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PREVENTIVE HEALTHCARE: TOPICAL ISSUES OF HEALTH RISK ANALYSIS

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SOCIAL AND EPIDEMIOLOGIC DETERMINANTS AND POTENTIAL FOR GROWTH IN LIFE EXPECTANCY OF THE POPULATION IN THE RUSSIAN FEDERATION TAKING INTO ACCOUNT REGIONAL DIFFERENTIATION

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The current work supplements the results obtained in previous research on a relation between leading parameters of living conditions and life expectancy of the RF population; it dwells on the results obtained via analyzing a role played by sanitary and epidemiologic determinants. A sanitary-epidemiologic situation in certain RF regions is unfavorable and it makes our research truly vital; it is also necessary to work out and implement activities aimed at eliminating or minimizing adverse environmental factors that can produce negative effects on demographic situation in the country. Our primary goal was to study impacts exerted by sanitary-epidemiologic parameters on life expectancy in the RF and to obtain predicted values for its growth taking into account regional and sex differentiation.

We examined domestic and foreign experience in researching relations between sanitary-epidemiologic welfare and life expectancy. All the RF regions were distributed into three clusters as per their sanitary-epidemiologic welfare. The third cluster that includes 11 regions is in much greater need for implementing activities aimed at reducing environmental contamination. Results obtained via regression and factor analysis revealed that should there be a scenario with an improvement in sanitary-epidemiologic parameters (by 10.0 %), the overall life expectancy for the RF population would increase by 140.39 days. An improvement in sanitary-epidemiologic situation taken as per sex differentiation indicated that a greater impact was expected on life expectancy growth among male population, as it would increase by 146.9 days (by 117.6 days for female population). We established that several parameters made the greatest contribution into life expectancy growth; they were "A share of population provided with high quality drinking water" (61.65 days); "Physical factors existing at workplaces" (35.83 days), "Sanitary-hygienic characteristics of objects under surveillance" (15.16 days), and "Sanitary-epidemiologic parameters of ambient air" (14.26 days).

The current work does not cover extreme sanitary-epidemiologic situations related to pandemic spread of new infectious agents causing highly contagious diseases (Coronavirus infection).

Key words: *life expectancy, sanitary-epidemiologic factors, environment, demographic policy, population, life quality, factor analysis, cluster analysis.*

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Introduction. In the 2nd half of the 20th century efforts made by the whole mankind allowed minimizing (variola eradication and elimination of measles, rubella, and poliomyelitis on certain territories) and almost completely getting rid of known communicable diseases; it resulted in the most favorable situation in public healthcare in history [1, 2]. During that period life span and life expectancy of a human being as a biological species achieved their record length and it is confirmed by mortality curve becoming rectangular [3–5]. Successes achieved in struggling against communicable diseases allowed decreasing their influence on public health as well as prolong life expectancy; but at the same time it led to changes in structure of morbidity and mortality. Nowadays non-communicable diseases (NCDs) are the leading cause of deaths all ever the world and in the Russian Federation (RF) as well [6]. Besides, such a wide spread of these diseases to a greater extent is determined by impacts exerted by scientific and technological progress on the environment and people.

Technological progress resulted in two most significant and interrelated processes in the society, namely industrialization and urbanization. Industrialization allowed significant increase in social and economic welfare of the population thus stimulating further society development. A change in social and economic relations led to a natural transition from a predominantly traditional (agricultural) society to a contemporary (urbanized) one with its peculiarities, including deteriorating ecological conditions in settlements due to intensifying industrial activities and growing transport flows. All the described processes are apparent in developing countries, such as China, India, or Pakistan [7–10].

Anthropogenic activities resulted in greater technogenic loads on the environment, namely great quantities of contaminants accumulated in environmental objects as well as wider spread of certain physical factors such as noise, vibration, electromagnetic irradiation, etc. [11–13].

As per WHO data, a contribution made by environmental factors may well reach 25.0 %. Despite several research works [14–17] stating that lifestyle and living standards of people exert more substantial influence on diseases occurrence and clinical course, qualitative properties of the environment where these people live can either make for deterioration of their health or, on the contrary, can be a source for its recovery and improvement via recreational opportunities or possibility to get good rest on a specific territory.

