surveillance, mathematical modeling, epidemic situation prediction, seasonality, morbidity dynamics.*

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Introduction. Enterovirus (non-polio) infection (EVnI) as a global issue is given a lot of attention [1, 2] and many research works by leading foreign and domestic scientists focus on examining it[1] [3, 4]. Experience gained worldwide and in Russia as well and accumulated knowledge call for their scientific and rational use to influence the epidemic process efficiently. EVnI is a significant issue due to its high intensity, overall prevalence, high share of outbreaks, contagiousness and lethality [5, 6, 7], high resistance of non-polio enteroviruses (NPEV) in the environment [8], and absence of any specific prevention measures [9, 10]. In the Russian Federation epidemiologic surveillance over EVnI is regulated by the Sanitary Rules "Prevention of enterovirus (non-polio) infection"[2] and methodical guidelines "Epidemiologic surveillance and prevention of enterovirus (non-polio) infection"[3]. Two departmental target programs were completed before 2017; at present a target program developed for 2018–2022 is being implemented.

However, dynamics of incidence with EVnI in the RF shows that it still apparently tends to grow. According to some authors, this trend occurs due to a change in dominating virulent NPEV variants [11, 12]; according to others, with incidence being revealed more efficiently due to improved laboratory diagnostics[4] [13].

Therefore, it becomes more vital in the RF to develop new approaches to examining incidence with EVnI [14–17]. Wide use of mathematical modeling is an apparent and vital task in improvement of epidemiologic surveillance regarding development of its information sub-system [18, 19].

Our research goal was to examine basic manifestations of the epidemic process for enterovirus (non-polio) infection in the Russian Federation over 14 years (2006–2019) using mathematical models.

Data and methods. Data for analysis of incidence with EVnI from 2006 to 2019 were taken from the Federal Statistic Report Form No. 1 "Data on infectious and parasitic diseases", submitted monthly; the Federal Statistic Report Form No. 2 "Data on infectious and parasitic diseases", submitted annually[5].

We used several descriptive and evaluative epidemiologic research techniques in the present work (statistic observation based on examining statistic reports and retrospective epidemiologic analysis). Retrospective epidemiologic analysis involved examining absolute incidence rates among population, intensive incidence rates for enterovirus infection (cases per 100 thousand people), intensity of the epidemic process, and long-term and annual dynamics. We used data

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[1] Organizatsiya i provedenie dezinfektsionnykh meropriyatii na razlichnykh ob"ektakh v period provedeniya massovykh meropriyatii: metodicheskie rekomendatsii [Organizing and implementing disinfection activities at various objects during mass events: Methodical guidelines]. Moscow, Rospotrebnadzor's Federal Center for Hygiene and Epidemiology Publ., 2013, 15 p. (in Russian).

[2] Ob utverzhdenii SP 3.1.2950-11 «Profilaktika enterovirusnoi (nepolio) infektsii» (vmeste s «SP 3.1.2950-11. Sanitarno-epidemiologicheskie pravila…»): Postanovlenie Glavnogo gosudarstvennogo sanitarnogo vracha RF ot 27.07.2011 g. № 106 [On approval of SR 3.1.2950-11 "Prevention of enterovirus (non-polio) infection (together with "SR 3.1.2950-11. Sanitary-epidemiologic rules..."): The Order by the RF Chief Sanitary Inspector issued on July 27, 2011 No. 106]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_122282/9fecd9944a61a84b2f22cfa5672a1a1a50e8cb11/ (December 19, 2020) (in Russian).

[3] Epidemiologicheskii nadzor i profilaktika enterovirusnoi (nepolio) infektsii: metodicheskie ukazaniya [Epidemiologic surveillance and prevention of enterovirus (non-polio) infection: Methodical guidelines]. Moscow, Rospotrebnadzor's Federal Center for Hygiene and Epidemiology Publ., 2009, 48 p. (in Russian).

[4] O sostoyanii sanitarno-epidemiologicheskogo blagopoluchiya naseleniya v Rossiiskoi Federatsii v 2018 godu: Gosudarstvennyi doklad [On sanitary-epidemiologic welfare of the population in the Russian Federation in 2018: The state Report]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing Publ., 2019, 254 p. (in Russian).

[5] Ob utverzhdenii form federal'nogo statisticheskogo nablyudeniya s ukazaniyami po ikh zapolneniyu dlya organizatsii Federal'noi sluzhboi po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka federal'nogo statisticheskogo nablyudeniya za sanitarnym sostoyaniem sub"ekta Rossiiskoi Federatsii: Prikaz Rosstata ot 29.12.2018 g. № 792 [On approval of federal statistic report forms with guidelines on filling in them for organization of federal statistic surveillance over a sanitary situation in RF regions by the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing: The Order by Rosstat issued on December 29, 2018 No. 792]. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_316110/ (December 19, 2020) (in Russian).

provided by the Federal State Statistic Service on population numbers to calculate intensive rates; the data were published in the Unified interdepartmental information statistic system (UIISS)[6].

We applied the least square method to fit coefficients in mathematical models and build up trends. The following approximations were used:

– linear: $y=a\cdot x+b$;
– logarithmic: $y=a\cdot Ln(x)+b$;
– power: $y=bx^a$;
– exponential: $y=b\cdot e^{a\cdot x}$.

An optimal mathematical model was selected using three statistical indicators: determination coefficient, Fischer's test, and standard error for *y* evaluation.

We took average annual growth rates to estimate intensity of the trends in the epidemic process in long-term dynamics. Since analyzed time series didn't exceed 14 observations (the observation period was 14 years), we used non-parametric statistic methods. Spearmen's correlation coefficient was used to determine occurrence and degree of linear correlation between two data sets. Periodicity of rises and falls in incidence was clearly shown through using periodograms and calculated by using Fourier's one-dimensional spectral analysis. If a correlation is strong (coefficient at a certain sine or cosine), we can conclude there is strict periodicity in data at a relevant frequency. Since sine and cosine functions are independent (or orthogonal), we can arithmetically sum the squares of coefficients for each frequency in order to obtain a required periodogram.

Periodogram values can be interpreted as data dispersion (variance) over a relevant period. Periodogram values (on *y*-axis) are calculated as:

$$P_k = \text{sin-coefficient}_k^2 + \text{cos-coefficient}_k^2 \cdot \frac{N}{2},$$

where sin and cos are functions of a cyclic component in a time series; P_k is periodogram values at frequency ν_k, and N is an overall length of a series. For better visualization, frequency on *x*-axis is transformed into a period: $T_k=1/\nu_k$. Coefficients were calculated by building up a linear multiple regression model where an observed time series were a dependent variable and independents variables or regressors were represented by sine functions of all possible (discrete) frequencies. Fast Fourier transform turned out to be the most efficient solution to the task.

A year dynamic of incidence with EVnI was estimated based on monthly distribution of the disease cases in the Russian Federation during the observation period from 2013 to 2019. We applied a procedure stipulated by the Methodical guidelines MR 3.1.2.0118-17.3.1.2[7] to calculate epidemic thresholds. Classic seasonal decomposition, or Census I method, was applied to analyze time series. The method is based on an assumption that a time series includes four different components: seasonal (usually given as *St* where *t* means a moment of time), trend (*Tt*), cyclic (*Ct*), and irregular one or fluctuation (*It*). A difference between a cyclic and a seasonal component is that the latter has regular (seasonal) periodicity whereas cyclic factors usually have a longer effect which also changes from cycle to cycle. Within Census I method, a trend and a cyclic component are usually combined into one trend-cyclic component (*TCt*). Specific functional interrelations between these two components can be quite variable. However, we can spot out two basic ways for them to interact with each other, an additive and a multiplicative one:

– additive model: Xt = TCt + St + It,
– multiplicative model: $Xt = Tt\cdot Ct\cdot St\cdot It$,

where *Xt* is a value of a time series at a time moment *t*.

[6] Ofitsial'nye statisticheskie pokazateli: sistema vvedena sovmestnym Prikazom Minkomsvyazi Rossii i Rosstata ot 16.11.2011 g. № 318/461 [Official statistical indicators. Introduced by the joint Order by the RF Ministry of Digital Development, Communications and Mass Media of the Russian Federation and Rosstat issued on November 16, 2011 No. 318/461]. *UIISS. State Statistics*. Available at: https://www.fedstat.ru// (December 19, 2020) (in Russian).

[7] MR 3.1.2.0118-17.3.1.2. Metodika rascheta epidemicheskikh porogov po grippu i ostrym respiratornym virusnym infektsiyam po sub"ektam Rossiiskoi Federatsii: metodicheskie rekomendatsii [MR 3.1.2.0118-17.3.1.2. The procedure for calculating epidemic thresholds for influenza and acute respiratory viral infections as per RF regions: methodical guidelines]. Moscow, The Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing Publ., 2018, 95 p. (in Russian).

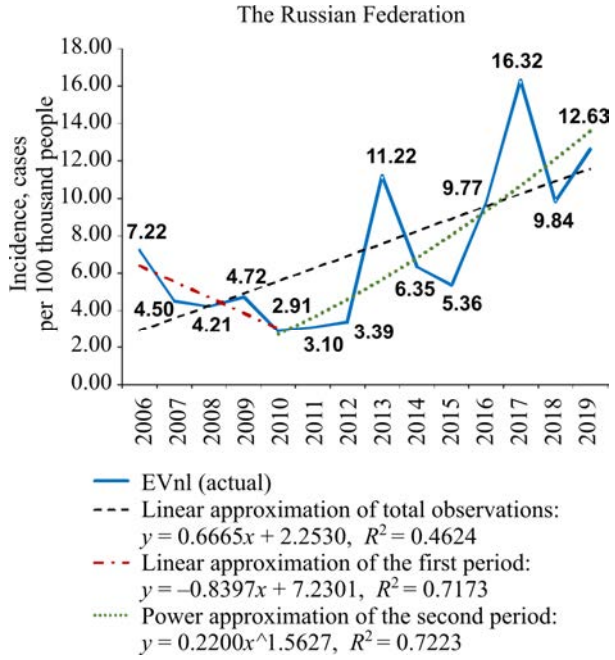

Figure 1. Long-term dynamics of incidence with enterovirus (non-polio) infection in the Russian Federation (2006–2019), cases / 100 thousand people

As for computations, the seasonal decomposition procedure (Census I) complies with the standard formulas [20].

Results and discussion. According to official statistic data, in the RF incidence with EVnI taken over 14 years (2006–2019) was distributed unevenly and tended to grow (Figure 1).

Average annual incidence (AAI) amounted to 7.25 $^{0}/_{0000}$ (standard deviation = 4.1 $^{0}/_{0000}$, median = 5.9 $^{0}/_{0000}$, interquartile range varied from 4.28 $^{0}/_{0000}$ to 9.82 $^{0}/_{0000}$), average annual growth rate was 4.4 % (a linear trend slope was 34°). Rises in incidence were detected in 2009 (4.72 $^{0}/_{0000}$), 2013 (11.22 $^{0}/_{0000}$), and 2017 (16.32 $^{0}/_{0000}$); each next rise was higher than the previous one. Local minimums were registered in 2010 (2.91 $^{0}/_{0000}$), 2014 (6.35 $^{0}/_{0000}$), and 2015 (5.36 $^{0}/_{0000}$).

Use of standard epidemiologic approach to estimating incidence for the analyzed period of time indicated that applied mathematical models (linear, logarithmic, power, or exponential approximation) were unsatisfactory for the given time series (Table 1) and that there were at the least two periods with multidirectional trends.

In our opinion, this was due to changes in EVnI cases registration that occurred in 2009 in the RF since Methodical guidelines MU 3.1.1.2363-08[8] came into force and stipulated the necessity to run laboratory tests to confirm not only group cases but also sporadic ones; this had its influence on quality of EVnI laboratory diagnostics. Given that, we can obtain a relevant mathematical model that determines both a trend and a growth rate for incidence only by dividing a time series into two periods (against the minimal rate in 2010 that was equal to 2.91 $^{0}/_{0000}$). The first period is from 2006 to 2010; the second one, from 2010 to 2019. We selected a time period from 2010 to 2019 to estimate incidence since it was the most relevant to incidence levels according to power approximation (Table 2).

Given that the observation period was not long enough (only 9 years, 2010–2019), we took median value of incidence that was equal to 6.35 $^{0}/_{0000}$ and interquartile range (IQR) being 3.38–9.84 $^{0}/_{0000}$ as exponential values in descriptive statistics.

Table 1

Mathematical models applied to analyze dynamics of incidence with enterovirus (non-polio) infection in the Russian Federation (2006–2019)

Trend	Mathematical model	Determination coefficient R^2	*F*-criterion	Standard error for *y* evaluation
Linear approximation	$y = 0.6665x + 2.2530$	0.4624	10.3216	3.1292
Logarithmic approximation	$y = 2.6436\text{Ln}(x) + 2.4950$	0.2491	3.9807	3.6982
Power approximation	$y = 3.5140x^{0.3228}$	0.2027	3.0514	0.5158
Exponential approximation	$y = 3.2716e^{0.0870x}$	0.4298	9.0461	0.4362

[8] MU 3.1.1.2363-08. Epidemiologicheskii nadzor i profilaktika enterovirusnoi (nepolio) infektsii: metodicheskie ukazaniya [MU 3.1.1.2363-08. Epidemiologic surveillance and prevention of enterovirus (non-polio) infection: methodical guidelines]. Moscow, Rospotrebnadzor's Federal Center for Hygiene and Epidemiology Publ., 2009, 48 p. (in Russian).

Table 2

Mathematical models applied to analyze dynamics of incidence with enterovirus (non-polio) infection in the Russian Federation (2010–2019)

Trend	Mathematical model	Determination coefficient R^2	*F*-criterion	Standard error for *y* evaluation
Linear approximation	$y = 1.1757x - 3.0816$	0.6055	12.2809	3.0473
Logarithmic approximation	$y = 10.4144\text{Ln}(x) - 14.8374$	0.6054	12.2759	3.0477
Power approximation	$y = 0.2200x^{1.5627}$	0.7223	20.8058	0.3513
Exponential approximation	$y = 1.3230e^{0.1732x}$	0.6966	18.3699	0.3671

Analysis of incidence performed by using this approached revealed that average annual incidence with EVnI in the RF amounted to 8.09 $^0/_{0000}$; average annual growth rate, 17.7 % (linear trend slope was 50°). According to the power function, EVnI epidemic process developed uncontrollably and forecasts were unfavorable.

Having compared incidence rates for the whole time series (2006–2019) and 2010–2019 time series, we detected statistically significant differences ($\chi^2 = 11.08$, *d.f.* = 1, $p = 0.0009$) in annual average growth rates (4.4 % against 17.7 %) and linear trend slopes (34° against 50°).

Fourier's spectral analysis was applied to examine periodicity in the time series of incidence with EVnI; this was done to determine correlations between sine and cosine functions with different frequencies and observed data (Figure 2).

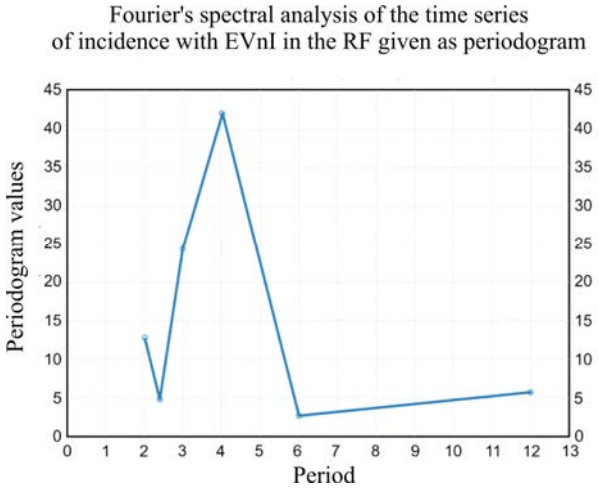

Figure 2. Periodogram showing incidence with enterovirus (non-polio) infection in the Russian Federation (2009–2019): *OX* axis shows periodicity in years, *OY* axis is data dispersion over a relevant period

Fourier's spectral analysis revealed 4-year periodicity when average long-term incidence with EVnI (2006–2019) was examined. Local minimums followed local maximums with the same 4-year periodicity.

Incidence with EVnI had apparent summer-autumn seasonality (Figure 3).

A seasonal rise in incidence began in July each year and ended in November with peaks usually reached in August and September (Figure 4). According to average annual long-term data, 89.5 % cases occurred during these seasonal rises in incidence with EVnI (from July to November). Specific weight of patients who got infected due to influence by seasonal factors amounted to 82.0 %.

Fourier's one-dimensional spectral analysis of a time series for monthly incidence revealed apparent 12-month periodicity; this indicated there were no significant shifts in the beginning and the end of an epidemic rise (Figure 5).

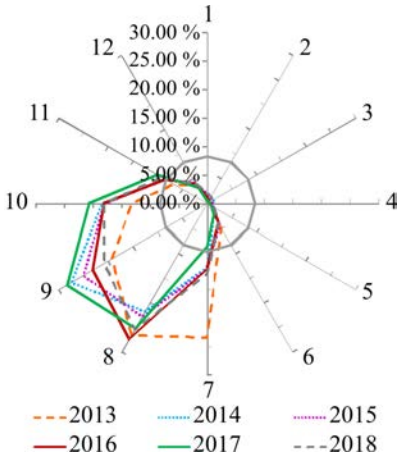

Figure 3. A year dynamics of enterovirus (non-polio) infection in the Russian Federation, shares of total annual incidence (2013–2018, rays of circle graph show months from January (1) to December (12)

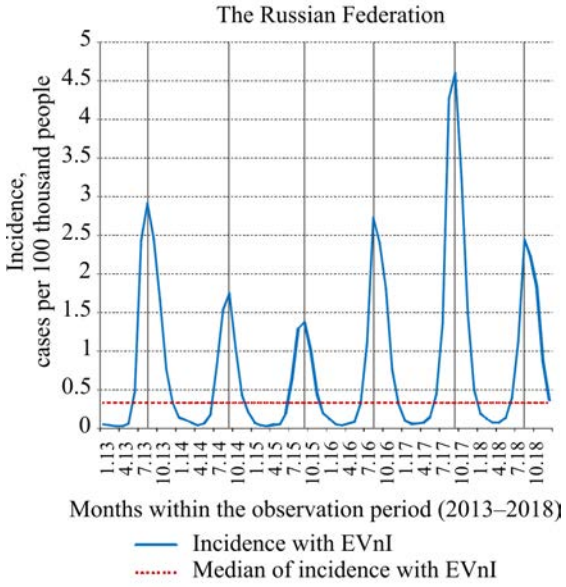

Figure 4. Annual dynamics of incidence with enterovirus (non-polio) infection in the Russian Federation (2013–2018), cases / 100 thousand people

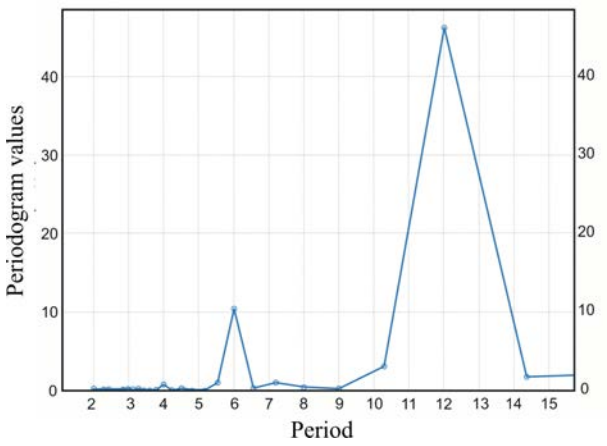

Figure 5. Periodogram showing monthly incidence with enterovirus (non-polio) infection in the Russian Federation (2013–2018): *OX* axis is periodicity in years, *OY* axis is data dispersion over a relevant period

Table 3

Seasonal components in monthly incidence with enterovirus (non-polio) infection in the Russian Federation (2013–2018)

Months	Seasonal component for the multiplicative model	Seasonal component for the additive model
January	14.5887	-0.708005977
February	8.8814	-0.73590166
March	6.4100	-0.746689643
April	7.4807	-0.74039556
May	11.0901	-0.708328827
June	39.1541	-0.498259943
July	**143.0773**	**0.413094759**
August	**307.2373**	**1.71443414**
September	**314.4177**	**1.68170537**
October	**216.7095**	**0.913272973**
November	92.1674	-0.05959021
December	38.7857	-0.525335427

Seasonal component in classical seasonal decomposition of this time series (Census I method) showed annual maximum influence exerted by seasonal factors on incidence growth in July, August, September, and October, both for the additive and multiplicative model (Table 3).

The detected periodicity turned out to be universal regarding the whole time series; however, each year could have individual peculiarities when it came down to the beginning and the end of an epidemic rise. We took retrospective values beyond seasonal epidemic rises in incidence to calculate epidemic thresholds for the analyzed periods. We accomplished primary division of time series as per incidence rate into all-year incidence and seasonal rises; the division was done as per the median for the whole data array. This procedure is widely spread in analyzing time series of incidence and often used by the World Health Organization since the median is not significantly influenced by a peak in incidence, as opposed to other mean values (simple mean, geometric mean, or harmonic mean).

Having calculated upper tolerance limits (Table 4), we established that the epidemic threshold values didn't exceed 0.443 $^{0}/_{0000}$ from January to May. Minimum value was detected in April and amounted to 0.178 $^{0}/_{0000}$. The maximum value was detected in June and amounted to 0.575 $^{0}/_{0000}$. It should be noted that incidence in November was higher than not only the overall median value but also than the maximum epidemic threshold in 4 cases out of 6. Given that, we concluded that November should be among months of seasonal rises in incidence despite low values of its seasonal component.