Drinking water sources, together with ambient air and soils, are gravely contaminated due to anthropogenic activities. Primary contaminants in water are heavy metals, microbiological agents, emergent contaminants, and other chemicals that penetrate water with sewage discharges from industrial and communal objects, diffusely enter from soils cultivated by agricultural enterprises, or from precipitations. We should take into account that new chemical compounds that can be found in drinking water sources occur faster than any potential hazardous effects produced by them on a human body are determined and that water is often supplied to an end customer via old water supply systems, and it, in one way or another, results in urban population facing a problem related to drinking water contamination [18, 19].

Existing water treatment facilities are able to prevent acute intoxications caused by drinking water contamination; however, the above mentioned factors gradually increase probability of various diseases such as urolithiasis and oncologic diseases and in case of any emergencies even communicable diseases [20–22].

About 90.0 % people are exposed to ambient air that doesn't conform to the WHO requirements regarding ambient air quality[1]. As per data provided by Global Health Observatory (GHO/WHO) [23] in 2016 approximately 7 million deaths all over the world were caused by ambient air contamination (4.2 million deaths) and contaminated air inside premises; ambient air contamination accounts for approximately 25.0 % deaths due to ischemic

[1] WHO recommendations fix average annual limit for $PM_{2.5}$ particles amounting to 10 μg/m^3.

heart disease and 24.0 % deaths due to cardiac infarction as well as about 246,000 deaths of children younger than 5 [24]. As per research data, in the RF overall number of deaths caused by exposure to particulate matter (PM_{10}[2]; $PM_{2.5}$) amounted to 140,851 cases among people of both sexes (UI[3]: 59,079–192,348), 83,938 out of them being due to ischemic heart disease, and 46,216 due to cardiac infarction. This quantity of death cases related to particulate matter contents in ambient air is one of the highest in the world as higher figures are registered only in India, 621,138 death cases (UI: 515,242–744,416), and China, 1,032,833 death cases (UI: 869,033–1,212,034) [25].

Environmental contamination leads to substantial losses in the world economy as well as certain national economies. Data provided by the Global Commission on Pollution and Health indicate that there are 2.0 % losses in GDP in middle-income countries (MIC) and low-income countries (LIC) due to diseases caused by environmental contamination. Besides, there is a growth in annual expenses on public healthcare that varies from 1.7 % in high-income countries (HIC) and up to 7.0 % in MIC and LIC where industrialization and urbanization are developing more intensely at the moment. Overall world economic losses caused by environmental contamination are 4.6 trillion USD [26].

As per data provided by S.A. Sarkodie et al., a growth in industrial emissions that contain $PM_{2.5}$ results in 0.004 % decrease in life expectancy (LE) (CI[4]: 95.0 %) [27]. The author also showed in that work that an increase in incomes as a process which was closely connected with growing environmental contamination led to a 0.02 % increase in LE (CI: 95,0 %) and a 0.01 % decrease in overall mortality. This phenomenon is explained with a hypothesis known as the Environmental Kuznets Curve [28]. According to this hypothesis a relation between the parameters is an upturned U and it shows that growing incomes go together with growing environmental contamination; however, in future, after certain population welfare has been reached, a society starts to implement activities aimed at reducing anthropogenic loads on the environment (ecological taxes, hygienic standards, transition to ecologically friendly technologies in energy production and manufacturing). A similar research work accomplished in the USA revealed that, firstly, there was an inverse correlation between LE and ambient air contamination with particulate matter; and, secondly, the correlation became even stronger in case there was inequality in population incomes [29].

As per data obtained by researchers from Denmark, life expectancy goes down not only due to ambient air being contaminated with ultra-dispersed particles, but also with nitrogen dioxide emitted by transport. Should NO_2 emissions from transport decrease up to levels that are typical for rural areas or by 20.0 %, it will result in LE increase by 2040 in Denmark, by 2.0 and 0.6 years for men and by 0.4 and 0.1 years for women accordingly [30].

Employable people are exposed to occupational factors during the biggest part of their lives. Impacts exerted by various occupational factors (chemical, physical, or biological ones) can lead to deterioration in one's health; various occupational pathologies, either mild or grave; a decrease in workers' life span; more frequent oncologic diseases; deterioration in emotional and mental state caused by various factors; as well as to other disorders [31–36].

In 2015 The UN General Assembly approved on a program called Sustainable Development Goals; it contains 17 global goals that the mankind is to achieve in order to support sustainable development up to 2030. The program describes urgent issues existing in the world in great detail; these issues are to be resolved in the next two decades. The program also sets certain tasks which are to be solved for achieving these goals. A pressing issue in public healthcare is to reduce mortality caused by NCDs by third; tasks which are to be

[2] Particulate matter (PM) are solid particles with their diameter being less than 10.0 nm and 2.5 μm.
[3] Uncertainty Interval.
[4] CI means confidence interval.

solved in the sphere involve reducing mortality and morbidity due to effects produced by hazardous chemicals and environmental contamination. Key instruments here are early prevention of hazardous effects on health and relevant policy aimed at reducing population health risks. Tasks in the sphere of ecology and environmental protection involve transition to ecologically friendly power production as well as rational use of chemicals and wastes together with a reduction in their volumes that penetrate the environment[5].

Despite the Russian Federation being among countries with high incomes (HIC), life expectancy in our country is significantly lower than in other economically developed countries. According to official statistic data, in 2018 life expectancy in Russia amounted to 72.91 years while in other European countries it was equal to 80 years or even more[6]. An important role here belongs to quantity of people exposed to negative impacts exerted by environmental factors. Data obtained via social and hygienic monitoring in 2018 revealed that more than 62 % RF population were exposed to adverse effects produced by contaminated drinking water, ambient air, and soils as well as to hazardous physical factors[7].

Taking into account world trends in environmental protection as well as specific demographic situation in the Russian Federation, state authorities have developed and are now implementing such national projects as "Demography" "Public healthcare", and "Ecology" which are aimed at preserving and multiplying human capital as well as creating a comfortable environment for country population [37].

Our research goal was to study sanitary and epidemiologic determinants and related potential for a growth in life expectancy in the Russian Federation.

Data and methods. This research work continues studying relations between environmental factors and life expectancy in the RF. To gain comparable results, just as it was the case with the first research work [8] that dwelled on impacts exerted on life expectancy for the whole country population by social and economic factors, we applied the same methodical approach in the present work. A basic distinction is that this study focuses on other influencing determinants or sanitary and epidemiologic parameters. We put forward a hypothesis that there is a direct or inverse regular cause-and-effect relation between life expectancy at birth (overall population, men and women separately, dependent variables) and sanitary-epidemiologic determinants (risk factors or independent variables, predictors or regressors).

Our analysis and model creation was based on statistical data collected in 2010–2018 in 85 RF regions (and data for the whole country) which were obtained from official sources such as reports issued by the Federal Statistics Service (emissions from stationary an mobile sources, life expectancy); Reports as per N 18 Statistic Form "Data on sanitary situation in a region in the RF" (sanitary-epidemiologic parameters).

Overall, we included 111 sanitary-epidemiologic parameters in our research. They were distributed into several groups according to sections in Statistic Form N 18: situation with drinking water supply; data on population being provided with drinking water that conformed to safety standards; a situation with water objects which people used to obtain water; ambient air contamination; situation with soils; hygienic properties of raw materials and food products; air inside premises and in working areas; a study on physical factors; sanitary-hygienic properties of objects applied by economic agents under surveillance in their activities.

We took Statistica 10.0 software for statistic analysis as our basic tool for testing adequacy and calculating parameters included into obtained models. Statistical hypotheses were

[5] Sustainable development goals. The United Nations Organization. Available at: https://www.un.org/sustainabledevelopment/ru/sustainable-development-goals/ (22.01.2020).

[6] Russian Regions. Social and economic parameters. 2019: P32 Statistic data collection. *Rosstat*. Moscow, 2019, vol. 1, 204 p.

[7] 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 Well-being, 2019, 254 p.

checked against regression quotients with Student's t-test in case parameters distribution was normal. Models were checked for their adequacy via dispersion analysis with Fischer's test and significance level being 0.05.

We applied factor analysis to solve tasks related to studying multiple correlations between sanitary-epidemiologic parameters and LEB as well as to reduce initial data dimension. Statistical models for correlations were built as per the following chain: "sanitary-epidemiologic parameters – generalized factors – life expectancy". We applied Kaiser Criterion (eigenvalues criterion) to reduce a number of common factors obtained via sequential building of correlation variables matrix with subsequent factors extraction with the least square technique. It allowed us to reduce a number of common factors to 12. Mutual influence exerted by factors on each other was excluded via orthogonal radiation with obtaining values for loads of variables on factors. These approaches allowed calculating quantitative changes in LEB under preset changes in examined sanitary-epidemiologic parameters.