Table 4

Epidemic thresholds of incidence with enterovirus (non-polio) infection calculated as per data on monthly incidence in the Russian Federation in 2013–2018 (cases / 100 thousand people)

Month	2013	2014	2015	2016	2017	2018	Average monthly incidence	Upper tolerance limit	Median
January	0.052	0.138	0.074	0.117	0.101	0.192	0.112		0.10937
February	0.038	0.106	0.037	0.049	0.057	0.126	0.069		0.05281
March	0.03	0.077	0.029	0.042	0.059	0.075	0.052		0.05076
April	0.031	0.041	0.045	0.058	0.076	0.075	0.054		0.052
May	0.064	0.061	0.049	0.083	0.144	0.132	0.089		0.074
June	0.476	0.173	0.192	0.327	0.443	0.394	0.334		0.36
July	2.426	0.808	0.637	1.1	1.356	1.118	1.241		1.109
August	2.913	1.528	1.288	2.722	4.271	2.444	2.528		2.583
September	2.449	1.744	1.374	2.4	4.598	2.222	2.465		2.311
October	1.654	1.036	1.001	1.805	3.231	1.834	1.76		1.729
November	0.757	0.432	0.443	0.753	1.481	0.862	0.788		0.755
December	0.33	0.21	0.193	0.312	0.498	0.361	0.317		0.321
Monthly incidence with threshold as per median for the whole data array:							Median (all months, 2013–2018)		0.328
January	0.052	0.138	0.074	0.117	0.101	0.192		0.443	
February	0.038	0.106	0.037	0.049	0.057	0.126		0.322	
March	0.03	0.077	0.029	0.042	0.059	0.075		0.195	
April	0.031	0.041	0.045	0.058	0.076	0.075		0.178	
May	0.064	0.061	0.049	0.083	0.144	0.132		0.355	
June	ex	0.173	0.192	0.327	ex	ex		0.575	
July	ex	ex	ex	ex	ex	ex			
August	ex	ex	ex	ex	ex	ex			
September	ex	ex	ex	ex	ex	ex			
October	ex	ex	ex	ex	ex	ex			
November	ex	0.432	0.443	ex	ex	ex			
December	ex	0.21	0.193	0.312	ex	ex		0.503	

Note: ex means incidence exceeded epidemic threshold in a given month.

Conclusions. Therefore, in the RF incidence with EVnI was characterized with two opposite trends over the examined period (2006–2019): had 4-year periodicity and apparent summer–autumn seasonality (seasonality coefficient was equal to 82 %). The epidemic process was uncontrollable and forecasts for its development were unfavorable. We analyzed manifestations of EVnI epidemic process in long-term dynamics given its uneven spread over years and established that it was advisable to apply mathematical models approximated as per separate time periods. Average annual long-term incidence taken over the observation period 2010–2019 amounted to 8.09 $^{0}/_{0000}$; average annual growth rate, 17.7 %; linear trend slope, 50°. Rates that characterized intensity of the trend in long-term incidence dynamics that were calculated based on mathematical modeling were statistically significantly different from those obtained by calculating mean values ($\chi^2 = 11.08$, $d.f. = 1$, $p = 0.0009$).

Epidemiologic diagnostics of enterovirus infection at the population level can become more qualitative due to wide use of mathematical models in epidemiologic research procedures when estimating manifestations of the epidemic process in long-term dynamics as well as when estimating its spatial characteristics.

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Conflict of interests. The authors declare there is no any conflict of interests.

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Research article

SOCIETAL DETERMINANTS OF HIV-INFECTION SPREAD IN REGIONS IN THE RUSSIAN FEDERATION

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The paper dwells on the results obtained via examining dependence between HIV-infection spread and factors related to social environmental and social structure of population in RF regions. These factors are considered to be potential health risk ones. The authors tested a hypothesis about influence exerted by demographic, economic, cultural and behavioral determinants and public healthcare availability on differences in territorial spread of the disease within social epidemiologic approach.

To solve the set task, data that characterized 85 RF regions were taken from official statistical reports. Descriptive statistic analysis was performed and regression models were built up; it allowed testing whether the analyzed factors had their influence in RF regions and selecting the most significant ones to be included into the overall regression model.

The research revealed significant contextual differences in HIV-infection spread. Regression analysis showed that 22.0 % differences in a number of HIV-infected people detected in RF regions occurred due to differences in urban population numbers, provision with ambulatories and polyclinics, and unemployment rate. Moreover, a number of registered crimes committed by minors determined 32.5 % difference in a number of patients with the first diagnosed HIV-infection between the examined regions.

These results allow assuming that the greatest influence on spread of the disease in RF regions is exerted by consequences of urbanization; this process is usually accompanied with a growth in a share of urban population in a given region, instability on the labor market there as well as related migration processes within the country and wider opportunities to pursue individual behavioral strategies including those that involve law violations and/or are destructive for people's health.

Key words: *inequalities in health, societal determinants, HIV-infection spread, regions in the Russian Federation, mathematical modeling, regression analysis.*

Introduction. Spread of socially significant diseases, just as health in general, is influenced by inequalities appearing at any level in society organization; institutional factors, material world, cultural, behavioral, individual socio-psychological and status determinants; the most significant effects are usually produced by income, education, and employment. Higher social status secures better access to vital resources that are necessary to preserve health and results in health inequalities and uneven distribution of risks related to morbidity and premature mortality in a society [1].

There are two basic approaches to examining social inequalities in health that are usually described in foreign theoretical reviews. The first one (1) is an objectivist approach that is being developed predominantly within social epidemiology and social sciences. This approach assumes that differences in people' health are determined by the environment with its material, cultural, and institutional parameters and not by people's individual psychological and behavioral peculiarities. The second approach (2) is subjectivist and is based on opinions by medical experts and experts in psychology who claim that there are differences in psychological and behavioral reactions and resistance that help people to cope with stresses and diseases successfully using

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their individual psychological resources and support provided by social environment. Explanatory mathematical models have been developed within these two approaches; they provide an opportunity to analyze influence exerted on health by material factors, cultural and behavioral parameters as well as individual socio-psychological ones, specific procedures to estimate social status; these models also allow analyzing how inequalities in health occur and reproduce themselves both in a cross-sectional measuring in specific social groups and in longitude one over a whole life time [2].

Experts who accomplished their research within these approaches put forward a hypothesis that was based on accumulated knowledge and concentrated on a role played by biological (genetic) environmental, social-structural and individual factors in spread of diseases in various social groups. The hypothesis was repeatedly corroborated in social and epidemiological studies that focused on analyzing existing material and cultural factors; social processes that create conditions for health inequalities to occur; and factors of economic well- (ill) -being (first of all, poverty and related issue of medical aid availability), living conditions, nutrition, negative psychological influences and related behavioral patterns regarding health. Social epidemiologic approach provided convincing evidence that health inequalities depended on the aforementioned reasons and thus dominated in studies focusing on health inequalities approximately up to late 1990s – early 2000s[1] [1, 3–6] and is still relevant. Social production of health / diseases and multilevel ecosocial theory of disease have been developed within it.

Multilevel methodology that is now applied by researchers helps estimate influences exerted on health by both individual determinants and structural and contextual ones. It has been repeatedly corroborated in empirical research that differences in morbidity and mortality are caused not so much by individual psychological and behavioral peculiarities as by material, cultural and institutional parameters of the environment [7]. And still most research papers that have been published by Western scientists over the last 15 years focus on examining social-structural and behavioral factors. Effects produced by social context factors have not been studied so profoundly although they are the most significant ones when it comes down to an insight into social mechanisms of diseases occurrence and their dependence on global social processes[2].

Although significance of issues related to social inequalities in health is hardly disputable, in Russia there have also been few studies on health being dependent on social parameters of macrocontext; it calls for a necessity to reveal factors that exert the greatest influence on spread of socially significant diseases.

According to statistic data provided by the RF Public Healthcare Ministry, The RF Labor Ministry, and Rosstat, HIV-infection caused by human immunodeficiency virus has been among the most frequently registered socially significant diseases in Russia over the last decades)[3]. A number of registered people with HIV-infection at the end of a calendar year minus those who have died over the same year is an indicator that shows prevalence of the disease in a given region and it is a significant analytical parameter used to comparatively assess territorial peculiarities related to spread of the disease.

Test of basic hypotheses formulated within social epidemiologic approach regarding peculiarities of territorial HIV-infection prevalence reveals common regularities in spread of the disease in the Russian Federation and allows making assumptions on reasons for existing differences between regions. This can give grounds for further development of target measures within social policy aimed at optimizing an epidemic situation in regions.

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Data and methods. A possibility to perform analysis that would correspond to the fixed tasks was limited by available statistical data on relevant macrocontext parameters in regions in Russia. Therefore, we examined data on these parameters collected in 2017 since they were the most completed in a database created by the Federal State Statistic Service.

In the RF over the last 15 years epidemiological situation regarding HIV-infection has been deteriorating [8]. As of December 31, 2017 a cumulative number of registered HIV-infection cases amounted to 1,220,659 people[4] among the RF citizens. At the end of 2017 more than 943,999 people in Russia had HIV-infection, 276,660 deceased patients excluded. In 2017 morbidity amounted to 71.1 cases per 100 thousand people (it was 69.6 in 2016). There were several regions where morbidity was higher than on average in the country and they became leaders as per this parameter. These regions included Kemerovo region (203.0 new registered HIV cases per 100 thousand people), Irkutsk region (160.7), Sverdlovsk region (157.2), Chelyabinsk region (154.0), Novosibirsk region (142.8) Perm region (140.8), Tyumen region (138.7), Tomsk region (128.2), Kurgan region (117.3), Orenburg region (114.7), Krasnoyarsk region (114.1), Khanty-Mansi Autonomous Area (109.2), Samara region (105.0), Omsk region (103.9), Altai region (101.5), Ulyanovsk region (93.9), Crimea (88.1), Udmurtia (87.4), Khakass Republic (84.4), Sevastopol (73.5), Moscow region (73.4), and Nizhniy Novgorod region (72.3) области.

Preliminary analysis of descriptive statistics on all regions in Russia in 2017 allows stating there are apparent territorial differences in HIV-infection spread (Figure 1).

We reviewed research papers that focused on explaining differences in morbidity with socially significant diseases among population in different countries in the world; it allowed us to spot out several key determinants at macro-level influencing spread of socially significant diseases. These are demographic factors, factors related to socioeconomic development including GDP (gross domestic product) and Gini coefficient (it shows income

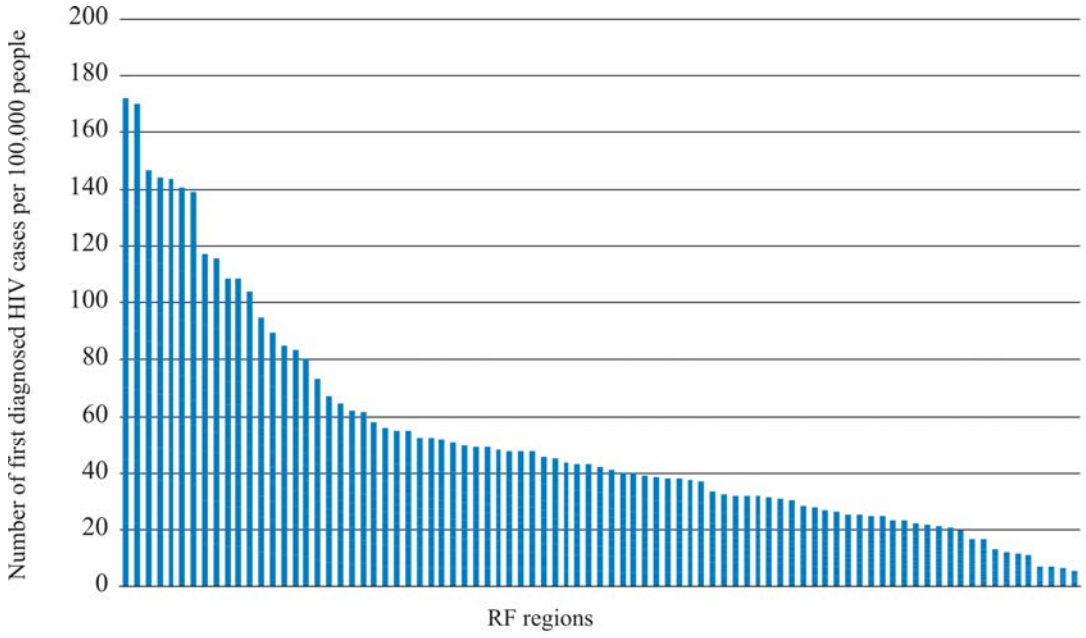

Figure 1. RF regions distributed as per a number of patients with first diagnosed HIV

[4] Spravka VICh-infektsiya v Rossiiskoi Federatsii v pervom polugodii 2019 g. [Reference to HIV-infection in the Russian Federation in the first half of 2019]. Available at: https://aids-centr.perm.ru/images/4/hiv_in_russia/hiv_in_rf_30.06.2019.pdf (March 01, 2021) (in Russian).

inequality), labor market parameters; social and political parameters of a society in a country and its functioning including social policies in education and public healthcare, expenses on public healthcare and provision with medical aid; factors related to social structure of a society including incomes, education, and occupation; cultural and behavioral determinants like prevalence of health-destructive behavioral patterns, crime rate, etc.

These and some other parameters can be used to estimate reasons for spread of socially significant diseases in regions in the Russian Federation and to examine regional differences [9].

To solve the task, we took regional statistic data on 85 RF regions from official statistic sources. When creating a database, we selected the following groups of parameters: demographic (population number in a given region, men to women ratio, specific weight of urban and rural population in the overall population number, people older than working age, migration growth rate); socioeconomic (Gini coefficient, gross regional product per capita, average floor area per person, a number of private passenger cars per 1,000 people, number of unemployed among people aged 15–72, unemployment rate); social and political (medical aid availability (number of in-patient beds per 10,000 people, a number of people per an in-patient bed, capacities of out-patient polyclinics, number of physicians with all specialties)); social and structural (number of people with incomes lower than living wage, number of specialists with higher education who chose teaching at a higher education establishment as a career after graduation in 2017); cultural and behavioral (number of registered crimes per 100 thousand people, a number of registered crimes committed by minors per 100 thousand people, number of patients with first diagnosed drug addiction who were subject to obligatory dispensary observation per 100 thousand people).

We applied a multiple linear regression to statistically analyze the selected data; the regression was calculated as per the following formula:

$$\gamma = \beta_0 + \beta_1 X_1 + ... + \beta_n X_n + e,$$

where γ is the dependent variable showing "a number of patients with first diagnosed HIV per 100 thousand people"; β_0 is the constant (intercept); $X_1 ... X_n$ are the independent variables showing regional parameters that probably influence morbidity with HIV-infection in a given region; e is unexplained residuals in regression. We analyzed correlations between HIV-infection prevalence and demographic, socioeconomic, social and political, social and structural, and cultural and behavioral parameters of the RF regions. Several regression models were built up to test influence exerted by each group of regional parameters and the most significant factors were selected among them to be included into the generalized linear model. Regression analysis involved step-by-step testing of influence exerted by different groups of parameters on HIV-prevalence in regions and building up the generalized model. To facilitate analysis, all the variables were standardized (transformed into Z-score) to provide correct comparison of regression coefficients. This procedure for data analysis allows selecting factors that are stronger correlated with spread of the disease and revealing key regional parameters that determine health inequalities among population living there.

Results and discussion. Table 1 contains minimum, maximum and average values and standard deviations for regional parameters that turned out to be statistically significant in regression analysis. Other factors also had certain influence; it was examined in regression analysis and turned out to be insignificant, so, descriptive statistic values for such factors were not included into the Table[5]. *A number*

[5] These parameters in 2017 included the following: men to women ratio (estimated at the end of the year; a number of women per 1,000 men) in a region, a share of population in their working age; a number of population of their income being lower than living wage; Gini coefficient; gross regional product per capita, a number of physicians per 10 thousand people; a number of people per one physician; a number of in-patient beds per 10 thousand people; migration growth rate; a number of registered crimes per 100 thousand people; a number of private passenger cars per 1,000 people; a number of drug addicts per 100 thousand people in a corresponding population group.

Table 1

Descriptive statistic values for parameters characterizing regions in the RF

Parameter	Minimum	Maximum	Average	Standard deviation
Number of patients with first diagnosed HIV per 100 thousand people in 2017	5.4	172.3	52.8	39.1
A share of urban population in a region in 2017, %	29.0	100.0	70.5	13.2
A number of unemployed people aged 15–72, thousand people	1.0	163.0	46.6	35.3
A number of registered crimes committed by minors per 100 thousand people	14.0	2107.0	530.1	428.7
Provision with out-patient polyclinics per 10 thousand people	124.9	507.6	277.0	54.2

Table 2

A correlation between a number of patients with first diagnosed HIV per 100 thousand people in 2017 and regional parameters

Parameter	Demographic factors Model 1	Economic factors Model 2	Medical aid availability Model 3	Cultural and behavioral factors Model 4
Constant (intercept)	5.285E-16 (0.104)	2.025E-16 (105)	-3.725E-16 (107)	5.5031E-16 (0.088) ***
Specific weight of urban population in total population	0.31 (0.104) ***			
A number of unemployed people aged 15–72		0.27 (0.106) *		
Provision with out-patient polyclinics per 10,000 people			0.21 (0.108)	
A number of registered crimes committed by minors per 100 thousand people				0.59 (0.088) ***
R^2	**0.096**	**0.071**	**0.041**	**0.352**
Adjusted R Square	**0.085**	**0.06**	**0.029**	**0.344**
F	**8.846*** **	**6.379***	**3.51.**	**45.11*** **

N o t e : *** means $p < 0.001$; ** means $p < 0.01$; * means $p < 0.05$;. means $p < 0.1$. β (SE) for each factor are given in the Table.

of patients with first diagnosed HIV per 100 thousand people differed in different regions in 2017 starting from 5.4 and up to 172.3 cases per 100 thousand people. This value is a dependent variable. *A share of urban population* varied from 29 % to 100 %. For example, federal cities (Saint Petersburg, Moscow, and Sevastopol) and northern regions (Magadan region and Murmansk region) had the greatest share of urban population. This share was minimal in such regions as Altai Republic, Chechnya, and Karachai-Cherkess Republic. *A number of unemployed people aged 15–72* varied from 1,000 to 163,000. The lowest numbers were detected in Chukotka, Nenets Autonomous Area, and Magadan region; the greatest numbers, in Dagestan, Moscow region, and Krasnodar region. *A number of registered crimes committed by minors per 100 thousand people* was the highest in Irkutsk region, Chelyabinsk region, and Sverdlovsk region. The lowest numbers of such crimes were registered in Ingushetia, Chechnya, and Chukotka. The minimum value of the parameter was 14 cases, and the maximum, 2,107 cases per 100 thousand people. *Provision with out-patient polyclinics per 10,000 people* was the best in Chukotka, Magadan region, and Komi Republic. The smallest numbers of such polyclinics per 10,000 people were detected in Chechnya, Ingushetia, and Dagestan.

Results obtained at the first stage in regression analysis are given in Table 2.

Model 1 was built to test an assumption that demographic parameters in a region had certain influence on a number of patients with

first diagnosed HIV per 100 thousand people (Figure 2).

The model included the following independent variables: specific weight of urban population in total population, men to women ration (estimated at the end of the year), a share of population in working age, and migration growth rate. Significant positive correlation was detected only with specific weight of urban population (0.31; $p < 0.001$) and this indicates that HIV-infections spreads predominantly among urban population.

Model 2 was created to test correlations between parameters related to medical aid availability in regions and a number of patients with first diagnosed HIV-infection. To test influence exerted by medical aid availability in regions on the dependent variable, we used the following parameters of regional healthcare systems: provision with out-patient polyclinics per 10 thousand people, a number of physicians per 10 thousand people, a number of people per one physician, and a number of inpatients bed per 10 thousand people. We revealed that there was only one statistically significant correlation in the model, between HIV prevalence and a number of out-patient polyclinics per 10 thousand people (0.21; $p < 0.1$) (Figure 3).

Model 3 was used to test correlations between economic factors and HIV-infection prevalence. We tested correlations between prevalence of the infection and a number of unemployed people aged 15–72, number of people with their incomes being lower than living wage, Gini coefficient, gross regional product per capita, a number of private passenger cars per 1,000 people, and average floor area per person. A statistically significant correlation was detected between HIV prevalence in a region and a number of unemployed people aged 15–72 (0.27; $p < 0.05$) (Figure 4).