An algorithm for building cause-and-effects relations as per "sanitary-epidemiologic parameters – generalized factors – life expectancy" chain included the following basic stages:

– recovering data for specific data series as per an algorithm that is given below;

– calculating mean values of a parameter and standard deviation for observation series;

– setting predicted sanitary-epidemiologic parameters via making changes into initial values by a scenario per cent (for example, 10 %, 5 %, or 1 %);

– calculating a difference between predicted and actual value of a sanitary-epidemiologic parameter;

– calculating standardized difference between predicted and actual value of a sanitary-epidemiologic parameter;

– calculating changes in generalized factors associated with changes in a sanitary-epidemiologic parameter, taking into account multiple regression coefficient "sanitary-epidemiologic parameters – life expectancy";

– ranking all the sanitary-epidemiologic parameters as per their contributions made into changes in LEB;

– summing up all the values of changes in LEB obtained at the previous stage associated with changes in a sanitary-epidemiologic parameter.

The difference between predicted and actual values of a sanitary-epidemiologic parameter was calculated as per the formula (1):

$$\Delta D = D' - D, \tag{1}$$

where ΔD is the difference between a predicted value of a sanitary-epidemiologic parameter (D') and its actual value (D).

Standardized difference between a predicted and an actual value of a parameter was determined as per the following formula (2):

$$\Delta d = d' - d = \frac{D' - \overline{D}}{D_s} - \frac{D - \overline{D}}{D_s} = \frac{D' - D}{D_s} = \frac{\Delta D}{D_s}, \tag{2}$$

where Δd is the standardized difference between a predicted and an actual value of a parameter; d' is a standardized predicted value of a parameter; d is a standardized actual value of a parameter; $\overline{D}$ is an average value of a parameter with recovered data; D_s is a standard deviation in a parameter with recovered data.

We calculated changes in generalized factors associated with changes in a sanitary-epidemiologic parameter as per the following formula (3):

$$\Delta F_i = \Delta d \cdot k_i, \tag{3}$$

where ΔF_i is a change in the i-th generalized factor associated with a change in a sanitary-epidemiologic parameter; k_i is a factor coefficient for the i-th generalized factor (determined as per factor analysis results).

A change in life expectancy associated with a change in a sanitary-epidemiologic parameter was determined via summing up all the products of changes in generalized factors multiplied by relevant "sanitary-epidemiologic parameters – LEB" multiple regression coefficients as per the following formula (4):

$$\Delta Z = \Delta F_i \cdot b_i, \tag{4}$$

where ΔZ is a change in life expectancy associated with a change in a sanitary-epidemiologic parameter given in years; b_i is a coefficient before the *i*-th factor in "sanitary-epidemiologic factors – LEB" multiple regression.

Exploratory factor analysis allowed obtaining a factor burden matrix that totally included 12 factors. Cumulative per cent of explained variance gained from these 12 factors amounted to 68.73 %. Formulas (3) and (4) were applied to calculate quantitative changes in LEB associated with a change in each examined sanitary-epidemiologic parameter. We give an example of calculating a change in life expectancy depending on a 10.0 % change in values of sanitary-epidemiologic parameters; in this calculation parameters that led to an increase in LEB were raised by 10.0 %, and those that decreased LEB were reduced by 10.0 %.

We divided RF regions into several clusters according to their sanitary-epidemiologic parameters and LEB applying a multi-dimensional statistical procedure, namely cluster analysis with k-medians clustering. Parameter values in clusters were compared as per their average cluster values.

Results and discussion. Linear correlation-regression analysis allowed us to obtain 195 authentically significant models and 134 out of them didn't contradict the hypothesis on cause-and-effect relations between population health and a sanitary-epidemiologic situation with environmental factors. We analyzed a correlation between sanitary-epidemiologic parameters and LEB of overall population and revealed the most significant factors as per explained variance coefficient (R^2); these factors given in the descending order are as follows: "A share of workplaces not conforming to sanitary standards as per illumination" ($a_x = –0.239$; $b = 72.59$; $p < 0.05$; $r = –0.495$; $R^2 = 0.245$); "A share of workplaces not conforming to sanitary standards as per microclimate" ($a_x = –0.195$; $b = 71.68$; $p < 0.05$; $r = –0.473$; $R^2 = 0.224$); "A share of workplaces not conforming to sanitary standards as per noise" ($a_x = –0.105$; $b = 71.98$; $p < 0.05$; $r = –0.367$; $R^2 = 0.135$); "A share of air samples with dust and aerosol contents exceeding MPC[8]" ($a_x = –0.158$; $b = 71.27$; $p < 0.05$; $r = –0.35$; $R^2 = 0.122$).