To test correlation between HIV prevalence and cultural and behavioral factors, we analyzed the following variables in the model: a number of specialists with higher education who chose teaching at a higher education establishment as a career after graduation, a number of registered crimes per 100 thousand

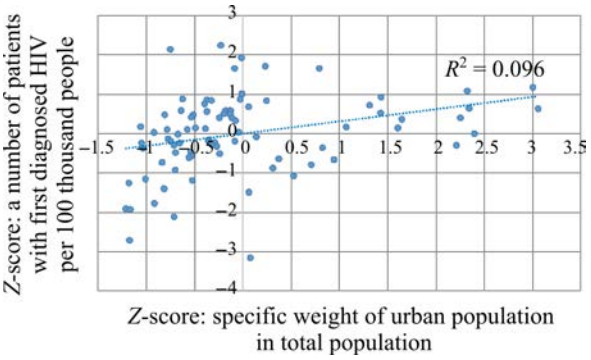

Figure 2. A correlation between a number of patients with first diagnosed HIV per 100 thousand people and specific weight of urban population in total population

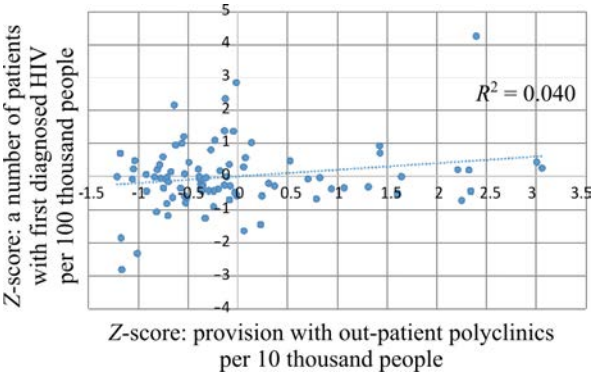

Figure 3. A correlation between number of patients with first diagnosed HIV per 100 thousand people and medical aid availability in RF regions

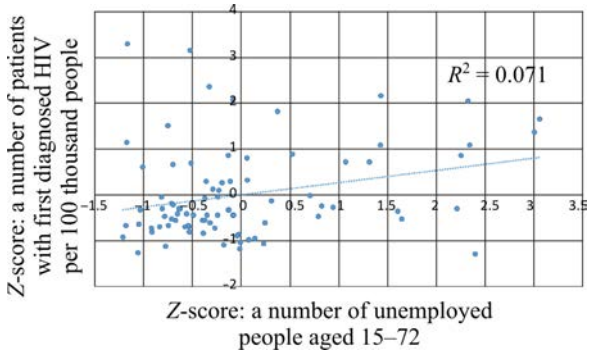

Figure 4. A correlation between number of patients with first diagnosed HIV per 100 thousand people and a number of unemployed people aged 15–72

people, a number of registered crimes committed by minors per 100 thousand people, a number of patients with first diagnosed drug addiction who were subject to obligatory dispensary observation per 100 thousand people). We detected that a number of drug addicts correlated with a number of HIV-infected patients.

However, this factor loses its significance when it is included into regression together with unemployment rate. So, we can assume that unemployment can cause both HIV spread and a growing number of patients with drug addiction. As a result, only a number of registered crimes committed by minors per 100 thousand people turned out to be significant in the created model (0.59; $p < 0.001$) (Figure 5).

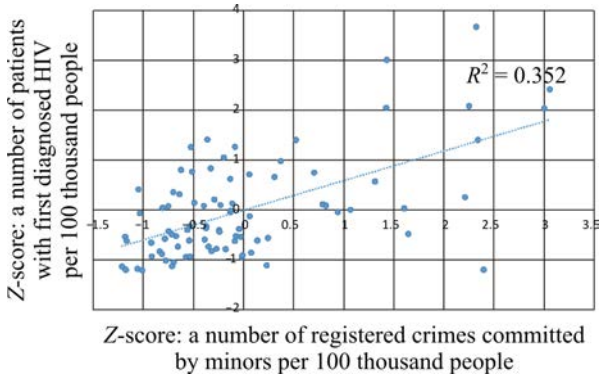

Figure 5. A correlation between a number of patients with first diagnosed HIV per 100 thousand people and a number of registered crimes committed by minors

A number of registered crimes committed by minors plays an important role in HIV prevalence in a region. When this parameter is included into the generalized linear model simultaneously with other factors (a share of urban population, a number of unemployed people, and a number of out-patient polyclinics), it results in occurring multicollinearity. This occurs due to a strong correlation between crimes committed by minors and a number of unemployed people aged 15–72 (correlation coefficient amounts to 0.685 for these two parameters; $p < 0.001$). To resolve the issue with multicollinearity, two models were built; one of them included unemployment rate, and another, a number of crimes committed by minors (Table 3).

Generalized linear model 1 included such regional parameters as specific weight of urban population in total population (0.24; $p < 0.05$), a number of unemployed aged 15–72 (0.36; $p < 0.001$), and provision with out-patient polyclinics (0.25; $p < 0.05$). They were all significantly correlated to the dependent variable: there were higher numbers of patients with first diagnosed HIV in regions with high shares of urban population, unemployment rate, and a number of out-patient polyclinics. It is these factors that explain 22.0 % of variations between regions in a number of patients with first diagnosed HIV.

Generalized linear model 2 included only one parameter, a number of registered crimes committed by minors per 100 thousand people. A share of explained variations in a number of patients with first diagnosed HIV amounted to 35.2 % in this model.

Regression analysis revealed that a number of HIV-infected people registered in RF regions correlated with a share of urban population in a given region, provision with out-patient polyclinics, a number of unemployed, and a number of registered crimes committed by minors (parameters are given in an ascending order for correlation strength). The latter

Table 3

Generalized linear models

Parameter	Generalized model 1	Generalized model 2
Constant (intercept)	9.6514E-17 (0.098)	5.5031E-16***
Specific weight of urban population in total population	0.24* (0.104)	
A number of unemployed people aged 15–72	0.36*** (0.105)	
Provision with out-patient polyclinics per 10,000 people	0.25* (0.111)	
A number of registered crimes committed by minors per 100 thousand people		0.59 (0.088) ***
R²	**0.22**	**0.352**
Adjusted R Square	**0.19**	**0.344**
F	**7.58*** **	**45.11*** **

Note: *** means $p < 0.001$; ** means $p < 0.01$; * means $p < 0.05$;. means $p < 0.1$. β (SE) are given for each factor in the Table.

two parameters (unemployment and a number of crimes committed by minors) are interrelated and it allows assuming that both HIV-infection spread and growing crime rate among young people result from unfavorable situation on regional labor markets.

Accomplished cross-sectional studies on correlations between macroeconomic factors and HIV-infection spread usually yield contradictory results, primarily due to variability of examined economic parameters and uninterrupted dynamics of parameters that characterize HIV-infection burden on a given territory (prevalence, morbidity, and mortality). In particular, in some works there are confirmations that average income per capita has its positive influence on morbidity with HIV-infection since an increase in income results in a decrease in morbidity with HIV [10]. But other research works provide evidence that this correlation is inverse since their authors state that higher gross regional product per capita, lower unemployment rate and smaller numbers of people with their incomes below living wage lead to an increase in a number of new HIV cases. At the same time there is a positive correlation between detection of patients with HIV and crime rates. Authors believe it occurs due to specific economic features of drug sales such as a region being rich, available drug distribution networks, and considerable resources of individual consumers; all this results in a growing number of relevant crimes [11].

Economic development of a region, availability of well-paid jobs and social security for unemployed people influence individual labor strategies pursued by economically active population groups. Young people have to start a career at younger ages and are forced to migrate to find a better job; all this leads to wider spread of forced deviant and delinquent behavior practices which, in their turn, make for HIV-infection spread in relevant demographic groups.

Contemporary studies confirm that HIV-infected people more often live in urban areas; it can be explained with a proven correlation between spread of the disease and provision with out-patient polyclinics and greater availability of public healthcare resources, consequently, a greater number of detected diseases cases [12, 13].

At the same time this trend may be due to occurring demographic processes such as labor migration by representatives from age and sex groups where HIV is most frequently detected from rural settlements to urban ones [10]; however, we haven't been able to detect any statistically significant correlations with growing migration rate in our research. As it is confirmed by works that focus on examining social and structural parameters of labor migration, young men aged from 25 to 50 tend to prevail among labor migrants in small towns in Russia [14–16]. Most HIV-infected people are also from these demographic groups. Over the last years there has been a growth in a number of infected people among those older than 25. In 2000 86.7 % HIV-infected people were diagnosed at 15–29 but this share went down to 44.3 % by 2010. In the first half of 2019 84.5 % of detected patients with first diagnosed HIV were older than 30. In 2019 HIV-infection was predominantly detected in people aged 30–49 (71.5 % new cases). A share of infected men was significantly higher than a share of infected women in all age groups[4].

In our research, we detected the strong correlation between HIV prevalence and a number of crimes committed by minors which in its turn was correlated to a number of unemployed people in a region. This correlation may occur due to trends revealed by criminologists in drug trafficking. Overall number of registered crimes committed by minors and related to drug trafficking grew by 28.6 % from 2010 to 2015 [17]. It is partly due to barriers on labor markets and difficulties in finding a job, especially in economically underdeveloped regions. In case there are no barriers to enter a dealer network and available developed intra- and inter-regional relations between organized crime groups who deal with drug trafficking, young people become involved into these criminal circles and commit crimes related to producing, sorting, and taking drugs. Young people participate actively

not only in producing and selling synthetic substances but also in taking them: a share of young people aged from 14 to 30 amounts to 76 % among drug addicts; a number of young people who take injection drugs is also growing and it results in greater risks of HIV-infection [18–21].

Our research results are consistent with conclusions made by authors who stated that men younger than 35 who lived in urban areas, including those currently or permanently unemployed, took drugs, violated the laws and were prone to risky behavior regarding their health prevailed among HIV-infected patients.

The current situation with growing numbers of HIV-infected people in RF regions and its correlation with social context factors is being discussed in works by social epidemiologists, sociologists, and experts in demography. Partially HIV-infection spread is explained by growing numbers of people in vulnerable population groups and their behavioral peculiarities. Accomplished studies reveal that people who take injection drugs, homosexuals, and commercial sex workers are primary risk groups regarding HIV [22]. At the same time contemporary social and epidemiological trends in HIV spread indicate that over the last years the infection has gone beyond these vulnerable population groups and started to spread actively among overall population[6] [23]. The process is supported not only by cultural and behavioral peculiarities typical for specific population groups, risky sexual behavior by commercial sex workers and drug addicts [24] but also by specific economic parameters in regions and demographic processes occurring in them.

Conclusion. Existing regional trends in HIV spread in the Russian Federation have apparent differences as per many macrocontext parameters including demographic, socioeconomic, social and political (medical aid availability), and cultural and behavioral ones. Consequences of urbanization create significant influence on the disease spread since the process is accompanied with a growing share of urban population, unstable labor markets, and growing demands for available free medical aid. We built up the regression model that included relevant regional parameters (specific weight of urban population in total population, a number of unemployed people aged 15–72, provision with out-patient polyclinics per 10,000 people) and it allowed us to reveal that it was exactly these factors that explained 22.0 % of regional differences in a number of patients with first diagnosed HIV.

But at the same time urbanization and intensified labor migration result in changing individual cultural and behavioral strategies including criminal ones and behavioral practices that are health-destructive. The greatest number of crimes committed by young people is registered in cities and this occurs due to pendulum labor migration, as well as contact-free drug distribution and intake becoming more and more popular. Having built the regression model that included a number of registered crimes committed by minors per 100 thousand people we revealed that this factor explained 35 % of regional differences in a number of patients with first diagnosed HIV.

Our results give grounds for assuming that HIV-infection spreads in RF regions primarily due to demographic and socioeconomic parameters and processes that provoke growing influence of specific cultural and behavioral determinants of the disease. Complex effects produced by the aforementioned factors result in apparent regional health inequalities and contribute to persistence of this issue related to morbidity with communicable diseases among population in Russia

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Conflict of interests. The authors declare there is no any conflict of interests.

[6] Spravka VICh-infektsiya v Rossiiskoi Federatsii v 2017 g. [Reference on HIV-infection in Russia in 2017]. Federal'nyi nauchno-metodicheskii tsentr po profilaktike i bor'be so SPIDom FBUN Tsentral'nogo NII epidemiologii Rospotrebnadzora. Available at: http://aids-centr.perm.ru/images/4/hiv_in_russia/hiv_in_rf_31.12.2017.pdf (March 05, 2021) (in Russian).

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Research article

MODIFICATION OF IMMUNOCYTES VIABLE PARAMETERS IN CHILDREN ASSOCIATED WITH COMBINED EXPOSURE TO CHEMICAL TECHNOGENIC AND EXTREME CLIMATIC FACTORS

I.N. Alikina, O.V. Dolgikh

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The article dwells on results obtained via immunologic parameters of immunocytes death among children living and attending educational establishments in regions beyond the Polar circle where industry was developing rather intensely.

Our research goal was to examine early disorders in immunologic profile as per immunocytes death among children living in Polar Regions under combined exposure to adverse chemical technogenic and extreme climatic factors.

75 children took part in the research (a test group); they lived and attended educational establishments in regions beyond the Polar circle where industry was developing rather intensely. Benzpyrene is a priority chemical exogenous factor in this region and a climatic one is extremely low temperature in winter (average temperature is -33.8 ^oC in this season). A reference group was made up of children (n = 35) who lived and attended educational facilities in Polar Regions where there was no exposure to technogenic chemical factors. The authors analyzed several immunogram parameters including $CD3^+CD95^+$, Annexin-V presenting cells, TNFRα, $CD3^+HLA\text{-}DR^+$, bax and p53. Cell death parameters were examined with fluorescent analysis via flow cytometry. Also, the authors assessed specific sensitivity of IgG to benzpyrene via allergosorbent testing with enzyme marker.

The research revealed hyperexpression of lymphocytes-cellular profile parameters in children from the test group in comparison with the reference one. They had 1.4 times higher expression of immunocytes stained with AnnexinV and a number of cells stained with PI (Propidium Iodide) was considerably higher than a number of cells stained with AnnexinV as well as the same parameters in the reference group(by 1.5 times) thus indicating that immunocytes predominantly die due to necrosis. There was hyperexpression of $HLA\text{-}DR^+$ receptor on lymphocytes (both its relative and absolute quantity in 12.4–13.7 % children). Expression of $CD95^+$ receptor (a membrane marker of immunocytes apoptosis) was 1.3 and 1.4 times higher (relative and absolute value accordingly).The authors detected an authentically elevated contents of tumor necrosis factor receptor (TNFR) as well as intracellular anti-tumor antigen p53, and antiapoptotic protein bax that were by 1.5, 1.2 and 1.3 times higher accordingly ($p < 0.05$) against the reference group. There was a significant difference in production of IgG specific to benzpyrene in children from the test group since its expression was 2.4 times higher than in children from the reference group ($p < 0.05$). The authors detected elevated risks of excessive expression both for membrane factors of cellular death TNFR (RR = 12.17), $CD3^+CD95^+$(RR = 5.42), HLA^-DR^+ (RR = 4.80) that were apoptosis affectors and for intracellular transcription factors bax (RR = 4.55) and p53 (RR = 3.71) that modulated apoptogenic signals. This risk was associated with combined exposure to chemical tehcnigenic and extreme climatic conditions.

It wasestablished that children living in the Polar Regions under combined exposure to chemical technogenic and extreme climatic conditions had imbalance in the immune status that became apparent via excessive expression of membrane ($HLA\text{-}DR^+$, $CD95^+$, TNFR) and intracellular (p53, bax) parameters with cell death program shifting towards necrosis (as opposed to the reference group that was exposed only to extreme climatic factors). These parameters indicate there is immune deficiency and a significant probability of viral infections and their complications.

Key words: *cell death, immunogram, children, extreme climatic conditions, benzpyrene.*

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Introduction. Population health is greatly influenced by living conditions, state of immune protection and an extent to which a body can adapt to adverse environmental conditions. Climatic conditions in Polar Regions are uncomfortable for people and social conditions there are much worse than those in central and southern regions in the country. Large industrial complexes located in the northern Siberia contaminate the environment and create threats of adverse impacts on human health, in particular for children population groups that are the most sensitive to changes in the environment [1–4]. There are available data in literature on changes in both levels of morbidity and its structure in different children groups who live under adverse technogenic exposure [5, 6]. A huge diversity of chemicals that are haptens in their essence occurs in the environment. Carcinogens are able to interact with each other, become active in favorable chemical conditions, transform and/or persist in any organic or non-organic medium over a long time [5, 7]. Aromatic and polyaromatic hydrocarbons have a significant place among toxicants that are ambient air pollutants. They are contained in exhaust gases, emissions from industrial enterprises and from large and small heating systems. Benzpyrene is a widely spread carcinogen in northern regions in Russia. Benzpyrene can enter a body via different ways and inhalation is among them. The substance is chemically and thermally stable, able to accumulate in biological media, and becomes carcinogenic in a concentration that is higher than 0.1 μg/100 m³. Apart from carcinogenic effects, benzpyrene produces mutagenic, embryotoxic, and hematotoxic ones [6].

In Polar Regions combined exposure to climatic and exogenous environmental factors exerts negative influence on a human body, induces grave stress responses, and creates additional difficulties for a developing (child) body. This set of factors influences the children's immune system significantly resulting in deadaptation, a decrease in reserve capabilities and a growth in morbidity. The immune system is highly sensitive to many environmental factors. In case ambient air is contaminated heavily, changes in health often become apparent via occurring secondary immune deficiency that makes for prevalence of sensitization [8–10].

Studies on health of population living in industrially developed Polar Regions in Russia are especially vital; such studies should concentrate on determining peculiarities of effect markers that characterize early disorders in immunologic health of children under climatic conditions existing beyond the Polar circle.

Our research goal was to examine early changes in immunologic profile indicators (apoptosis markers) among children living in Polar Regions under combined exposure to negative chemical technogenic and climatic factors.

Data and methods. The research involved examining 75 children (a test group) who lived and attended educational establishments on a territory with intensely developing industry located beyond the Polar circle (Krasnoyarsk region). Benzpyrene is a priority chemical exogenous factor in this region and a leading climatic factor is extremely low temperature in winter (average temperature is –33.8 °C). A reference group was made up of children (n = 35) who lived and attended educational establishments on territories beyond the Polar circle where there was no exposure to technogenic chemical factors.

An examination of children's immune state included analyzing the total leucocytes number, and relative and absolute lymphocytes quantity with conventional clinical analysis procedures accomplished with Drew-3 (D3) Drew Scientific hematologic analyzer (Great Britain, USA). Membrane and transcriptional indicators of lymphocytes cell death as well as quantity of IgG antibodies specific to benzpyrene were examined in children's peripheral blood.

The research included determining quantity of the following lymphocytes subpopula-

tions: activated T-lymphocytes $CD3^+CD95^+$, $CD3^+HLA\text{-}DR^+$. Levels of lymphocytes cell death were determined via staining with *Annexin V-FITC* (USA) and simultaneously with *Propidium Iodium PI* (USA); it allowed identifying cells at early stages in apoptosis. To assess a system of programmed cell death, we examined intracellular expression of Bax protein, intracellular apoptosis marker p53-portein, and surface expression of tumor necrosis factor receptor (TNFR1). Lymphocytes were extracted from peripheral blood via centrifuging in Ficoll-Urografin gradient solution. Gradient density for human blood corpuscle fractioning is equal to 1.077 g/cm^3. These indicators were selected basing on a hypothesis that programmed cell death was modified due to combined exposure (extreme climatic temperatures and contamination with exogenous chemical haptens) occurring under combined impacts exerted by various risk factors.

Lymphocytes immunophenotyping was performed via using monoclonal antibodies to surface differential antigens on immune system cells; it was done via flow laser cytofluorometry with *BDFACS Calibur flow cytofluorometer* (USA). Lymphocytes suspension was added with monoclonal antibodies (MAT) to a certain lymphocytes marker with these MAT being stained with fluorochrome. Then these antibodies bound to lymphocytes that expressed a marker relevant to a specific monoclonal antibody. Cell suspensions were washed via centrifuging in *Cell Wash solution* (BD, USA) thus removing antibodies that remained unbound to cells.

IgG specific to benzpyrene was identified with using reagins that were conjugated with peroxidase; it was done via allergosorbent testing procedure with an allergen being sorbed on cellulose substrates. Photometric measuring of optical density was performed with *Sunrise ELISA reader* (Tecan, Austria).

Results were estimated with Statistica, Statsoft, Inc. (USA), a universal software package for data analysis. Significance of differences was estimated with Student's t-test; differences between groups were considered valid at $p < 0.05$.

Results and discussion. Having performed immunocytes phenotyping, we revealed the following in the test group: hyperexpression of absolute and relative lymphocytes levels of activation phenotypes such as activated T-killers $CD3^+HLA\text{-}DR^+$, $CD3^+CD95^+$ lymphocytes (relative value), TNFR1 receptor 1, as well as p53, an intracellular protein that induced apoptosis. Values that deviated from reference ones were detected in 15.3 %, 12.1 %, 47.9 % and 18.8 % children accordingly.

Examined children from the test group had 1.4 times higher activation of cells stained with *Annexin V* against the reference group ($p < 0.05$). Cells stained with *PI* occurred in much greater quantities than phenotypes stained with *Annexin V* as well as the same parameters in the reference group (1.5 times higher). It is due to cells reaching the later stage in cell death faster under stress or due to them being already dead (necrosis). Cells lose cellular membrane integrity at later stages in apoptosis and absorb *PI* [11, 12]. However, this indicator remained within reference levels.

The research also revealed elevated $HLA\text{-}DR^+$ expression on lymphocytes in the test group since it was by 12.4–13.7 % higher than in the reference one. Expression of later activation marker $HLA\text{-}DR^+$ indicates how intense an immune response is. Excessive T-lymphocytes expression can occur due to many diseases that involve chronic inflammation (autoimmune diseases, hepatitis C, pneumonia, etc.) [12, 13]. We also established elevated levels of immunocytes $CD95^+$ apoptosis receptor that was 1.3 and 1.4 times higher than in the reference group (relative and absolute value accordingly). This marker is expressed on all cells in the immune system and plays an important role in control over the immune system functioning (immunocytes life cycle). An increase in a number of lymphocytes that

Table

Immunologic profile parameters of children from the examined groups

Parameter	Reference level	Test group, $M \pm m$, $n = 75$	Reference group, $M \pm m$, $n = 35$
$CD3^+HLA^-DR^+$, %	8–20	$28.347 \pm 1.199^{*/**}$	$27.359 \pm 2.242^{*}$
$CD3^+HLA^-DR^+$ lymphocytes, abs.,$10^9/dm^3$	0.1–0.5	$0.775 \pm 0.042^{*/**}$	$0.548 \pm 0.052^{*}$
$CD3^+CD95^+$ lymphocytes, %	15–25	$27.658 \pm 0.953^{*/**}$	20.200 ± 2.263
$CD3^+CD95^+$ lymphocytes, abs., $10^9/dm^3$	0.4–0.7	$0.765 \pm 0.038^{**}$	0.605 ± 0.087
Annexin V-$FITC^+7AAD^-$, %	0.5–1.0	$0.828 \pm 0.128^{**}$	0.609 ± 0.109
Annexin V-FITC+$7AAD^+$, %	5.0–7.0	$7.730 \pm 1.237^{**}$	5.090 ± 0.494
TNFR1, %	1–1.5	$6.246 \pm 0.430^{*/**}$	$4.118 \pm 0.384^{*}$
Bax, %	5–9	$8.318 \pm 0.645^{**}$	6.312 ± 0.998
p53, %	1.2–1.8	$5.570 \pm 0.448^{*/**}$	$4.808 \pm 0.425^{*}$
IgG specific to benzpyrene, arb.units	0–0.2	$0.198 \pm 0.029^{**}$	0.081 ± 0.008

Note: * means there is statistically authentic difference from reference level as per independent Student's t-test at $p < 0.05$; ** means there is statistically authentic difference from reference group as per independent Student's t-test at $p < 0.05$.

present $CD95^+$ is a natural sign that the immune system has been activated and it is typical for an immune-inflammatory process [14–16]. Also, there was an authentic increase in tumor necrosis factor receptor (TNFR) and cellular tumor antigen p53 in blood of children from the test group, by 1.5 and 1.2 times accordingly against the reference group. We also noted that there were no significant differences in pro-apoptotic protein Bax contents from reference levels but this indicator was significantly (by 1.3 times) higher in the test group than in the reference one. Exposure to cold is known to induce significant shifts in a body that become apparent via changes in a quantity of proteins, re-distribution of their fractions, and an increase in quantities of protein metabolism products [17].

There was an authentic difference between two groups regarding antibodies to benzpyrene since production of specific IgG was 2.4 times higher among children from the test group than among those from the reference one ($p < 0.05$). When polycyclic hydrocarbons enter a human body, they create an epoxy-compound under impacts exerted by enzymes; this compound enters a reaction with guanine and it disrupts DNA synthesis, leads to disorders or mutations that make for oncologic diseases development including such types of cancer as carcinoma and sarcoma [16]. Stress-dependent protein p53 inhibits a change in cellular cycle phases as a response to DNA damage thus inducing cell apoptosis [18–20]. In this research expression of phosphoprotein p53, as well as expression of tumor necrosis factor receptor TNFR, was established to be higher than the upper reference level in blood of children from the test group and was authentically higher than the same parameter in the reference one. Therefore, we can conclude that extreme climatic conditions and excessive quantities of exogenous hapten benzpyrene (evidenced by hyperexpression of specific IgG) act together as apoptosis stimulators making for excessive quantities of proteins that speed up immunocytes death in children living in industrially developed areas beyond the Polar circle.

Having assessed relative risks (RR)[1] of disorders in apoptosis development under exposure to excessive contamination with

[1] Rukovodstvo po otsenke riska dlya zdorov'ya naseleniya pri vozdeistvii khimicheskikh veshchestv, zagryaznyayushchikh okruzhayushchuyu sredu [The Guide on assessing population health risks under exposure to chemicals that pollute the environment]. Moscow, The Federal Center for State Sanitary Epidemiologic Surveillance of the RF Public Healthcare Ministry Publ., 2004, 143 p. (in Russian).

benzpyrene, we established that elevated benzpyrene concentrations in blood of the examined children led to elevated risks of excessive expression, both regarding membrane cell death factors such as TNFR (*RR* = 12.17), $CD3^+CD95^+$ (*RR* = 5.42), and HLA^-DR^+ (*RR* = 4.80) that were apoptosis affectors, and intracellular transcription factors that modulated apoptogenic signals such as Bax (*RR* = 4.55) and p53 (*RR* = 3.71). And here there was no risk of disrupted effector structures of apoptotic scenario that were bound to *Annexin V*.

Therefore, an additive effect produced by extreme climatic conditions together with exogenous hapten stimulation leads to an imbalance in immune state of the examined children living in Polar Regions; this imbalance becomes apparent via hyperexpression of cell death indicators such as an increase in oncosuppression transcription factors Bax and p53 as well as membrane factors TNFR, $CD3^+$HLA-DR^+, and $CD3^+CD95^+$ responsible for induction and transcription stages in apoptosis scenario.

It should be noted that this research focuses on analyzing peculiarities of programmed cell death modification in children under combined exposure to climatic (extremely cold temperatures) and technogenic (exogenous chemical) adverse factors and its effects on apoptosis; the research results allow predicting risks of early health disorders.

Conclusion. Peculiarities of immunocytes viability parameters in children living under combined exposure to technogenic and extreme climatic factors are hyperproduction of IgG specific to benzpyrene as well as excessive expression of cell death indicators such as transcription and oncosuppression factors Bax and p53 and membrane phenotypes $CD3^+$HLA-DR^+, $CD3^+CD95^+$, and TNFR. The latter are responsible for apoptosis realization, both at a stage when it is induced and at a stage of intracellular modulation of its program that is modified negatively under exposure to haptens in the environment (benzpyrene) and it predetermines immune deficiency in children living under these conditions. Having assessed relative risks (*RR*) of disorders in apoptosis under exposure to excessive contamination with benzpyrene, we revealed that elevated benzpyrene concentration in the examined children's blood resulted in elevated risks of excessive expression, both regarding membrane cell death factors TNFR (*RR* = 12.17), $CD3^+CD95^+$ (*RR* = 5.42), and HLA^-DR^+ (*RR* = 4.80) that were apoptosis affectors, and intracellular transcription factors Bax (*RR* = 4.55) and p53 (*RR* = 3.71) that modulated apoptogenic signals.

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Research article

GEOMAGNETIC CHANGES AS A RISK FACTOR CAUSING OXIDATIVE STRESS IN HUMAN ERYTHROCYTES

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At present impacts exerted by heliogeophysic factors on different living organisms are being examined more and more intensely. Over the last decade, it has been detected that meteorological factors play an important role in formation of adaptation mechanisms in living systems. Sun and earth interaction is also known to directly influence rheological properties of human blood, both in a healthy body and in case there are chronic non-communicable diseases. Given that, impacts exerted by "cosmic weather" on living organisms, people in particular, are a stimulus to perform profound studies on reactions occurring as a response to effects produced by solar and geomagnetic activity, first of all, solar flares and magnetic storms.

At present the most widely used approach to determining influences exerted by the Earth magnetic field on biological systems involves searching for correlations between different parameters of living organisms functioning and geomagnetic indexes.

Our research goal was to assess dependence between enzymatic activity of antioxidant protection system, exemplified by superoxide dismutase (SOD) and catalase, and geomagnetic field disturbances.

The research focused on examining blood samples taken from conditionally healthy volunteers living in Nizhny Novgorod region. SOD and catalase activity were determined in blood erythrocytes with spectrophotometry. Geomagnetic disturbances force was determined as per planetary Kp-index value.

The research established a statistically significant correlation between SOD and catalase activity and a value of geomagnetic field disturbance; this correlation allows making an indirect assumption that geomagnetic conditions directly influence superoxide radical production in a body.

Therefore, a change in superoxide radical production is a way for the geomagnetic field to influence living organisms. Activation of free radical oxidation can make for both occurrence and more intense clinical course of several diseases (especially cardiovascular and neuropsychic ones). So, in future it is advisable to assess reactivity of antioxidant protection system as a response to geomagnetic fluctuations in case there are pathologic changes in a body.

Key words: *geomagnetic disturbances, solar activity, heliobiology, Kp-index, superoxide dismutase, catalase, magnetic sensitivity, human body.*

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At present, sufficient data have been accumulated on influence exerted by space factors on the biosphere but their action mechanisms have not been studied enough [1–8]. Such ecological impacts have apparent biotropic effects that result in biochemical and physiological transformations in living organisms [9] and become obvious not only through changes in individual development cycle but also through effects produced on development of next generations as a whole [10]. Better insight into these fundamental mechanisms is a necessary basis of predictive and preventive programs in medicine.

The Sun is known to be the primary source of space weather on the Earth since this star is the closest to our planet. Influence by the Sun occurs due to electromagnetic radiation in various ranges, stellar wind flows, solar cosmic rays, solar flares and corona mass ejections. The Earth has its own shield to protect from destructive effects produced by these phenomena; this shield is the planetary magnetic field and the ozone layer in the atmosphere. However, some high-speed charged particles can penetrate into the Earth magnetosphere thus leading to changes in the atmosphere and ionosphere. Quantity and intensity of influencing solar factors grows as the star becomes more active, and rises in solar activity repeat periodically at the beginning of each new cycle which is conditionally called "11-year one" [11]. According to official data provided by the Space Weather Prediction Center, the USA, the 24th solar cycle ended relatively not long ago (minimal activity was registered in December 2019), and then the 25th cycle started with maximum activity predicted in between November 2024 and March 2026. More intense radiation at various frequencies and corpuscular fluxes lead to ellipticity of magnetosphere and an increase in the geomagnetic field intensity. Thus a geomagnetic storm is born as global disturbance in the Earth magnetosphere [12].

We should keep in mind that there is a certain time gap between occurring heliogeophysical disturbances and such responses to them as changes in physiological state of the body. These responses occur in most people at the beginning of global disturbance in the Earth magnetosphere, 2–3 days after a solar flare. However, some people become sensitive even 1–2 days prior to a geomagnetic storm arises. This effect is partially due to solar influence being multi-component and these components moving at different speed: electromagnetic radiation in any range covers the distance from the Sun to the Earth in 8–10 minutes while it takes corpuscular fluxes 24–30 hours to reach the planet [13].

It should be noted that influence exerted by solar activity on processes in the living nature at all levels of biological systems organization, including human health and population morbidity and even social and historical events, was established as far back as in 20ties last century by A.L. Chizhevsky, an outstanding Soviet scientist and the founder of contemporary heliobiology. Since then, multiple facts have been accumulated on impacts exerted by fluctuations in physical fields in the Near-Earth space that disrupt functioning of various physiological systems in the human body [12, 14]. According to up-to-date concepts, the cardiovascular system and the nervous system are the most sensitive to changes in space weather and the fact is confirmed by observed rises in morbidity and mortality among patients with pathologies of these systems during disturbances in the magnetosphere [15–19].

Moreover, it was established that healthy people had different blood system parameters during years with maximum and minimum solar activity [20–21]. When it comes down to practical medicine, it is important to keep in mind influence exerted on the body not only by fluctuations in the solar activity that are averaged over a long period of time but also individual ones such as flares and falls in separate days. We should note that such changes in the solar activity result in abnormal strengthening of solar wind that creates geomagnetic storms. Geomagnetic field fluc-

tuations may become health risk factors since they make for disorders in coordination between rhythms of biological processes in living organisms and environmental cycle recurrence [22].

We can also mention that intense magnetic disturbances sometimes occur due to unknown reasons; in this case it is difficult to detected a source of these fluctuations since there are no active events on the Sun that are directed at the Earth. A magnetic storm of G3 level registered on May 12, 2021 that was the greatest since 2019 can be a good example. According to data provided by the laboratory of X-ray Astronomy of the Sun at the Institute of Physics, the Russian Academy of Sciences [23], geomagnetic disturbance intensity reached Kp-index value equal to 7 as per a 9-score scale at the peak of the storm that occurred in the evening, between 3 and 9 pm Moscow time. Actually it was just one but very powerful space strike on the Earth. Solar flares that were registered during those days occurred on such spots on the Sun that were remote from direction at the Earth and the storm was not predicted at all.

It is noted that various molecules, for example, paramagnetic free radicals, can serve as "receiving elements for an external magnetic signal. Paramagnetic properties of these reaction-active particles are determined by an unpaired electron on the external valent layer and this makes them unstable. These very molecules are intermediate participants in such biochemical reaction as one-electron reduction. Therefore, geomagnetic variations follow this mechanism to manage free radical processes of substance transformation." [24].

Various physical indicators (Kp-, Ap-, C-index and some others) are applied to determine geomagnetic activity. Kp-index is the most widely used one; it measures geomagnetic field fluctuations. This indicator shows a deviation in the Earth magnetic field from its normal state during a 3-hour interval. Its values vary from 0 to 9 and are calculated as an average for values provided by several geomagnetic observatories located between 44 and 60 degrees of northern and southern latitudes. Geomagnetic storms are relatively short (from 6–7 hours to several days) and after their end physical parameters of the geomagnetic field return to their initial values. G-index characterizes intensity of variations in a geomagnetic storm as per a 5-score scale with storm gradation starting from G1 (weak storm) to G5 (extremely strong storm) [25, 26].

At present, mechanisms of influence exerted by geomagnetic field disturbances on biological systems, people included, have not been studied enough. Search for magnetosensitive markers can be a promising approach to revealing mechanisms of such influence [27].

Our research goal was to assess dependence between enzymatic activity of antioxidant protection system and geomagnetic field disturbances.

Materials and methods. We examined blood samples taken from conditionally healthy volunteers who lived in Nizhny Novgorod region (n = 37). Biological materials were taken from September 2019 to February 2020, in the morning hours from 8 am to 9 am. Donors' age varied from 19 to 58 years. To assess influence exerted by geomagnetic situation on antioxidant protection system, we focused on superoxide dismutase (SOD) and catalase since these enzymes belong to the first section in intracellular protection from reactive oxygen species. SOD activity was determined as per inhibition of recovery rate detected for nitroblue tetrazolium in non-enzymatic system of phenazine methosulfate and nicotinamide adenine dinucleotide (NADN)[1]. Catalase activity was determined by spectrophotometry in erythrocytes as per

[1] Arutyunan A.V., Dubinina E.E., Zybina N.N. Metody otsenki svobodnoradikal'nogo okisleniya i antioksidantnoi sistemy organizma [Procedures for estimating free radical oxidation and antioxidant system in the body]. Saint Petersburg: IKF "Foliant", 2000, 104 p. (in Russian).

changes in optical density in a region of hydrogen peroxide absorption [28]. Intensity of geomagnetic disturbances was estimated as per planet Kp-index; its values for Nizhny Novgorod region on a day when biological materials were taken were available at www.tesis.lebedev.ru [23]. It is the official web-site of the laboratory of X-ray Astronomy of the Sun at the Institute of Physics, the Russian Academy of Sciences where uninterrupted monitoring over the conditions of the Sun is conducted. Correlations between data were estimated with correlation analysis according to Spearman.

Results and discussion. The research revealed a statistically significant correlation between activity of anti-oxidant enzymes in erythrocytes and planet Kp-index (Figures 1 and 2).

On days when Kp-index values were high, SOD and catalase enzymes became more active in erythrocytes of healthy volunteers. Superoxide dismutase turned out to be more sensitive to geomagnetic situation since we detected statistically significant positive correlations between its activity and planet Kp-index values, both 2.5–3.0 hours prior to blood sampling (Figure 1A) and values at the moment blood was being sampled (Figure 1B), as well as average planet Kp-index values over the whole day of blood sampling (Figure 1C). Correlation between catalase activity and Kp-index values was detected only for Kp-index values 2.5–3.0 hours prior to blood sampling (Figure 2).

SOD and catalase are known to be high-specialized antioxidant enzymes. Superoxide dismutase serves as a catalyst in a reaction of superoxide dismutation into hydrogen peroxide and oxygen. Catalase participates in hydrogen peroxide decomposition thus preventing formation of hydroxyl radicals when hydrogen peroxide interacts with iron or copper ions as per the Fenton reaction [29].

Detected correlations between geomagnetic activity and activity of the analyzed enzymes can give grounds for an implicit assumption that geomagnetic situation has direct influence on production of superoxide radical; and, as it is well known, non-enzymatic

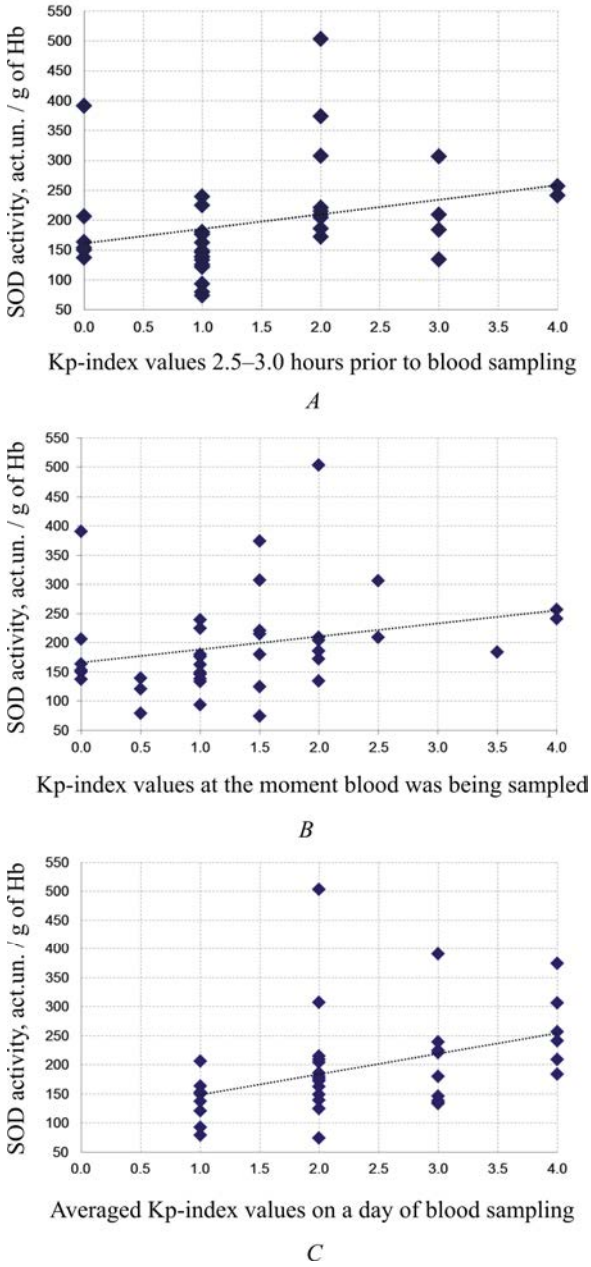

Figure 1. Correlaiton field for dependence between antioxidant enzyme superoxide dismutase and geomagnetic situation: **A**, 2.5–3.0 hours prior to blood sampling (Spearman's correlation $r = 0.44$, $p = 0.007$); **B**, at the moment blood was being sampled (Spearman's correlation $r = 0.37$, $p = 0.013$); **C**, average value on a day of blood sampling (Spearman's correlation $r = 0.50$, $p = 0.002$)

spontaneous hemoglobin oxidation into methemoglobin is a basic source of superoxide anion in erythrocytes (Figure 3). It is in line with the concept by V.P. Reutov [1] according to which Fe^{2+}-containing proteins are targets for effects

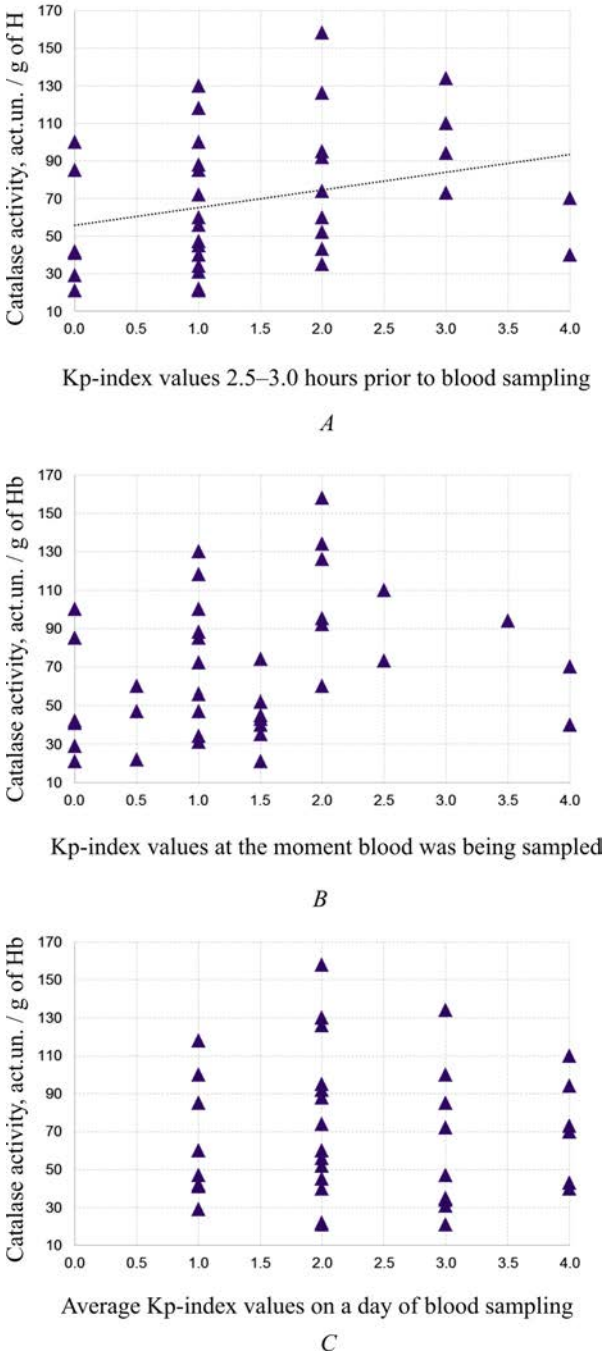

Figure 2. Correlaiton field for dependence between antioxidant enzyme catalase and geomagnetic situation: **A**, 2.5–3.0 hours prior to blood sampling (Spearman's correlation $r = 0.34$, $p = 0.041$); **B**, at the moment blood was being sampled (Spearman's correlation $r = 0.32$, $p > 0.05$); **C**, average value on a day of blood sampling (Spearman's correlation $r = –0.01$, $p > 0.05$)

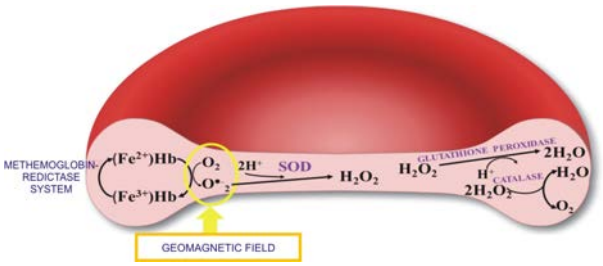

Figure 3. Scheme showing generation and neutralization of superoxide radical and hydrogen peroxide in erythrocytes

produced by geomagnetic field. The fact that the detected correlation is moderate is probably due to, first of all, absence of strong geomagnetic storms during the observation period, and, secondly, individual peculiarities of participating volunteers.