The most significant parameters for LE among male population were the following: "A share of workplaces not conforming to sanitary standards as per illumination" ($a_x = –0.262$; $b = 67.25$; $p < 0.05$; $r = –0.468$; $R^2 = 0.219$); "A share of workplaces not conforming to sanitary standards as per microclimate" ($a_x = –0.201$; $b = 66.17$; $p < 0.05$; $r = –0.423$; $R^2 = 0.179$); "A share of workplaces not conforming to sanitary standards as per noise" ($a_x = –0.119$; $b = 66.66$; $p < 0.05$; $r = –0.362$; $R^2 = 0.131$); "A share of air samples with contents of substanes belonging to the 1st and 2nd hazard category exceeding MPC (vapor and gases)" ($a_x = –0.231$; $b = 65.43$; $p < 0.05$; $r = –0.33$; $R^2 = 0.109$).

The most significant parameters for LE among female population were the following: "A share of workplaces not conforming to sanitary standards as per illumination" ($a_x = –0.202$; $b = 77.79$; $p < 0.05$; $r = –0.51$; $R^2 = 0.261$); "A hare of workplaces not conforming to sanitary standards as per microclimate" ($a_x = –0.171$; $b = 77.08$; $p < 0.05$; $r = –0.511$; $R^2 = 0.261$); A hare of air samples with dust and aerosol contents exceeding MPC ($a_x = –0.134$; $b = 76.73$; $p < 0.05$; $r = –0.37$; $R^2 = 0.137$); "A share of workplaces not conforming to sanitary standards as per noise" ($a_x = –0.082$; $b = 77.17$; $p < 0.05$; $r = –0.35$; $R^2 = 0.123$).

As per cluster analysis results, all the RF regions were distributed into 3 clusters, each having its peculiarities related to a sanitary-epidemiologic situation there. The 1st cluster included 24 regions; the 2nd, 50 regions; the 3rd, 11 regions (Figure 1).

The 1st cluster includes 24 RF regions, namely Belgorod, Bryansk, Vladimir, Ivanovo, Kaluga, Kostroma, Smolensk, Tambov, Tula, Yaroslavl, Arkhangelsk, Leningrad, Murmansk, Rostov, Kirov, Samara, Ulyanovsk, Sverdlovsk, Chelyabinsk, and Magadan regions, the Nenets Autonomous Area, Mordovia, Primorye, and the Jewish Autonomous Region. Average cluster LE value for the overall population amounted to 71.8 years; male population, 66.4 ears (the lowest LE among all clusters);

[8] MPC means maximum permissible concentration.

female population, 77.1 years. Drinking water supply was analyzed and assessed as per a share of samples not conforming to requirements as per sanitary-chemical properties; those shares amounted to 24.3 % for centralized water supply networks and to 18.8 % for distribution networks thus making average cluster values higher than in the country on average (16.97 % and 13.0 % accordingly). A share of air samples with MPC deviating from standards in urban settlements was the lowest among all three clusters (0.56 %). Sanitary-epidemiologic situation with soil in regions belonging to the 1st cluster is relatively unfavorable (certain parameters being higher than in the country on average): a share of soils samples not conforming to standards as per sanitary-chemical parameters amounts to 7.98 %, including those as per metals, 7.97 %; a share of soil samples deviating from standards as per microbiological parameters amounts to 9.98 % (the highest value among all three clusters). Parameters related to food products safety and quality also deviated from standards more frequently in this cluster than in the country on average. Thus, a share of samples not conforming to requirements as per sanitary-epidemiologic parameters amounts to 0.45 % (0.39 % on average in the RF); as per microbiological parameters, 4.25 % (3.88 % on average in the RF); a share of samples not corresponding to standards as per calorie contents and chemical structure amounts to 9.75 % (6.73 % on average in the RF). Certain parameters of air in working areas at economic entities operating in regions form the 1st cluster are also higher than in the country on average; a share of air samples with vapor and gases contents exceeding MPC amounts to 1.44 % (1.38 % on average in the RF), and a share of air samples with contents of substances belonging to the 1st and 2nd hazard category exceeding MPC (vapor and gases) amounts to 1.9 % (1.77 % on average in the RF). Workplaces were assessed as per physical factors occurring there; the assessment revealed that the cluster had the highest share of samples deviating from standards as per "IR[9]" as it amounted to 0.67 %; a share of deviating samples as per "noise" factors amounted to 13.86 %; "microclimate", 5.22 %; "EMF[10]", 5.87 %, "illumination", 8.76 %; all these values are higher than on average in the country (12.76 %, 4.7 %, 4.49 %, and 8.31 % accordingly). The cluster also has the lowest share of objects belonging to the 3rd hazard category as per their sanitary-hygienic characteristics (3.01 %); a share of objects belonging to the 1st category amounts to 48.1 %.