The detected correlation between catalase activity and geomagnetic situation several hours prior to blood sampling might be weak due to absence of any direct effects produced by the Earth magnetic field on generation of hydrogen peroxide H_2O_2 in erythrocytes. Instead, we can see only indirect influence through dismutation reaction catalyzed by SOD that results in hydrogen peroxide formation and catalase activity changes accordingly (Figure 3).

Conclusion. Therefore, there is indirect evidence that a change in production of superoxide radical is a mechanism of influence exerted by the geomagnetic filed on living organisms. Activation of free radical oxidation can make for development and aggravation of many diseases (especially cardiovascular and neuropsychic ones). Therefore it seems advisable to estimate responses by antioxidant protection system to geomagnetic fluctuations in case there are pathological changes in the body.

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Research article

THE RISK OF COVID-19 SEVERITY IN PATIENTS WITH MS APPEARS TO BE ASSOCIATED WITH IMMUNOTHERAPY

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Immunomodulatory drugs are important to control disease activity in relapsing-remitting multiple sclerosis (MS). Anti-CD20-therapy is one of such medications. In Sweden, extensive off label prescription of rituximab (RTX) in MS has been documented; it is presently prescribed for more than half of all treated MS patients. The rationale for the increasing prescription of RTX was previous data from phase II and observational studies supporting high efficacy and safety, in addition to the financial aspect. We report national data on usage of disease modifying therapies in MS patients and risk of severe COVID-19 in association with RTX exposure within this group.

The Swedish National MS Registry (SMSreg) aims to cover all patients with MS in the country, (n = approximately 18,000). After COVID-19 pandemic started in Sweden, a new section was established in it to register clinical and demographic parameters in COVID-19-infected patients. Data presented in the current report were obtained from the SMSreg.

A total of 85 out of approximately 6,000 RTX-treated Swedish MS patients had been hospitalized with COVID-19 (as reported from the SMSreg, June 16, 2021) and adjusted analyses showed a 2–3 fold increase in a risk (OR = 2.89; p = 0.001) of hospitalization for anti-CD20 treated patients. A change of praxis was introduced in Sweden in spring 2020, resulting in a majority of patients receiving RTX infusions with extended intervals in order to reduce the risk of severe COVID-19 infection.

Current Swedish registry data suggest that exposure to RTX in MS may affect the clinical outcome of COVID-19 infection. These observations have rapidly impacted use of immunomodulatory drugs in Swedish MS patients.

Key words: *multiple sclerosis, immunomodulatory therapies, infection, COVID-19, anti-CD20-therapy, rituximab, national quality health registries, Swedish Multiple Sclerosis registry.*

The current COVID-19 (SARS-CoV-2) pandemic has affected various aspects of life and healthcare all over the world. Multiple sclerosis (MS) is a chronic demyelinating neuro-inflammatory disease of the central nervous system. The incidence rates of MS are greater in countries located at higher latitudes, such as Sweden and Russia [1–3]. Since MS is an autoimmune disease, patients within this group often adhere to immunomodulatory treatment but available therapies are quite diverse and have multiple targets [4]. Regarding relapsing-remitting MS patients (RRMS), both first- and second-line

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drugs are used depending on disease activity and response to treatment. One of the second line treatments, rituximab (RTX), which is primarily used for rheumatoid arthritis (RA), has developed extensive off-label prescription use in Sweden for MS [4–8]. The mechanism of RTX is based on anti-CD20 monoclonal antibodies (mABs), which mediate destruction of CD20-expressing cells through apoptosis, complement activation and antibody dependent-cell-mediated cytotoxicity [7, 9]. A Swedish study reported a rising trend for ongoing immunotherapy with RTX from 2011 to 2016 in MS population in the country [5]. In Sweden, RTX was prescribed to 53.3 % of all MS patients that started MS disease modifying therapy (DMT) during 2017 [5]. This trend was partially explained by both efficacy of the drug, its relative safety in use as well as cost effectiveness as documented in both RA and MS cases [5, 7]. At least within the Swedish healthcare system, the difference in cost is astounding. Compared to fingolimod and natalizumab with a cost of approximately 200,000 SEK per patient per year, the same cost for RTX corresponds to 25,000 SEK per patient per year [10]. Sweden is not alone in its off-label use, RTX is also prescribed in Norway, Denmark and less frequently in Russia [8, 11, 12].

The potential impact on humoral immunity with RTX has raised concerns. A few studies have shown that subsets of patients treated with RTX develop hypogammaglobulinemia, which may be associated with an increased risk of severe infections [13]. In 2020 a Swedish original investigation by Luna et al. [14] studied RTX and other highly effective immune therapies used for treating MS and concluded that exposure to RTX was associated with the highest rate of serious infections. Infections may trigger both clinical exacerbations and pseudo relapses with developing reversible or irreversible deterioration of neurological performance in MS patients. Along with further data linking neurological manifestations and COVID-19, there is an interesting review over intervention of immunomodulatory therapy and whether exposure to different treatments may impact COVID-19 outcome in the current situation of the pandemic [14, 15].

The aim of this investigation was to present the current prescription of DMTs in the Swedish MS population and to report data of COVID-19 outcome in association with the use of DMTs in COVID-19 infected Swedish MS patients.

Method. The Swedish MS registry (SMSreg) was established with the purpose of ensuring high quality neurological care and treatment [16]. It has been applied in neurological departments across the country for over 20 years and covers approximately 80 % of the Swedish MS population which corresponds to a total number of about 18,000 patients. The SMSreg contains data on clinical and demographic variables, disease activity measures and immunomodulatory therapies prescribed for MS patients. The registry is regularly used by physicians and nurses in clinical patient care and often provides a basis for decision making regarding treatments. A registration module to collect data on clinical parameters related to COVID-19 infection in MS patients was put into practice in the SMSreg just after the pandemic started. The COVID-19 module was developed to gain increased knowledge about the impact produced by COVID-19 infection on the Swedish MS population [17]. The COVID-19 module allows neurologists to register data on MS patients with confirmed (positive COVID-19 PCR test or positive antibody serology) or suspected COVID-19 disease (according to the WHO criteria). The variables for registration include gender, age, date of COVID-19 disease onset, clinical symptoms, disease duration of COVID-19, current immunotherapy, last known lymphocyte count, need for hospital care / intensive care / assisted ventilation and outcome (survival / death). The data described in the present report has been obtained from the webpage of the SMSreg and from the

Visualization and Analyses Platform (VAP) in the SMSreg [16].

Results. A total number of 10,962 out of approximately 18,000 MS patients registered within the SMSreg received DMTs of which more than half of the patients were treated with RTX (52 %). The proportions of other prescribed DMTs were as follows: dimethylfumarate (11 %), natalizumab (12 %), interferon-beta (7 %), fingolimod (7 %), glatiramer acetate (4 %), teriflunomide (4 %), cladribine (1 %), alemtuzumab (1 %) and ocrelizumab (1 %), (data retrieved from the VAP, SMSreg, June, 2021) (Figure 1).

As of June 16, 2021, 971 of the nearly 18,000 MS patients within the SMSreg, were registered as COVID-19-infected, of which 127 were admitted for hospital care [16]. Of those admitted, 26 patients required intensive care and five patients had died. Furthermore, a total of 85 out of nearly 6,000 RTX-treated Swedish MS patients had been hospitalized with COVID-19. Adjusted data analyses (for age, gender, disease duration, expanded disability status scale (EDSS) and progressive disease course) showed a 2–3-fold increased risk of hospitalization (OR = 2.89, $p < 0.001$) for patients treated with anti-CD20-therapy (including RTX and ocrelizumab), (data retrieved from the SMSreg webpage) [16].

The proportions of DMT usage in the Swedish MS population have changed over time. A national trend towards a decrease in RTX usage, and an increased use of natalizumab has been observed in connection with the pandemic, (data retrieved from the VAP, SMSreg, June, 2021) (Figure 2).

It was anticipated that the current highly effective immunomodulatory therapies, such as anti-CD20-therapy, might impact the COVID-19 outcome amongst MS patients. The association between anti-CD20-therapy and risk for hospitalization observed in the Swedish MS population according to the SMSreg was corroborated in an international study covering registry data

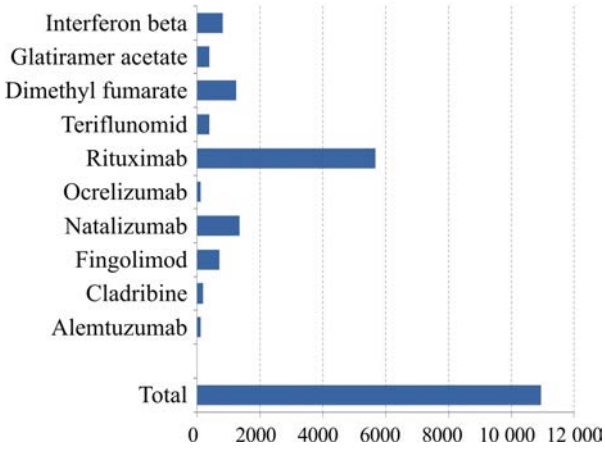

Figure 1. Number of ongoing disease-modifying therapies for MS patients in Sweden. Data retrieved from the Visualization and Analyses Platform (VAP), Swedish MS register (SMSreg), June, 2021

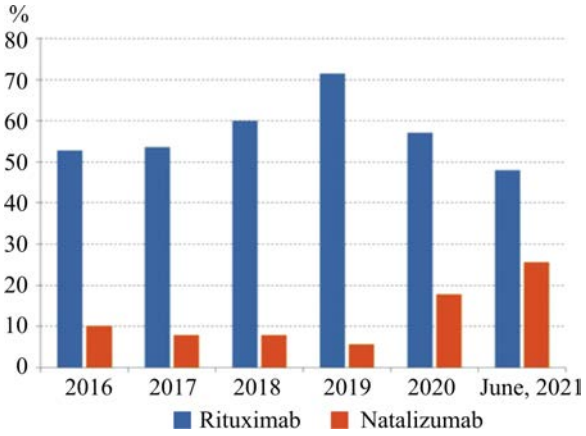

Figure 2. The proportion of MS patients that initiated treatment with rituximab and natalizumab in relation to all disease-modifying drugs during the time period January 2016 to June 2021. Data retrieved from the Visualization and Analyses Platform (VAP), Swedish MS register (SMSreg), June, 2021

from several countries [18]. A positive correlation between anti-CD20-treatment and risk of both hospital care and intensive care was described in this large international MS cohort of more than 2,000 patients with COVID-19 infection [18]. Comparable results were reported in an Italian MS study of 844 patients [19]. Another study from North America showed that MS patients treated with RTX ran an increased risk of

COVID-19 and suffering a worse disease course, compared to patients without immunomodulatory treatment [19, 20]. Similarly, a separate study showed that RTX treated MS patients ran a greater risk of severe COVID-19 compared to the general population on average during 2.5 months after recent RTX infusion [21]. A high dose of RTX (1,000 mg) was also correlated to worse COVID-19 outcomes [21].

Furthermore, in line with the results obtained via the international study using several national MS cohorts, a large global study on more than 2,800 COVID-19 infected RA patients showed that exposure to RTX correlated with worse COVID-19 outcome compared to patients treated with tumor necrosis factor inhibitors [22].

The accumulating support for an increased risk of worse COVID-19 outcome in MS patients that are treated with RTX has rapidly implicated clinical work for decision making regarding choice and timing of DMTs in this population. In Sweden, dosing intervals between RTX infusions were advised to be extended in spring 2020. The intention was to shorten the duration of B-cell depletion to possibly reduce the risk for severe COVID-19 infection. This strategy was supported by a Swedish report that described absence of recurrence in disease activity after discontinuation of RTX in MS patients [23]. In addition, data from the SMSreg have shown a recent change in the prescription of DMTs in MS including a trend of decreased usage of RTX and increased prescription of natalizumab in Sweden.

To sum up, current national data from the SMSreg and international combined registry data suggest an increased risk for a worse outcome of COVID-19 in patients exposed to anti-CD20-therapy. Choice on immunomodulatory therapy is to be made under careful consideration of other common and MS specific risk factors including disability status, to reduce the risk for severe COVID-19 infection.

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Research article

RISK OF CIRCULATORY DISEASES ON THE EXAMPLE OF HEART RHYTHM DISORDERS IN PATIENTS WITH SEROLOGICAL MARKERS OF EPSTEIN – BARR INFECTION

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To reduce a burden of circulatory diseases (CD) is among primary tasks the state has to solve. There are available data on a contribution made by chronic inflammation on occurrence of heart and vascular diseases. Given that, it seems especially interesting to examine impacts exerted by Epstein-Barr virus infection on CD development.

The paper focuses on analyzing morbidity with infectious mononucleosis and CD in the Russian Federation over 1995–2018. 103 patients with heart rhythm disorders and 92 blood donors were examined to determine whether they had immunoglobulins to EBV antigens.

The results were statistically processed involving calculation of Pearson's linear correlation coefficient, error of correlation coefficient, validity coefficient, determination coefficient, and frequencies of EBV markers detection per 100 examined people and their confidence intervals. Differences were considered to be authentic when confidence probability was equal to 95 % and confidence significance was $p < 0.05$. Relative risks of heart rhythm disorders in patients with active EBV-infection were calculated as per results obtained via creating a fourfold table.

EBV was established to make a significant contribution into circulatory pathology occurrence and it was confirmed by an overall ascending trend in morbidity with infectious mononucleosis and CD in the Russian Federation in 1995-2018 and an authentic strong direct correlation between these two processes ($r = 0.94$; $m = 0.02$; $t = 47$; $p < 0.01$) with determination being equal to 0.88. Risk of developing heart rhythm disorders was determined by active EBV-infection in case history combined with detected M-immunoglobulins to capsid EBV antigen and G-immunoglobulins to early EBV antigen, as well as an increase in concentration of G-immunoglobulins to capsid antigen in blood serum (RR = 5.8 and 2.3 accordingly).

These detected peculiarities require further more profound study and development of activities aimed at risk minimization.

Key words: *Epstein – Barr virus, circulatory diseases, infectious mononucleosis, morbidity, heart rhythm disorders, blood donors, relative risk, positivity rate.*

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High prevalence of circulatory diseases (CD) has been given a lot of attention by experts in different fields both in the Russian Federation and abroad [1–6]. In the Russian Federation CD prevalence grew by 20.7 % merely over 6 years, from 2011 to 2017 [7]. Researchers point out that there is a correlation between growing CD cases and negative effects produced by external factors including quality of food products, drinking water and ambient air contamination, and factors related to lifestyle such as smoking alcohol abuse, hypodynamia, emotional overstrain, etc. [3, 6, 8–10]. Influence exerted by aforementioned factors on the human body is accompanied with metabolic disorders and changes in key biochemical parameters, lipid profile included [11–13]. In 2017 Russian researchers analyzed contributions made by specific biochemical parameters into a risk of ischemic heart disease; the analysis revealed that in most cases clinical specialists failed to take into account results of laboratory tests indicating that oxidative processes were disrupted and inflammation occurred [14].

At present, there is scientific evidence that it is oxidative processes that lead to changes in redox balance in internal environment of the body [15, 16]; chronic inflammation is a typical sign of cardiovascular diseases and it leads to more severe outcomes regardless of cholesterol contents in blood serum [17].

Polish scientists published a systematic review that focused on analyzing a great number of research works; they established that pro-inflammatory cytokines had direct influence on health of the cardiovascular system. Expression of such cytokines grows over the whole lifetime and correlates with prevalence of this pathology and mortality caused by it. Growing electrical instability of cardiac muscle accompanied with heart rhythm disorders occupies a special place among other effects produced by pro-inflammatory cytokines [18].

Elevated concentrations of pro-inflammatory cytokines are markers of low-grade chronic inflammation typical for reactivation of latent viral infections including one caused by Epstein – Barr virus (EBV) [19]. A study that involved examining 222 elderly people living in the USA (their average age was 64.1 ± 14.1) revealed that higher titers of antibodies to EBV among people who were seropositive simultaneously to EBV and cytomegalovirus were associated with elevated contents of C-reactive protein and interleukin-6 and were accompanied with deteriorating health [20]. Another research work accomplished by American scientists revealed a correlation between interleukin-6 contents in blood plasma and titers of neutralizing antibodies to EBV-coded deoxyuridine triphosphate nucleotidohydrolase. This protein is synthesized at an early stage in virus reproduction and induces human monocytes to produce interleukin-6 and tumor necrosis factor alpha which, in their turn, play a key role in chronic inflammation development and cardiovascular pathology [21].

Analysis of the epidemiological situation regarding EBV-infection indicates it is widely spread and occurs everywhere [22–26]; there has also been an ascending trend in incidence with infectious mononucleosis in the Russian Federation over the recent years [27–29].

Given that, it seems vital to examine influence exerted by EBV-infection on cardiovascular diseases in general and heart rhythm disorders in particular.

Our research goal was to assess contribution made by EBV-infection to development of circulatory diseases on the example of heart rhythm disorders.

Materials and methods. We performed retrospective epidemiologic analysis of incidence with infectious mononucleosis and cardiovascular diseases in the Russian Federation in 1995–2018. Incidence rates were calculated per 100 thousand people. Data were taken from the Statistical Report Form No. 2 “Data on infectious and parasitic diseases” and statistical data collections “Public Healthcare in Russia” (Rosstat)[1].

[1] Zdravookhranenie v Rossii – 2019 [Public Healthcare in Russia – 2019]. *Rosstat*. Available at: http://gks.ru/bgd/regl/b19_34/ (September 10, 2020) (in Russian).

We calculated Pearson's linear correlation coefficient (r) and standards error for correlation coefficient (m). Correlation authenticity was checked with authenticity test (t). Differences were considered to be authentic at the significance level being $p < 0.01$ and confidence probability being 99 %. Calculation of determination coefficient (r^2) allowed determining a share belonging to influence of incidence with infectious mononucleosis on CD prevalence.

We conducted a randomized controlled trial of blood serum from February 10, 2020 to June 01, 2020 to determine immunoglobulins M (IgM) to capsid antigen (VCA) and G (IgG) to capsid early (EA) and nuclear antigens (EBNA) to EBV; the trial was performed on two groups of patients through enzyme immunoassay using "Vector best" reagent kit. The first or test group was made up of 103 patients with heart rhythm disorders (there were 60 men or 58.25 % (95 %; CI 48.49 ÷ 68.01) and 43 women or 41.75 % (95 %; CI 31.99 ÷ 51.51) aged from 21 to 65, average age being 50.27 (95 %; CI 48.41 ÷ 52.13)). The second or reference group consisted of 92 conditionally healthy people who were active blood donors, 61 men (66.3 % (95 %; CI 56.39 ÷ 76.21)) and 31 women (33.7 % (95 %; CI 23.79 ÷ 43.61)) aged from 19 to 60, average age being 41.03 (95 %; CI 33.55 ÷ 48.51). The analyzed groups were comparable in terms of sex, age, region of living, and a period when the trial took place.

Patients with heart rhythm disorders (the test group) were selected to participate according to the following criteria:

– they were being treated in hospitals due to an acute event involving heart rhythm disorders;

– they had a diagnosed heart rhythm disorder (paroxysmal tachycardia, atrial fibrillation, atrioventricular blocks, bundle branch blocks, premature ventricular contractions with extrasystole contribution exceeding 5 % of all contractions a day) confirmed with ECG and 24-hour Holter monitoring;

– they had negative results of tests aimed at determining serological and molecular-biological markers of contagion with syphilis agent, human immunodeficiency virus, hepatitis *B* and *C*;

– their age was from 18 to 65, both men and women were included;

– they gave their informed consent to take part in the trial.

The test group didn't include patients treated in out-patient clinics; patients with heart rhythm disorders in their case history who were in remission at the moment the trial was being conducted; patients with heart rhythm disorders not being confirmed with ECG or 24-hour Holter monitoring; patients who had myocardial infarction during 30 days prior to the trial; patients younger than 18 or older than 65; patients who didn't give their consent to take part in the trial; patients with positive results of molecular-biological test aimed at determining COVID-19 genetic material or CT-signs of COVID-19.