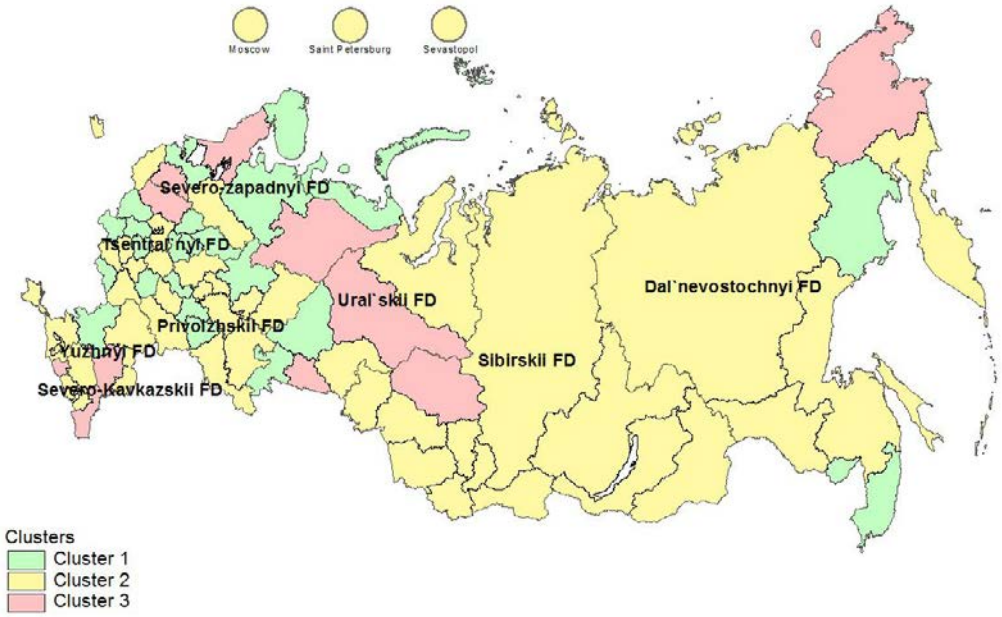

Figure 1. RF regions distributed into 3 clusters as per sanitary-epidemiologic parameters

[9] IR means ionizing radiation.
[10] EMF – Electromagnetic fields.

The 2nd cluster includes 50 RF regions, namely Voronezh, Kursk, Lipetsk, Moscow, Orel, Ryazan, Vologda, Kaliningrad, Pskov, Astrakhan, Volgograd, Nizhniy Novgorod, Orenburg, Penza, Saratov, Tyumen, Irkutsk, Kemerovo, Novosibirsk, Omsk, Amur, Sakhalin, Moscow City, Saint Petersburg, Adygei Republic, Crimea, Krasnodar, Sevastopol, Ingushetia, Kabardino-Balkarskaya Republic, North Ossetia, Chechnya, Stavropol, Bashkortostan, Mari El Republic, Tatarstan, Udmurtia, Chuvashskaya Republic, Perm region, Yamal-Nenets Autonomous Area, Altai Republic, Buryatia, Tyva Republic, Khakassia, Altai region, Transbaikalia, Krasnoyarsk, Yakut Republic, Kamchatka, and Khabarovsk region. This cluster has LE comparable with average Russian one for the overall population, male and female population as well (72.46, 67.29, and 77.45 years accordingly). Drinking water quality in this sector is better than on average in the country; a share of samples from supply and distribution networks that deviate from standards as per sanitary-chemical parameters amounts to 9.79 % (16.97 % on average in the RF) and 9.81 % (9.81 % on average in the RF). Sanitary-epidemiologic situation with soils in regions in this cluster is also comparatively better than on average in the country; a share of soil samples deviating from standards as per sanitary-chemical parameters amounts to 4.75 % (5.06 % on average in the country); as per microbiological parameters, 5.95 % (5.95 % on average in the country). A situation with food products safety and quality is also quite favorable in the cluster as a share of samples not conforming to standards as per microbiological parameters amounts to 3.66 %; a share of samples not conforming to standards as per calorie contents and chemical structure amounts to 6.08 %; a share of samples deviating from standards as per sanitary-chemical parameters amounts to 0.41 %; all these values are close to average country levels (3.88 %, 6.73 %, and 0.39 % accordingly). Quality of air in working areas at economic entities operating in the cluster is the highest among all three clusters as per a share of air samples with dust and aerosol contents exceeding MPC (3.52 %) and a share of air samples with contents of substances belonging to the 1st and 2nd hazard category exceeding MPC (dust and aerosols) (2.91 %). The cluster has the lowest number of workplaces that don't conform to sanitary standards as per physical factors; as per noise, 9.57 %; vibration, 4.04 %; microclimate, 4.09 %; EMF, 2.93 %; illumination, 7.19 %; IR, 0.4 %.