People for the second (reference) group, or conditionally healthy people, were selected from active donors of blood and its components according to the following inclusion criteria:

– they were men and women aged from 18 to 65;

– they gave their informed written consent to participate;

– they had a medical examination that included body weight measuring, body temperature taking, blood pressure measuring, determining heart rate and heart rhythm (provided there were no heart rhythm disorders); they didn't have absolute contraindications to be donors and were given a therapeutic report on absence of any therapeutic pathology at the moment their blood was taken;

– they had negative results of tests aimed at determining serological and molecular-biological markers of contagion with syphilis agent, human immunodeficiency virus, viral hepatitis *B* and *C*;

– contents of alanine amine transferase, hemoglobin, hematocrit, erythrocytes sedimentation rate, total protein and protein

fractions, and leukocytes count in their blood were within reference ranges determined by the RF Public Healthcare Ministry Order issued on September 14, 2001 (last edited on June 06, 2008) No. 364 "On Approval of the procedure for medical examination obligatory for a donor of blood and its components";

– they had a medical reference from their polyclinic that during 6 months prior to donorship they hadn't had any diseases or contacts with contagious patients.

People were not included into the reference group if:

– they had a disease or a contact with contagious patients during 6 months prior to the trial;

– they had positive results of tests aimed at determining syphilis, HIV-infection, viral hepatitis *B* and *C*;

– their blood parameters determined through laboratory tests were beyond the reference ranges;

– they had at least one absolute contraindication to being a donor[2] including 2–3 degree hypertension, ischemic heart disease, atherosclerosis, atherosclerotic cardiosclerosis, obliterative endarteritis, non-specific aortoarteritis, recurrent trombophlebitis, endocarditis, myocarditis, or cardiac defects;

– they were younger than 18 or older than 65;

– they didn't give their written informed consent to take part in the trial;

– they had positive results of molecular-biological tests aimed at determining COVID-19 genetic material or CT-signs of COVID-19.

People were excluded from either the test or reference group in case they refused to cooperate after the trial had begun as well as in case a sample of a biological material was missing or at least one sample of a biological material taken from an examined person was not suitable for analysis.

Statistical analysis of data obtained through laboratory tests involved calculating frequency of detecting IgM and IgG to EBV antigens (%), average signal-to-cutoff ratios of IgM VCA and IgG EBNA for each examined group. A signal-to-cutoff ratio (S/Co) was determined by dividing optical density value of the sample being tested by the optical density values of the assay cutoff calculated according to the instruction provided with a relevant reagent kit. The result was considered positive at S/Co > 1. In case the test result was positive, S/Co value was used to estimate concentrations of specific antibodies in analyzed samples.

IgM VCA and / or IgG EA detection indicated there was active EBV-infection (either acute primary or reactivated chronic one).

Validity of differences was estimated with confidence intervals (CI). Margin of error for confidence interval was calculated as per the formula:

$$Z \cdot \frac{\delta}{\sqrt{n}},$$

where Z is confidence coefficient equal to 1.96 at confidence probability being equal to 95 % and confidence significance being $p < 0.05$; δ is standard deviation; n is size of a sampling.

Relative risk (*RR*) of heart rhythm disorders in case there was active EBV-infection and high IgG VCA concentration was calculated based on results obtained through building up a fourfold table as per the formula A:(A+B)/C:(C+D). The rate was considered positive in case its value was > 1. Differences were considered authentic if a confidence interval for this rate didn't include 1. The upper limit of a confidence interval for average S/Co of donors' IgG VCA was taken as the upper limit of a reference interval; when this limit was exceeded, this meant IgG VCA concentration was high (high S/Co).

[2] Contraindications are stipulated by the Order of the RF Public Healthcare Ministry issued on September 14, 2001 (last edited on June 06, 2008) No. 364 "On Approval of the procedure for medical examination obligatory for a donor of blood and its components".

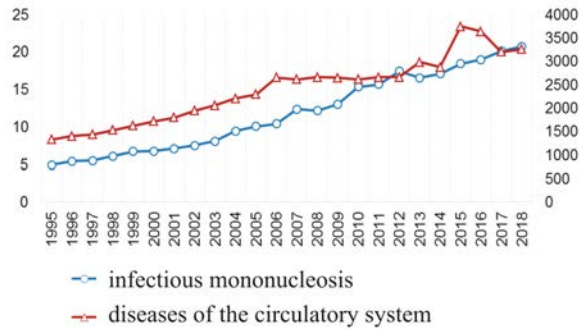

Figure. Incidence with infectious mononucleosis and prevalence of circulatory diseases in the Russian Federation in 1995–2018 (per 100 thousand people)

Results and discussion. Both incidence with infectious mononucleosis and prevalence of circulatory diseases tended to grow in the Russian Federation from 1995 to 2018 (Figure).

Correlation analysis of incidence with infectious mononucleosis in and CD prevalence in the Russian Federation in 1995–2018 revealed a strong direct correlation between them ($r = 0.94$; $m = 0.02$); this correlation was authentic according to authenticity test being $t = 47$; $p < 0.01$.

Determination coefficient r^2 amounted to 0.88 and this indicated that contribution made by EBV into CD prevalence reached 88 % and only 12 % were determined by other factors.

Blood serums taken from patients with heart rhythm disorders and conditionally healthy donors were analyzed comparatively to determine immunoglobulins G and M to Epstein – Barr virus antigens in them; authentic IgM VCA prevalence was detected in the test group (Table 1). We should note that frequency of detecting IgG EA and IgG VCA was also higher in the first (test) group but those differences were not authentic.

Overall, active EBV-infection was more frequently detected by serological tests among patients with heart rhythm disorders, 12.62 (95 %; CI 6.18 ÷ 19.06) against 2.17 (95 %; CI 0 ÷ 5.16) among donors (the reference group).

Relative risk (*RR*) of heart rhythm disorders in case a patient had active EBV-infection amounted to 5.8 (95 %; CI 1.3 ÷ 25.0) and difference between the groups was authentic (Table 2).

Table 1

Frequency of detecting immunoglobulins M and G to Epstein – Barr virus antigens in patients with heart rhythm disorders and donors, %

Ig		Patients with heart rhythm disorders ($n = 103$)	Donors ($n = 92$)
IgM VCA	abs.	5	0
	%	4.85	0
	95 %; CI	0.68 ÷ 9.02	0 ÷ 0
IgG VCA	abs.	103	90
	%	100	97.82
	95 %; CI	100 ÷ 100	94.83 ÷ 100.81
IgG EA	abs.	8	2
	%	7.77	2.17
	95 %; CI	2.58 ÷ 12.96	0 ÷ 5.16
IgG EBNA	abs.	98	88
	%	95.15	95.65
	95 %; CI	90.98 ÷ 99.32	91.46 ÷ 99.84

Table 2

Distribution of examined people depending on detection of active EBV-infection markers

Examined people	Active EBV-infection markers	
	yes	no
Patients with heart rhythm disorders ($n = 103$)	13	90
Donors ($n = 92$)	2	90

Analysis of average signal-to-cutoff ratios in patients with heart rhythm disorders revealed authentic prevalence of IgG VCA S/Co over IgG EBNA S/Co (Table 3). For reference, IgG VCA and IgG EBNA signal-to-cutoff ratios were comparable in donors with no authentic differences between them. IgG EBNA S/Co in patients with heart rhythm disorders didn't differ from the same ratio in donors whereas IgG VCA S/Co was authentically lower in donors than in patients with heart rhythm disorders.

Bearing in mind that donors participating in this research were considered to be conditionally healthy population and IgG VCA usually occur in blood serum during the first week since the acute primary infection started and persist there during the whole lifetime, we took a confidence interval of average IgG

VCA S/Co for donors as a reference value in this specific research. Having compared IgG VCA S/Co for blood serums of patients with heart rhythm disorders and selected reference values, we detected that these values were exceeded in 57 cases (55.34 % (95 %; CI 45.69 ÷ 64.99)) and this indicated that IgG VCA concentrations were high in blood serum of these patients. For reference, only 22 people among donors had elevated values of this ratio (higher than the reference level) (23.91 % (95 %; CI 15.15 ÷ 32.67)).

Table 3

Signal-to-cutoff ratios of analyzed IgG VCA and IgG EBNA samples in with heart rhythm disorders (n = 103) and donors (n = 92)

Ig	Group	Average signal-to-cutoff ratio	Confidence interval 95 %; CI
IgG VCA	Patients with heart rhythm disorders	78.1	69.3 ÷ 86.9
	Donors	40.65	34.6 ÷ 58.4
IgG EBNA	Patients with heart rhythm disorders	38.4	35.1 ÷ 41.7
	Donors	35.6	27.4 ÷ 43.8

Relative risk (*RR*) of heart rhythm disorders in case IgG VCA concentration was high amounted to 2.3 (95 %; CI 1.5 ÷ 3.5) and the differences between the groups were authentic (Table 4).

Table 4

Distribution of examined people depending on IgG VCA concentration being higher or lower than reference values

Examined people	Active EBV-infection markers	
	yes	no
Patients with heart rhythm disorders (n = 103)	57	46
Donors (n = 92)	22	70

An assumption on possible contribution made by EBV-infection into circulatory pathology in general and heart rhythm disorders in particular were based on results obtained in previous research works. Thus, myocarditis was established to be among the most widely spread clinical symptoms of infectious mononucleosis. The most typical features of myocarditis combined with EBV included damage to the heart conduction system (atrioventricular blocks, ventricular arrhythmia) and sinus tachycardia that were detected by ECG as a shift in ST segment and changes in T peak (flattening, two-phase, or inversion) as well as atrioventricular conduction disorders (atrioventricular blocks, bundle brunch blocks) [30–34].

At the same time, the overwhelming majority of previous research works that focused on EBV contribution to arrhythmia development was clinical in their essence and dealt with heart conduction disorders in children [35–38]. This population group was most likely chosen due to high rates of incidence with infectious mononucleosis among people younger than 18 [27, 29]. It was shown that children with heart rhythm disorders in their case history also more frequently had viral infections in it (infectious mononucleosis, chicken pox, rubella, or B19 parvovirus infection) than children without this pathology [38–41]. And there were cases when patients with heart rhythm disorders had genetic EBV materials detected in their serous pericardial effusion, endomyocardial tissue sampling, and venous blood [34, 36, 39, 41–43]; they also had IgM VCA in blood serum together with antibodies to antigens of working myocardium and conduction system [37, 39, 44, 45].

People older than 18 have infectious mononucleosis much less frequently than younger ones; however, they are not excluded from the epidemic process of EBV-infection which becomes a chronic latent disease with periodical reactivations in most cases after the first contact with the virus [22, 46]. And we should also remember that circulatory diseases including those that involve heart rhythm disorders are a widely spread pathology among adult population [7, 11, 13].

A distinctive feature of the present research is assessment of the correlation between incidence with infectious mononucleosis with EBV being its primary infectious agent and

CD prevalence in the overall population in the Russian Federation over a long period of time (23 years) with subsequent analysis of influence exerted by frequency of detecting active EBV-infection markers and IgG VCA concentration on heart rhythm disorders by the randomized controlled trial.

The research results allow making a well-grounded conclusion that active EBV-infection makes a substantial contribution to cardiovascular system pathology in general and that involving heart rhythm disorders in particular. It gives grounds for more profound studies on the issue by experts in various spheres.

Conclusions. The accomplished research allowed making the following conclusions:

1. EBV makes a significant contribution to occurrence of circulatory pathology and this was confirmed by the common ascending trend in incidence with infectious mononucleosis and CD prevalence in the Russian Federation in 1995–2018 and the strong authentic direct correlation between these two processes ($r = 0.94$; $m = 0.02$; $t = 47$; $p < 0.01$) with determination coefficient being equal to 0.88.

2. Active EBV-infection in case history combined with IgM VCA and IgG EA detection and elevated IgG VCA concentration in blood serum determines a risk of heart rhythm disorders ($RR = 5.8$ (95 %; CI 1.3 ÷ 25.0) and 2.3 (95 %; CI 1.5 ÷ 3.5) accordingly).

3. The detected phenomena require further profound examination and development of activities aimed at risk minimization.

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Research article

RISK FACTORS PROFILE FOR MYOCARDIAL INFARCTION: FOCUS AT A YOUNG AGE

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The paper focuses on examining peculiarities of risk factors causing cardiac infarction at a young age. Although cardiac infarction primarily occurs among patients older than 45, its frequency at a young age has been growing recently. Risk factors that cause cardiac infarction at a young and old age are quite different. Examining risk factors profiles in different age groups provides wider opportunities for implementing primary and secondary prevention strategies aimed at reducing frequency and negative outcomes of ischemic heart disease.

108 patients aged from 18 to 45 and 35 patients aged from 60 to 75 took part in the research; they all had confirmed cardiac infarction with or without rise in ST segment and were treated in a regional center for cardiovascular pathology treatment in a period from January 01, 2017 to January 01, 2019. Basic risk factors of cardiac infarction were assessed when a patient was admitted to a clinic for treatment.

The research results indicate high prevalence of risk factors that could cause ischemic heart disease among young patients. 92.2 % young patients have dyslipidemia, 70.2 % smoke, 68.5 % have low physical activity, 68.2 % suffer from overweight and obesity, 58.8 % have arterial hypertension, 7.4 % suffer from type II pancreatic diabetes, and disorders in tolerance to carbohydrates was reveled in 15.7 % cases. Such factors as male sex (85.2 vs. 37.1 %, p = 0,000), smoking (70.2 vs. 20.6 %, p = 0.000) and burdened heredity as per early ischemic heart disease occurrence (54.6 vs. 16.0 %, p = 0.001) were significantly more frequent among young patients than among older ones.

Data obtained via the present research allowed creating risk factors profile for cardiac infarction associated with cardiac infarction occurrence at a young age; this profile included such factors as male sex, early ischemic heart disease occurrence in family history, and smoking

Key words: *ischemic heart disease, cardiac infarction, young age, risk factors, smoking, male sex, burdened heredity, primary prevention.*

Introduction. Ischemic heart disease (IHD) still occupies a leading position in the structure of morbidity and mortality in the population all over the world [1]. Myocardial infarction (MI) is the most lethal form of IHD and can manifest, among other things, as sudden cardiac death. Despite the fact that MI mainly occurs in patients older than 45 years, in recent years its frequency at a young age has been increasing [2]. The consequences of MI at a young age can be devastating because of the potential impact on psychosocial status and work ability. The pathogenesis of cardiovascular diseases is among the most complex ones due to the interactions between numerous factors including genetics, lifestyle and environment which have a direct impact on a moment of time when the disease starts to develop, its manifestation, localization, severity of the lesion and outcome. The INTERHEART study showed that nine traditional risk factors such as dyslipidemia, smoking, arterial

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hypertension, diabetes mellitus, obesity, unhealthy diet, low physical activity, alcohol abuse and psychosocial stress explain the development of more than 90 % of all MI cases [3]. However there is evidence that the risk factors that cause MI in young and old age differ [4]. Studies focusing on investigating the characteristics of myocardial infarction in young patients are scattered and few. Studies on risk factor profiles in different age groups provide wider possibilities for implementing primary and secondary prevention strategies aimed at reducing the burden of IHD.

The aim of the study is to identify the characteristics of the risk factors profile for MI at a young age.

Materials and methods. The study included 143 patients with a confirmed diagnosis of MI with and without ST segment elevation who were admitted to the regional vascular center from January 01, 2017 to January 01, 2019. The patients were divided into two groups. The group of young people consisted of 108 patients aged from 18 to 45, the group of elderly people, 35 patients aged from 60 to 75. The exclusion criteria were: acute diseases, exacerbation of chronic diseases, severe concomitant somatic pathology, type 1 diabetes mellitus, mental illness, and dementia. All patients included in the study gave their voluntary informed consent to participate in it. The characteristics of the patients included in the study are presented in Table 1.

All patients underwent a complete clinical examination including collection of anamnesis data, physical examination, complete blood count, biochemical analysis, coagulation testing, and assessment of urinary albumin excretion. An original questionnaire was used to study the prevalence of traditional risk factors.

Statistical data analysis was carried out using IBM SPSS Statistics v.23 software. Quantitative indicators were assessed for compliance with the normal distribution using the Kolmogorov – Smirnov test as well as indicators of asymmetry and kurtosis. Quantitative indicators that had a normal distribution were represented by the arithmetic mean (*M*) and standard deviation (*SD*). Quantitative indicators whose distribution differed from normal were represented by the median (*Me*) and lower and upper quartiles (*Q1–Q3*); nominal data, by absolute values and percentages. Mean values in the case of a normal distribution were compared by calculating the Student's t-test, in the absence of a normal distribution, the Mann – Whitney U-test. The nominal data were compared using the Pearson χ^2-test. Differences in indicators were considered statistically significant at the significance level being $p < 0.05$.

Results and discussion. Men prevailed among young patients with MI compared with the elderly ones. Young patients smoked 3.5 times more often, they had a family history of early-onset IHD 3.4 times more often than elderly patients. In Arterial hypertension was 1.5 times more common and type 2 diabetes mellitus 4 times more common among elderly people. In both groups the majority of patients led a sedentary lifestyle, were overweight and obese, almost all had dyslipidemia (Table 2).

According to the biochemical analysis of blood elderly patients had a lower glomerular filtration rate. The groups of young and elderly patients did not differ in terms of blood lipid spectrum indicators. In both groups there was an increase in the level of total cholesterol and low-density lipoproteins (LDLP) (Table 3).

Table 1

Characteristics of young and elderly patients with myocardial infarction

Parameter	Young patients ($n = 108$)	Elderly patients ($n = 35$)	p
ST-elevation myocardial infarction, *n* (%)	91/108 (84.3)	16/35 (48.5)	0.000
Non-ST-elevation myocardial infarction, *n* (%)	17/108 (15.7)	17/35 (51.5)	0.000
Age, years *Me* (*Q1–Q3*)	41.0 (38.0–43.0)	67.5 (64.0–71.3)	0.000

Table 2

Traditional risk factors for myocardial infarction in young and elderly patients

Parameter	Young patients (n = 108)	Elderly patients (n = 35)	p
Male, *n* (%)	92/108 (85.2)	13/35 (37.1)	0.000
Female, *n* (%)	16/108 (14.8)	22/35 (62.9)	0.000
Arterial hypertension, *n* (%)	57/97 (58.8)	30/34 (88.2)	0.002
Dyslipidemia, *n* (%)	83/90 (92.2)	31/31 (100.0)	0.110
Normal weight, *n* (%)	34/107 (31.8)	8/28 (28.6)	0.106
Overweight, *n* (%)	47/107 (43.9)	7/28 (25.0)	0.069
Obesity (Class I), *n* (%)	17/107 (15.9)	10/28 (35.7)	0.020
Obesity (Class II), *n* (%)	8/107 (7.5)	3/28 (10.7)	0.578
Obesity (Class III), *n* (%)	1/107 (0.9)	0/28 (0.0)	0.608
Premature coronary heart disease in family history, *n* (%)	53/97 (54.6)	4/25 (16.0)	0.001
Sedentary lifestyle, *n* (%)	74/108 (68.5)	22/33 (66.7)	0.842
Smoking, *n* (%)	73/104 (70.2)	7/34 (20.6)	0.000
Diabetes mellitus type 2, *n* (%)	8/108 (7.4)	10/34 (29.4)	0.000
Prediabetes, *n* (%)	17/108 (15.7)	1/34 (2.9)	0.051

Table 3

Biochemical parameters in young and elderly patients with myocardial infarction

Parameter	Young patients (n = 108)	Elderly patients (n = 35)	p
Glucose, mmol/L *Me (Q1–Q3)*	5.6 (5.2–6.2)	5.8 (5.2–7.1)	0.288
Creatinine, μmol/L *Me (Q1–Q3)*	81.0 (71.0–91.0)	81.5 (69.3–98.8)	0.445
Glomerular filtration rate, mL/min/1.73m² *Me (Q1-Q3)*	108.0 (96.0–116.0)	80.5 (66.0–89.8)	0.000
Total cholesterol, mmol/L ($M \pm SD$)	5.0 ± 1.2	5.3 ± 1.3	0.207
Low-density lipoproteins, mmol/L ($M \pm SD$)	3.1 ± 1.1	3.4 ± 1.0	0.179
High-density lipoproteins, mmol/L *Me (Q1–Q3*)	1.1 (0.8–1.3)	1.2 (0.9–1.4)	0.396
Triglycerides, mmol/L *Me (Q1–Q3)*	1.3 (0.9–2.1)	1.2 (0.8–1.6)	0.352

Table 4

Hemostatic system indicators in young and elderly patients with myocardial infarction

Parameter	Young patients (n = 108)	Elderly patients (n = 34)	p
Fibrinogen, g/L *Me (Q1–Q3)*	3.0 (2.5–3.6)	3.3 (2.7–4.0)	0.146
Adenosine diphosphate-induced platelet aggregation, sec *Me (Q1–Q3)*	8.0 (6.0–10.0)	7.0 (6.0–7.0)	0.355

High urinary albumin excretion was observed in 74.1 % of cases among young patients; among elderly ones, in 58.3 %; this difference was statistically insignificant (p = 0.164).

Examination of the haemostatic system revealed an increase of adenosine diphosphate-induced platelet aggregation in both young and elderly patients (Table 4).

Thus, the results of the study demonstrate high prevalence of risk factors for IHD at a young age: 92.2 % of young patients have dyslipidemia; 70.2 % smoke; 68.5 % lead

a sedentary lifestyle; 68.2 % are overweight and obese; they have arterial hypertension in 58.8 % of cases; type 2 diabetes mellitus, in 7.4 %; prediabetes, in 15.7 %. Also in 74.1 % of cases there is high excretion of albumin in urine, which is a marker of endothelial dysfunction and early vascular aging. An increase in adenosine diphosphate-induced platelet aggregation was noted upon admission of patients to the clinic which indicates a thrombophilic status in vascular-thrombocyte homeostasis.

Male gender (85.2 % vs. 37.1 %, $p = 0.000$), smoking (70.2 % vs. 20.6 %, $p = 0.000$) and family history of premature IHD (54.6 % vs. 16.0 %, $p = 0.001$) were more common among all risk factors in the cohort of young patients compared with the elderly.

Our data are consistent with the results by W.P. Zhang et al. They also rank smoking and a family history of premature IHD among the most significant risk factors for MI in young patients. The authors noted significantly lower prevalence of arterial hypertension and type 2 diabetes mellitus in the cohort of young patients compared with the elderly which was also consistent with our data. At the same time in this study young patients had higher levels of LDLP and triglycerides and lower levels of high density lipoprotein (HDLP) [5]. According to our data there were no statistically significant differences in lipid spectrum indicators between the groups of young and elderly patients. B.D. Hoit et al. also noted smoking, family history of premature IHD and male gender as the main risk factors for MI at a young age [6]. Some authors, in addition to the aforementioned risk factors, distinguished dyslipidemia, arterial hypertension, and type 2 diabetes mellitus, and this was different from our results [7–9].

MI at a young age is much more common in men than in women. According to numerous studies a share of males varies from 71.5 to 100 % in this cohort [10–14]. IHD in men manifests 7–10 years earlier than in women [15]. Women are less susceptible to developing MI at a young age due to the direct protective effect of estrogens on the coronary arteries [16, 17].

A family history of premature IHD is an important independent risk factor for MI [18]. Young patients in comparison with the elderly are twice as likely to have first-line relatives with early manifestation of IHD [6, 19]. A. Oliveira et al. established that the relative risk of developing MI in young patients with a family history of premature IHD was 1.84 (95 % CI 1.07–3.17) against their healthy peers [20]. Such patients were found out to have a more severe course of IHD and more pronounced violation of lipid metabolism, they also suffered from insulin resistance and obesity more frequently [21, 22]. The high prevalence of this risk factor among young patients included in the study makes it possible to discuss probable genetic predisposition to the early development of IHD in this cohort.

Smoking is the only one potentially fully modifiable risk factor for IHD. Smoking plays an important role not only in the occurrence of cardiovascular diseases but also significantly contributes to their progression and poor prognosis. Smoking accelerates the development of atherosclerosis, causing damage to the vascular endothelium, reducing tissue oxygenation and increasing the activity of the sympathetic nervous system. In addition smoking promotes an increase in platelet aggregation activity and a decrease in HDLP levels [23, 24]. Smoking increases the risk of developing MI at a young age by 3.33 times whereas there is only 2.44 time increase caused by it in the elderly people [3]. It was noted that young patients smoked more cigarettes per day [25]. In a study made by A. Oliveira et al. the relative risk of MI was 4.56 (95 % CI 2.32–9.0) among patients younger than 45 who smoked more than 15 cigarettes per day against young people who quit smoking [20]. These data confirm the persistent negative effects produced by smoking. Giving up smoking remains one of the most effective measures to prevent cardiovascular disease [24].

Conclusion. The data obtained in this study made it possible to form the risk factors profile of MI taking into account age characteristics. Risk factors associated with the development of MI at a young age include male gender, family history of premature IHD, and smoking. At the same time dyslipidemia, arterial hypertension, physical inactivity and obesity are also widespread among young patients and this is accompanied with developing endothelial dysfunction, early vascular aging and the formation of thrombophilic status in the hemostatic system.

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Review

ASSESSING RISKS CAUSED BY NICKEL-CONTAINING NANOMATERIALS: HAZARD CHARACTERIZATION *IN VIVO*

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Nanoparticles (NP) of nickel (Ni) and its compounds are promising materials for being used as catalysts in chemical, pharmaceutical and food industry; as construction materials in electronics and optoelectronics, in manufacturing current sources, medications, diagnostic preparations, and pesticides. Annual production volumes for these materials in their nanoform are equal to dozen tons and are expected to growth further. According to data obtained via multiple research nanoforms of Ni and its compounds are toxic to many types of cells; stimulate apoptosis; and can induce malignant transformation in vitro. It indicates that this group of nanomaterials can possibly be hazardous for human health. Risk assessment includes such a necessary stage as quantitative hazard characterization, that is, establishing toxic and maximum no-observed-adverse-effect levels (NOAEL) for a nanomaterial that penetrates into a body via inhalation, through undamaged skin, or the gastrointestinal tract. Experiments in vivo performed on laboratory animals with Ni-containing materials revealed overall toxic effects; toxicity to specific organs (including hepatoxoticity and cardiotoxicity); atherogenic, allergenic, and immune-toxic effects, as well as reproductive toxicity. There are multiple available data indicating that all Ni-containing nanomaterials are genotoxic and mutagenic, though data on their carcinogenic potential are rather scarce. Factors that determine toxicity of Ni and its compounds in nanoform are their ability to penetrate through biological barriers and to release free Ni++ ions in biological media.

The review focuses on analyzing and generalizing data on toxicity signs in vivo and effective toxic doses under various introductions of Ni and its compounds in nanoform into a body over a period starting predominantly from 2011.

Key words: *nickel, nickel oxide, nanoparticles, genotoxicity, allergenic capacity, reproductive toxicity, carcinogenicity, occupational exposure, risk assessment.*

Nanoparticles (NPs) of nickel and its compounds are used as catalysts in chemical, pharmaceutical, and food industry; construction materials production; electronics and optoelectronics; production of current sources, medications, diagnostic preparations, and pesticides. These substances in their nanoforms are produced in dozen tons and production volumes are only expected to grow in future [1].

Obviously, NPs of nickel and its compounds are among nanotechnological products and exposure to them is likely to grow in the nearest future for production workers, consumers of various products and population in general; this unavoidably calls for assessing risks related to this growing exposure [2]. Multiple experimental and epidemiological studies have revealed that metallic nickel and its compounds with common dispersity are carcinogens [3]. Based on this data, Internaitonal Agency for Research on Cancer (IARC) ranked Ni (II) compounds as belonging to

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Group I (carcinogenic for people) whereas metallic Ni was classified as belonging to Group 2B (probably carcinogenic for people). Ni compounds are also active allergens [4, 5].

We generalized data available in scientific literature in works published predominantly from 2011 to 2020 in our previous review and showed that NPs of metallic nickel and its compounds (NiO, $Ni(OH)_2$) as well as nickel nanofibers and nanorods were highly toxic for various cells including cells of bronchopulmonary epithelium, liver, kidneys, intestine, central nervous system and reproductive organs. Cytotoxicity mechanisms include oxidation stress development, functional disorders in cell membranes and mitochondria, expression of nuclear transcription factors, caspases, and proto-oncogenes. Overall, it indicates that Ni-containing nanomaterials are potentially greatly hazardous when they penetrate into the body. However, data of model studies *in vitro* performed on cellular cultures do not allow qualitative assessment of hazards since they don't take into account peculiarities related to how a nanomaterial penetrates through biological barriers as well as its bioaccumulation and biotransformation capabilities.

The aim of this review is to analyze and generalize data on effective toxic doses of Ni-containing nanomaterials under different introduction into laboratory animals' bodies and observed toxic effects as well as possible long-term adverse effects. Attention was primarily paid to data taken from works published in the last decade (starting from 2011); all these works conformed to conventional requirements regarding scientific authenticity and completeness and were found in international reference databases PubMed, WoS and Scopus.

Studies in vivo. Ni NPs are introduced into the body through airways, gastrointestinal tract, and, presumably, skin. When Ni-containing materials are used in medicine (in theranostics) they can possibly penetrate into the body through parenteral (intravenous) introduction. Besides, Ni nanoforms can also be released from implants. Therefore, it seems advisable to consider toxic effects produced by Ni-containing nanomaterials under all these ways of introduction.

Parenteral introduction. A single intravenous introduction of Ni NPs in doses equal to 1, 10 and 20 mg/kg into Sprague Dawley rats caused acute inflammatory damage to the lungs, liver, and spleen as well as acute cardiotoxicity. Histopathologic examination revealed changes in the liver with a growth in its mass depending on a dose as well as changes in the lungs, spleen and some other organs [6].

Marzban A. with colleagues [7] described an experiment involving male rats getting daily intraperitoneal injections of NiO NPs suspension or micronized NiO powder in doses equal to 0, 10, 25 and 50 mg/kg during 8 days. As a result, there was a decrease in recovered glutathione reserves and elevated malonic dialdehyde contents after either Ni-containing form was introduced but NPs introduction additionally led to increased activity of glutathione-S-transferase and catalase. Histopathologic examination revealed necrosis, hyperemia, and sponge-like changes in the brain tissue for both introduced preparations.

Katsnelson B.A. with colleagues [2, 8] described intraperitoneal introduction of NiO NPs suspension (particles diameter was 17 nm), Mn_3O_4 (18 nm) or their mixture into rats 3 times a week during 6 weeks. Introduction of both NPs resulted in functional and histological disorders in the brain, liver, spleen, and kidneys. Mathematical analysis of data based on Response Surface methodology revealed a variety of combined toxic effects that depended on nature, size, and concentrations of particles.

Male and female mice underwent intraperitoneal injections of NiO NPs in doses equal to 20 or 50 mg/kg of body weight during 14 days and this resulted in male mice having elevated urea contents in blood plasma, an increase in superoxide dismutase (SOD) activity and elevated malonic dialdehyde contents in the liver whereas catalase was less active in the heart and kidneys. Female mice had prevailing signs of decreased total protein and albumin in plasma, lower SOD activity in the lungs and elevated malonic dialdehyde (MDA) contents in the liver [9].

Experiments performed on male Sprague Dawley rats involved intraperitoneal injections of Ni NPs in a dose equal to 10 mg/kg of body weight during 90 days; this led to significant deviations in biochemical and hematological indicators, oxidation stress development, morphological disorders of the kidneys and liver. An extract from Cinnamomum cassia bark (Chinese cassia) in a dose equal to 175–225 mg/kg of body weight that was introduced orally inhibited toxic effects produced by NPs [10].

Introduction into airways. Intratracheal and oropharyngeal instillations of nanomaterials are frequently used in toxicological studies as a model of respiratory exposure. Compared with inhalation introduction, they allow introducing more exact doses of a nanomaterial into each animal although there are certain peculiarities related to distribution of particles with different sizes and chemical structure in the airways given this alternative way of exposure. Both acute and various subacute exposure can be modeled in such experiments.

There was an early study [11] during which NiO NOs sized 8 nm and aggregated into clusters sized 26 nm were introduced into male Wistar rats via single intratracheal administration in a dose equal to 0.33–0.66 mg/kg. Starting from the 3rd day in the experiment, bronchoalveolar lavage (BAL) contained elevated quantities of CINC-1, -2αβ and -3 cytokine-induced neutrophils chemoattractants. The changes persisted up to 6 months after the introduction. This research work seemed to be the first confirmation that Ni NPs were able to induce persistent inflammatory response in the lung even after a single introduction. There was a study similar in its design where elevated contents of CINC-2αβ and CINC-3 in BAL were detected 3 days, 1 week, 3 months, and 6 months after a single intratracheal introduction of nanostructured aggregated NiO with primary NPs sized 8.4 in a dose equal to 3.3 mg/kg into male Wistar rats [12]. In the following experiment, rats got a single intratracheal injection of NiO NOs sized 26 nm in a dose equal to 0.2 mg; after that, researchers observed alveolar macrophages infiltration, long-term increase in MIP-1a contents and transient interleukin (IL) – IL-1α, IL-1β and MCP-1 in lung tissue lysates and BAL within a period of time from 3 days to 6 months after the injection [13]. Therefore, chemokines play an important role in developing lung inflammation caused by Ni-containing NPs.

Spherical NiO NPs and those with irregular shape as well as NiO nanofibers were one-time introduced intratracheally into male F 344 rats in doses equal to 0.67–6.0 mg/kg of body weight; there was translocation into pectoral lymph nodes where the particles were partially dissolved and biologically degraded under exposure to oxidants produced by immune cells. Dissolution, oxidation, and clearance of Ni nanofibers were much faster than for two aforementioned types of NPs [14].

The research work [15] also focused on a relation between clearance of various Ni-containing nanomaterials from the lungs and their dissolubility. NiO NPs were shown to have greater lung toxicity than microparticles (MPs) since they dissolved faster releasing Ni++ ions; the process was reproduced *in vitro* with using artificial alveolar liquid. A peak in responses by inflammation markers in rats' lungs was detected *in vivo* within a period form 1 week to 1 month and to a much lesser extent during the first 3 days; this was in line with a typical period of time required for NPs dissolution *in vitro* that was equal approximately to 1 week. Data available in the work [16] also highlight a role played by dissolubility of Ni-containing nanomaterials and adsorption of surfactant components on their surface in their apparent lung toxicity. In this research [16] effects produced by NiO NPs on mice under pharyngeal aspiration differed depending on a structure of dispersion medium including phosphate-salt buffer, and solution of albumin, non-ion detergent and phospholipids that imitated a structure of surfactant.

NiO NPs were introduced into rats as a suspension through a single intratracheal introduction and induced persistent lung inflammation with inflammatory cell proliferation, alveolar proteinosis and cytokine production 3–28 days after the introduction. *Nlrp3* expres-

sion was elevated together with hyperexpression of caspase 1 (p20) and IL-1β production of which was suppressed due to effects produced by caspase inhibitor on macrophages *ex vivo*. NiO NPs are assumed to induce activation of *Nlrp3*-inflammasome which requires their absorption by cells and production of reactive oxygen species (ROS) [17].

14 days after Ni NPs sized 41 nm and corresponding MPs (350 nm) were introduced in dose equal to 4–20 mg/kg into Sprague Dawley rats via a single intratracheal introduction, inflammatory damage was detected in the lungs, liver, and kidneys, there were hyperplasic changes in the lungs and greater expression of homoxygenase-1 (HO-1) and Nrf2 there without any changes in expression of C-myc oncogene. There were signs confirming that NPs were more cytotoxic than MPs [18].

In the study [19] NiO NPs were one-time introduced intratracheally into mice and then inflammatory reactions were examined in the lungs within 1–28 days after the introduction. There was greater activity of lactate dehydrogenase (LDG), elevated total protein and IL-6 contents and decreased IL-10 contents in BAL. By the 24th hour in the experiment there was a growth in 8-oxo-2-deoxyguanosine (8-oxo-G) and caspase-3 contents in lung tissue and enhancing dose-dependent inflammation in the lower airways. By the 28th day lung tissue fibrosis occurred. Proteome analysis revealed that after 24 hours disorders in the metabolic ways of cell adhesion prevailed in inflammation mechanism whereas on the 28th day prevalence shifted to processes related to tissue glutathione depletion.

Authors of the research [20] introduced NiO NPs into rats through a single intratracheal introduction in doses equal to 0.2 and 1 mg/kg or through inhalation. NiO NPs persisted in the lungs longer than TiO_2, NPs in all tests and biological persistence correlated with histopathologic changes and levels of inflammatory biomarkers in BAL.

Three types of Ni NPs (with non-modified surface, oxide-passivated, and protected with monomolecular carbon layers) were introduced through single intratracheal instillation into common mice and mice with knocked-out micro-RNA (miR)-21 gene; the experiments revealed that expression of *Reck* gene (tumor dissemination inhibitor) that was a direct target for effects produced by miR-21 was inhibited only in wild mice under exposure to the first two types of NPs. These data highlight an important role played by miR-21 in inflammatory response induction *in vivo* and probably carcinogenicity of nickel NPs [21, 22]. There was a comparative study performed on mice who were exposed to 3 types of Ni-containing NPs through a single intratracheal instillation in doses varying from 10 to 100 μg; the exposure resulted in dose-dependent development of acute lung inflammation with elevated contents of neutrophils, CXCL1/KC, total proteins and greater LDG activity in BAL. Time dynamics of the inflammation was as follows: occurrence on the 1st day after introduction, a peak on the 3rd day and a decline after 7 days, although even after 42 days inflammation signs were still more apparent than in the reference group. Oxide-passivated NPs were characterized practically with the same lung toxicity as pure Ni NPs but when NPs were covered with carbon layer, it led to a significant decrease in their toxicity [23].

Macrophage degeneration and necrosis as well as inflammation and proliferation of type II pneumocytes were detected in lungs of Sprague Dawley rats after a single intratracheal instillation of NPs in doses equal to 0, 0.2, 0.67, and 2 mg/kg of body weight [24]. Inter-laboratory studies in 5 laboratories confirmed that the developed procedure was quite reproducible.

A comparison was made between a single and divided (two or four intakes with daily intervals) intratracheal introduction of NiO NPs into rats in a total dose equal to 2 mg/kg of body weight; the experiment revealed that resulting development of lung inflammation with such signs as NPs phagocytosis by alveolar macrophages, their degeneration and necrosis, a response by inflammatory markers in BAL after 3 and days after the last intake were practically the same after either a single or divided introduction of NPs [25].

Sub-acute experimental exposure was modeled in the research [26] where NiO NPs or MPs were introduced intratracheally into male Wistar rats in doses equal to 0.015–0.25 mg/kg twice a week during 6 weeks. Exposed animals had elevated contents of cells in apoptosis in their livers as well as higher expression of IRE-1α, X box protein-1S, pancreatic ER kinase (PERK), eukaryotic initiating factor-2 alpha (eIF-2α), their phosphorylated forms, caspase-3, 9 and 12, glucose-regulated 78 kDa protein and CCAAT-enhancer binding protein; all this indicated there was stress developing in endoplasmatic reticulum.

Histopathologic research by the same authors revealed lung fibrosis and elevated contents of hydroxyproline, collagen-1 and 3 in lung tissues after NiO NPs were introduced into rats in the aforementioned doses. All this was combined with elevated expression of fibrosis factors TGF-1β (transforming growth factor (TGF)), Smad2, Smad4, matrix metalloproteinase (MMP) and their tissue inhibitor of metalloproteinase (TIMP) [27]. Morphological studies revealed dose-dependent widening of alveoli, inflammatory infiltration, and NPs deposition in lung tissues. Exposure to the highest dose resulted in signs of nitrative stress including elevated NO contents and more active total (tNOS) and induced NO-synthase (iNOS); 8-oxo-G was produced in greater volumes, and there were elevated contents of IL-2, TGF-β and IFN-γ starting from a NPs dose equal to 0.06 mg/kg. These effects were authentically more apparent when NPs were introduced than after introduction of MPs in a quantity equivalent as per mass [28]. The central role in immunologic reaction developing in lung tissues as a response to NiO NPs introduction belonged to NF-κB activation and an increase in a relative share of Th2 lymphocytes against Th1 [29].

According to data available in the research by Yu S. [30], sub-acute intratracheal introduction of NiO NPs into male Wistar rats led to changes in the liver including growing mass of the organ, cellular edema, biliary ducts closing, and multinuclear cells occurrence. NO synthase became more active and NO contents in the liver grew under exposure to a dose of NiO equal to 0.25 mg/kg. Besides, there were growing concentrations of hydroxyl radical, lipid peroxides, catalase, glutathione peroxidase, and SOD. Therefore, intratracheal introduction of NiO NPs results in systemic effects including damage to the liver.

Ni NPs toxicity for male and female mice was compared under two oropharyngeal instillation regimes: as a single dose (an acute experiment) or 6 intakes during 3 weeks. Acute exposure resulted in elevated contents of CXCL1 and IL-6 and higher neutrophils quantity in BAL in male mice; there was also more intense STAT3 phosphorylation in lung tissues. Sub-acute exposure to Ni NPs caused greater monocytes quantities in lavage among male mice and there was also CXCL1 and CCL2 induction but STAT1 phosphorylation under such exposure was detected only in female mice. Male mice also tended to have greater IL-6 expression in the liver under acute exposure and greater CCL2 expression under sub-acute exposure than female mice. Differences in the lung responses to Ni NPs introduction were to a greater extent determined by animals' sex than introduction regime [31].

Zhang Q. and colleagues [32] performed experiments that involved introducing NiO NPs into male Wistar rats in doses varying from 0.015 to 0.25 mg/kg intratracheally two times a week during 9 weeks. Elevated accumulation of type I and III collagens was detected in the liver combined with elevated expression of TGF-β1, phosphorylated Smad2, Smad3, α-actin, MMP9, TIMP1, and a decrease in E-cadherin and Smad7 which indicated fibrosis was developing. The results were similar to those obtained by the same authors in experiments *in vitro* on liver cells culture and this indicates there is probably systemic NPs translocation after their introduction into the airways.

Inhalation exposure. Inhalation exposure to nanomaterials is modeled by placing an animal into an aerosol chamber or by putting on a specially designed helmet on its head. Such experiments are more suitable for reproducing occupational exposure than direct introduction into the airways and they are also

less traumatic. But still, there are certain problems related to them caused by a necessity to take respirable dose into account (that is, a dose that produces effects on the lower airways) as opposed to "inhaled one", or a dose of a nanomaterial that occurs direct in inhaled air. Effects that occur due to these two exposure types were described by Mizuguchi Y. and colleagues [33] who performed experiments involving NiO NPs introduction into male Wistar rats either through intratracheal instillation or sub-acute 4-week inhalation. Examinations were performed with a wide range of NPs doses. It was shown that both procedures could yield comparable and reproducible results as per polymorphonuclear leukocytes contents in BAL if a dose of a nanomaterial was measured as per surface area and not as per a mass or mass concentration.

As per data provided by Horie M. and colleagues [34] a response by oxidation stress markers in the lungs including HO-1, 8-isoprostaglandin-F2α, thioreodoxin, myeloperoxidase and iNOS developed more intensely at the first stage in inflammatory response under intratracheal introduction of NiO NPs as opposed to inhalation; however, later difference between these two introduction ways disappeared.

There were also experiments that involved comparing effects produced on rats by 4-week exposure to NiO NPs, Multi-Walled Carbon Nanotubes (MWCNTs) or fullerene in doses equal to 0.13–0.37 mg/m^3; it was revealed that NiO NPs caused the gravest and the most adverse changes in animals' lungs regarding contents of phospholipids and SP-D (surfactant-specific protein D). MWCNTs also produced certain effects, though less apparent; there were no any toxicity signs detected under exposure to fullerene [35]. Also, micron-sized NiO particles as well as TiO_2 particles turned out to have low lung toxicity for rats according to expression of MMP-1, TIMP, and type 1 collagen. These data indicate that inhalation toxicity of Ni-containing particles obviously grows when they are in nanoform.

The longest inhalation exposure (10 months) of rats to NiO NPs was accomplished by Sutunkova M.P. and others [36]. Nanomaterial concentration in aerosol amounted to 0.23 ± ± 0.01 mg/m^3. BAL examination revealed changes in cytological and some biochemical properties with paradoxically feebly apparent histopathologic picture of lung tissues and comparatively feebly apparent NPs accumulation in the lungs. But at the same time there were signs of systemic toxicity including damage to the liver and kidneys, allergic effects, transient stimulation of erythropoiesis and NiO NPs penetration into the brain through the olfactory pathway. Genotoxicity became obvious due to DNA fragmentation in nuclear cells in blood (RAPD test) with a tendency to grow as exposure became longer. It was also established that a majority of these adverse effects could be inhibited with some bioprotectors introduced orally into animals including vitamins C and E, fish oil, glycine, monosodium glutamate, etc. There are reviews focusing on issues related to using nutrients (antioxidants, polyunsaturated fatty acids, or amino acids) as biological protectors to eliminate toxic effects produced by nanomaterials including Ni-containing NPs [2, 37].

Cardiotoxic and atherogenic effects produced by Ni-containing NPs under inhalation exposure are especially interesting. In particular, it was shown that when C57BL/6 mice underwent 5-hour inhalation exposure to NPs of $Ni(OH)_2$ with their diameter being up to 40 nm in a dose equal to 100–900 μg/m^3, vasoconstriction went down in the exposed animals' carotid due to effects by phenylephrine and vasorelaxation decreased due to effects by acetylcholine. Thus, even relatively short-term inhalation exposure to Ni-containing NPs results in changes in endothelium of great vessels that are located far from a place where a nanomaterial penetrates into the body [38]. Later it was shown that inhalation exposure of mice to NPs of metallic Ni under similar conditions led to a growth in quantity of endothelial progenitor cells (EPCs) in bone marrow and blood flow and it indicated there was damage to vascular endothelium. Tubes formation and chemotaxis *ex vivo* of EPCs taken from mice exposed to Ni NPs were disturbed substantially. There was also a decrease in a

number of receptors for mRNA on EPCs that were responsible for mobilization and tissue fixation due to exposure to NPs [39]. Kang G.S. with colleagues [40] noted in their research work that inhalation exposure to NPs of $Ni(OH)_2$ in a dose equal 79 μg Ni/m^3 5 days a week during a period of time starting from 1 week and up to 5 months resulted in graver vascular atherosclerosis in sensitive mice with knocked-out gene of lipoprotein E (ApoE-/-).

Oral introduction. There are relatively few research works that focus on examining effects produced by orally introduced nickel-containing nanomaterials since such an exposure scenario is not considered as a priority one in most studies. Dumala N. and others accomplished an experiment [41] where acute oral toxicity of Ni NPs exceeded 2,000 mg/kg of body weight and it meant the substance was classified as belonging to the 5th hazard category according to Organization on Economic Cooperation and Development Guidelines Test No. 420. There were no lethal cases detected during 14 days after acute oral introduction of Ni NPs sized approximately 16 nm into female Wistar rats in doses varying from 5 to 2,000 mg/kg of body weight. Rats which were exposed to Ni NPs in a dose equal to 2,000 mg/kg became languid and irritated, they consumed food in slightly smaller quantities, and their body weight and relative masses of organs decreased insignificantly. According to Comet Assay data, exposure to the highest dose led to DNA damage in the liver and kidneys after 24 hours. Similar results were yielded in a micronucleus test. Use of this model to examine NiO NPs toxicity revealed an authentic decrease in a number of erythrocytes and AchE inhibition in rats' brains under exposure to high doses of NPs. Transaminases became more active in the liver and blood serum and less active in the kidneys. Exposure to NPs in high doses also involved disorders of enzyme balance in antioxidant protection [42].

Sub-acute oral toxicity and biological distribution of NiO NPs sized 13 nm was examined in a 28-day experiment performed on Wistar rats [43]. There were histopathologic changes in some organs, growing transaminases activity in the liver and kidney homogenates, less active SOD and more active catalase. Reserves of recovered glutathione depleted and there was a decrease in malonic dialdehyde contents indicating there was oxidative stress developing. The liver was the primary place where Ni accumulated; the kidneys followed. Ni was excreted mostly with feces and to a very small extent with urine.

Sub-acute oral toxicity of Ni NPs was examined in experiments performed on male and female rats exposed to doses varying from 5 to 45 mg/kg of body weight during 10 weeks (research design conformed to Organization on Economic Cooperation and Development Guidelines No. 415). The experiments revealed ultra-structural changes in the ovaries and testicles, oxidation stress development and expression of proteins that were associated with apoptosis [44, 45]. It was mentioned in the same works that exogenous ascorbic acid introduced with food protected animals from adverse effects produced by Ni NPs.

NiO NPs sized approximately 50 nm were introduced intragastrically into male Wistar rats during 7 or 14 days in doses from 1 to 4 mg/kg of the body weight. This resulted in a significant growth in a number of chromosome aberrations, micronuclei, and damage to DNA. Flow cytometry procedure revealed apoptosis, reactive oxygen species (ROS) generation and dysfunction of mitochondria membrane potential. There was antioxidant enzymes imbalance and histological changes in the liver. Immunoblotting revealed interaction between apoptosis stimulating factor (p53) and mitogen-activated protein kinase (MAPK)-signal pathway with activation of MAPAPK-2 (phosphorylation substrate for p38 MAP kinase), caspases 3 and 8, cytochrome C being released from mitochondria, expression of Bcl-associated X-protein (Bax) and inhibition of intracellular apoptosis regulator (Bcl-2) [46].

Antagonism between toxic effects as per some integral and biochemical parameters was revealed in rats after single combined oral exposure to 0.5 or 1 g of NiO NPs and Co_3O_4 NPs. Each nanomaterial was more toxic for rats when introduced separately than in combination with another [47].

Long-term effects produced by toxicity of Ni-containing nanomaterials. ***Carcinogenicity of Ni-containing NPs in vivo.*** Mechanisms of carcinogenesis induced by metals have not been studied enough. As it can been seen from all aforementioned data NPs of Ni and its compounds induce oxidative stress and inflammatory reactions and inhibit apoptosis factors, that is, they act as non-genetic pathologic factors that can be considered carcinogenic. Besides, experiments accomplished in model systems *in vitro* revealed that Ni and NiO NPs were highly genotoxic and mutagenic as well as able to induce malignant transformations of certain cell lines. Given that, it seems vital to examine carcinogenicity of various Ni-containing materials *in vivo*. However, there are hardly any research works on the subject available at the moment. There was a single study accomplished by Hansen T. and others [48] where authors observed development of rhabdomyosarcoma in rats with implants that contained Ni NPs in their spinal columns. Epigenetic mechanisms can play a significant role in carcinogenesis that is possibly induced by Ni compounds [3, 49]. It is assumed that absorption of Ni NPs by cells is a crucial component that determines their carcinogenicity [50]. Still it is widely known that absorption is greatly influenced by surface charge of a particle and it should be taken into account when planning experiments *in vivo*.

Therefore, additional studies are required to establish carcinogenicity of Ni NPs and NPs of other Ni-containing nanomaterials when they are into the body through natural ways

Immunotoxicity and allergenic capacity. Allergenic properties of Ni NPs are to a great extent determined by their immunotoxicity and they can become apparent both in a direct contact (when applied on skin) [51] and in indirect ones through the airways and gastrointestinal tract [4]. Single intratracheal introduction of NiO NPs into female Wistar rats in doses varying from 50 to 200 cm^2 recalculated as per particle surface resulted in growing inflammation markers in humoral and cellular fractions of BAL. Quantity of eosinophils didn't correlate with total IgE and anaphylatoxines but alveolar macrophage lysis and extracellular LDG activity positively correlated with eotaxin release. A conclusion was that NPs accumulation in phagolysosomes of immune cells induced their lysis that was accompanied with eotaxin production and eosinophilia. Allergenic capacities of NiO NPs were comparable to those of Ni salt as well as ovalbumin [52].

According to data provided by Glista-Baker E.E. with colleagues [53], transcription factor *Tbx21* (T-bet) has a significant role in preventing the immune response from switching from antigen Th1 type to Th2 type and, consequently, it is necessary for preventing development of allergic reactions such as bronchial asthma. A study was performed on mice with knocked-out gene *Tbx21* and with T-bet-/- genotype in comparison with wild animals. Animals were exposed to nanomaterials through oropharyngeal aspiration; the following histopathologic research revealed that cell metaplasia in alveoli mucosa was authentically higher in T-bet-/- mice on the 21st day after the experiment than in mice from the reference group. This effect was less apparent after exposure to Multi Walled Carbon Nanotubes (MWCNTs). Chronic alveolitis developed in T-bet-/- mice on the 21st day after the exposure to Ni NPs, but not to MWCNTs. Higher expression of MUC5AC and MUC5B was also observed in the test group against the reference one under exposure to Ni NPs. T-bet-/- mice had elevated levels of IL-13, CCL2 and elevated quantity of eosinophils in BAL (bronchoalveolar lavage) already 1 day after the exposure to Ni NPs. When T-bet-/- mice were treated with monoclonal antibodies to CCL2, this resulted in higher metaplasia in mucosa and MUC5AC expression. These data confirm a significant role that belongs to T-bet in protection from allergenic effects produced by Ni NPs.

Roach K.A. and others performed a study [54] where mice were twice exposed to NiO NPs (42 nm in diameter) or MPs (181 nm) through pharyngeal aspiration, on the 1st and 19th day in the experiment, in a dose equal to 3–40 μg and were simultaneously parenterally sensitized with ovalbumin. Exposure to NiO in doses that were comparable in particle surface resulted in changes in total IgE, cytokine con-

tents in blood and the lungs. When an introduced surface was low, the immune response developed predominantly as per Th2-pathway, but in case it was high, there was a switch to Th1 type. There was also a growth in lung eosinophilia under exposure to high doses of NPs.

Possibility that the immune response could switch to Th2 type was also mentioned in the work [29] where intratracheal introduction of NiO NPs into male Wistar rats led to greater expression of GATA-3 and T-bet against elevated levels of cytokines TNF-α, IL-2, IL-10 and neutorphil chemoattractants CINC-1, CINC-2αβ and CINC-3.

The research work [55] revealed there was a possibility of enhanced mice sensitization with Ni NPs combined with lipopolysaccharide when introduced subcutaneously. Silver NPs produced the same effects but that was not the case with Ag+ ions, NPs of gold and amorphous SiO_2.

Reproductive toxicity. Ni NPs (90 nm in diameter) were daily introduced into female rats through a gavage in a dose equal to 3–45 mg/kg during 14 days. As a result, there was mitochondria swelling in ovary tissue, mitochondrial cristae disappeared, and endoplasmatic reticulum grew in size. There was an authentic decrease in SOD and catalase activity and a growth in contents of ROS, malonic dialdehyde, and NO. There also was an authentic growth in expression of mRNA, caspase-3, 8 and 9, Fas, cytochrome C, Bax and Bid with a simultaneous decrease in Bcl-2 expression. Effects produced by Ni MPs were weaker as per several parameters than those produced by NPs [44]. The authors conducted the next research where they orally exposed male rats to Ni NPs (90 nm in diameter) in doses equal to 15–45 mg/kg of body weight every day during 10 weeks prior to coupling and estimated a number of fertilized female rates after it. There was lower SOD and catalase activity as well as lower contents of gonad-stimulating hormone GSH in testicles of the exposed animals with simultaneous elevated contents of NO, malonic dialdehyde, and ROS. Expression of caspases 3, 8 and 9 grew but there was a decline in expression of Bcl-2-associated X protein (Bax) and apoptosis-induced factor (AIF). The aforementioned effects could be partially inhibited by ascorbic acid introduced into animals in high doses and this indicates they were pro-oxidant in their essence [45].

Ni NPs sized 90 nm were one-time introduced through a gavage into male ICR mice in doses varying from 5 to 45 mg/kg of body weight; 30 days after the introduction there was apoptosis of cells in spermatophore tubes, a decrease in testicle mass index, lower activity of marker tissue enzymes in them and lower sperm mobility [56].

Sub-acute intratracheal introduction of NiO NPs into male Sprage Dawley rats in a dose equal to approximately 1 mg once every three days during 3 months resulted in a decrease in total number of sperm cells, number of live cells, and a growth in a number of morphologically abnormal sperm cells. Female rats who coupled with exposed male rats had greater number of dead fetuses. Ni concentration which correlated with lower spermatogenesis parameters grew in male rats' blood and sperm [57].

Table 1 contains a list of the most significant genetic and molecular markers showing toxicity of Ni-containing NPs according to data provided by research *in vivo*.

Table 2 provides data on experimental estimates for maximum non-effective dose (NOAEL) for Ni-containing NPs as per data available in several sources. In most cases authors failed to establish NOAEL; therefore, its estimates are given "from above", that is, according to detected toxic effects.

Toxicity in clinical observations. There are very few clinical or epidemiologic observations regarding adverse effects produced by NPs that contain Ni and its compounds despite occupational exposure to them is quite probable [2]. NPs of heavy metals including Ni were detected through retrospective analysis in bodies of patients who died from Hodgkin lymphoma; given that, they were considered a factor that might cause this neoplasm [58]. Another research revealed that workers who had contacts with Ni nanopowder at their workplaces had irritated throat and stuffy nose, red skin on their face and other skin reactions

Table 1

The most significant biomarkers of toxic effects produced by Ni-containing nanomaterials *in vivo*

No.	Biomarker	Abbreviation	Place of detection	Source
1	Interleukins	IL-1α, IL-1β, IL-2, IL-6, IL-8, INF-γ, TNF–α	Lungs BAL	[17, 28, 31] [13]
2	Matrix metalloproteinase	MMP 2, 9	Lungs Liver	[27] [32]
3	Matrix metalloproteinase inhibitors	TIMP 1,3	The same	[32]
4	Micro-RNA 210	miR210	Lungs	[21, 22]
5	8-oxo-2-deoxyguanosine	8-oxo-G	The same	[19]
6	Glutathione	GSH	Liver	[7]
7	Superoxide dismutase	SOD	Blood plasma, brains	[9]
8	Catalase	Cat	The same	[9]
9	Malonic dialdehyde	MDA	The same	[9]
10	Chemokines	MIP-1a, MCP-1, CINC-1, CINC-2αβ, CINC-3	Lungs, BAL	[11, 13]
11	Caspases 1, 3, 8, 9, 12	-	Lungs, liver, ovaries	[19, 26, 44, 46]
12	Heme oxygenase-1	HO-1	Lungs	[18]
13	Lactate dehydrogenase	LDH	Lungs	[19]
14	Nuclear transcription factor erythroid-2	NRF-2	Lungs	[18]
15	Nuclear transcription factor T-bet	Tbx21	Lungs	[29]

Note: LDH – lactate dehydrogenase; IL – interleukin; INF – interferon.

Table 2

Estimates of maximum non-effective doses (NOAEL) for NPs of Ni and its compounds as per data available in literature

Nanomaterial (composure and particle size)	Experimental model	Examined parameters	Estimate for NOAEL, meas. units	Source
Intratracheal and inhalation introduction				
NiO 8–26 nm	Wistar rats	CINC-1, CINC-2αβ, CINC-3 in BAL	< 0.33 mg/kg	[11]
NiO 8.4 nm	Wistar rats	CINC-1, CINC-2αβ, CINC-3 in BAL	< 3.3 mg/kg	[12]
NiO 26 nm	Rats	Cytokines in BAL	< 0,2 mg/kg	[14]
Ni 41 nm	Sprague Dawley rats	Histopathology, HO-1, NRF-2 (Lungs)	< 4 mg/kg	[18]
NiO	Rats	Histopathology of internal organs	< 0.2 mg/kg	[20]
Ni	Mice	Neutrophils, LDH, CXCL1/KC in BAL	< 0.4 mg/kg	[23]
NiO	Sprague Dawley rats	Histopathology, (Lungs)	< 2 mg/kg	[24]
NiO	Rats	Histopathology, (Lungs)	< 2 mg/kg	[25]
NiO	Rats	Apoptosis index (Liver)	0.015 mg/kg	[26]
NiO	Male Wistar rats	Histopathology, (Liver)	< 0.25 mg/kg	[30]
Ni 20 nm	Mice, males and females	Macrophages, LDH in BAL	4 mg/kg (females)	[31]
NiO 10–20 nm	Male Wistar rats	Polymorphonuclear leukocytes in BAL	200 cm^2 per 1 animal	[33]
NiO 15–35 nm	Male Fischer 344 rats	HO-1 (Lungs)	< 0.2 mg/kg	[34]
NiO 23 nm	Female rats **	Neutrophils and macrophages in BAL, histopathology of liver, kidneys, and spleen	< 1 mg/m^3	[36]
NiO 54 nm	Male Wistar rats	Surfactant components in BAL	< 0.2 mg/m^3	[35]
$Ni(OH)_2$	Male C57Bl/6J mice	Pharmacologically stimulated vasoconstriction and vasorelaxation	< 0.15 mg/m^3	[38]
Ni	ApoE(-/-) mice	Vascular atherosclerosis	< 0.08 mg/m^3	[40]
Oral introduction				
NiO 20 nm	Female Wistar rats ***	Genotoxicity (bone marrow), Enzyme activity (blood serum, liver, and kidneys)	125 mg/kg	[41, 42]
NiO 13 nm	Wistar rats, males and females	Hematologic parameters, histopathology (liver)	< 50 mg/kg	[43]
Ni 90 nm	Male Sprague Dawley rats	Glutathione (gonads)	< 5 mg/kg	[45]
NiO 50 nm	Male Wistar rats	Apoptosis (bone marrow)	< 1 mg/kg	[46]
NiO 50 nm	Male Wistar rats ***	Integral, biochemical, and hematologic parameters	< 1 g/kg	[47]

Note: * means abbreviations are given in Table 1; ** means sub-chronic experiment (10 months); *** means acute experiment (a single introduction); LDH – lactate dehydrogenase.

in case they were not properly protected [59]. There was a clinical case when a worker who dealt with metal arc welding involving inhalation exposure to Ni NPs died due to respiratory distress-syndrome. Autopsy revealed Ni NPs sized 25 nm in lung macrophages, elevated Ni contents in biological media and tubular necrosis [60].

Conclusions. Therefore, literature analysis reveals that NPs of metallic Ni and its compounds (NiO, $Ni(OH)_2$) as well as nickel nanofibers and nanorods produce local and systemic toxic effects when introduced into the body both parenterally and naturally, though the airways and gastrointestinal tract. Systemic effects occur due to these NPs being able to move into organs that are located far from the place where they penetrated the body, either with blood or lymph flow.

Toxicity of both Ni NPs and Ni-compounds with traditional dispersity is to a certain extent related to their ability to penetrate through membranes and biological barriers, dissolve and degrade biologically in the body. Other things being equal, better soluble NPs (NiO) turn out to be more toxic than poorer soluble NPs of metal Ni or Ni MPs. On the other hand, in some cases Ni-containing NPs turn out to be even more toxic than soluble nickel salts due to their greater ability to penetrate into cells through membranes. It further confirms an assumption that substances in their nanoform can be substantially different from their micro-dispersed analogues regarding their impacts on biological systems, and hazards caused by nanomaterials are to be described profoundly in each specific case [61].

Ni-containing nanomaterials are known to produce various adverse effects including overall toxic ones, toxic effects on specific organs (hepatotoxic and cardiotoxic included), atherogenic, fibrogenic, allergenic, and immunotoxic ones; they also have reproductive toxicity. But at the same time, data obtained via experiments *in vitro* and indicating that Ni-containing nanomaterials are genotoxic, mutagenic, and have transforming capabilities, have found practically no confirmations in chronic experiments *in vivo* that focused on establishing whether these materials were carcinogenic. Given that, carcinogenicity of Ni and its compounds in their nanoform remains rather disputable.

Experimental estimates of maximum non-effective doses and concentrations of Ni-containing nanomaterials show that such doses are within a range being lower than 0.2 mg/kg of body weight or 1 mg/m^3 when they are introduced into the airways regardless of particle size and structure of a nanomaterial. At present there are no reliable estimates of maximum non-effective or toxic doses for multiple (subacute and chronic) oral exposures to these nanomaterials.

A necessary stage in risk assessment procedure is to determine exposure to examined adverse factors at workplaces, due to environmental factors, and consumer products. Unfortunately, at present such data on NPs of Ni and its compounds are practically unavailable. The only exceptions are rare reports on detecting Ni-containing NPs in working area air at metallurgic enterprises. So, it is practically impossible to assess risks caused by Ni NPs including those caused by their occurrence in food products and water, as residual quantities of nickel catalysts or due to the environmental contamination with these nanomaterials.

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