The 3rd cluster includes 11 RF regions, namely Tver, Novgorod, Kurgan, Tomsk, Karelia, the Komi Republic, the Kalmyk Republic, Dagestan, Karachai-Cherkess, Khanti-Mansi Autonomous Area, and Chukotka. LE among the overall population and female population is the lowest here against the other two clusters (71.6 and 76.6 years accordingly). Quality of drinking water taken from centralized water supply systems in unsatisfactory as a share of water samples that deviate from standards as per sanitary-chemical parameters amounts to 44.6 % for water taken from water supply systems and to 36.6 % for water taken from distribution networks. There are similar problems with ambient air quality as a share of ambient air samples with exceeding MPC amounted to 3.92 % in urban settlements (0.66 % on average in the RF). But soil quality, on the contrary, is the highest in this cluster as a share of soil samples that deviate from standards as per sanitary-chemical parameters amounts to 4.38 % (2.99 % as per metals). Food products safety and quality is in general lower than on average in the country. A share of food products samples that don't conform to sanitary-epidemiologic requirements as per sanitary-chemical parameters amounts to 0.77 % (0.39 % on average in the RF). Certain food products have the lowest quality as per this parameter, for example, poultry and poultry products (0.17 %); butter and butter products (0.26 %); fish and fish products (2.21 %); culinary products (0.65 %): vegetables and greenery (1.51 %). A share of workplaces that don't correspond to sanitary standards as per vibration and microclimate is also the highest in the cluster (10.83 % and 12.62 % accordingly). A share of objects that belong to the 1st hazard category as per their sanitary-hygienic properties is the lowest in this clus-

ter (35.61 %); and a share of objects belonging to the 3rd hazard category is the highest (5.9 %).

Factor analysis results allowed us to establish predicted values of life expectancy at birth (overall population; make and female population separately) that are related to sanitary-epidemiologic parameters. Table contains LEB values under a scenario with a 10 % improvement in the given parameters.

Table

Potential growth (in days) in life expectancy of the RF population related to changes in sanitary-epidemiologic parameters (a scenario entails a 10.0 % improvement in the given parameters)

A group of parameters	Parameter	An increase in LEB (overall population), days	An increase in LEB (male population), days	An increase in LEB (female population), days
Sanitary-epidemiologic parameters related to ambient air quality	A share of ambient air samples with contaminants contents being higher than MPC (in urban settlements)	0.71	0.39	0.38
	Mobile monitoring posts. A share of ambient air samples with contaminants contents being higher than MPC (in urban settlements)	6.16	5.98	5.98
	Observations at stationary posts. A share of ambient air samples with contaminants contents being higher than MPC (in urban settlements)	3.92	–	4.19
	A share of ambient air samples with contaminants contents being higher than MPC (in rural settlements)	3.47	3.64	3.64
A parameters showing sanitary-epidemiologic situation with drinking water – a share of population provided with qualitative drinking water		61.65	74.71	37.83
Sanitary-epidemiologic parameters related to soil quality	A share of samples not conforming to SER* (microbiological parameters)	4.04	5.95	2.66
	A share of samples not conforming to SER (sanitary-chemical parameters)	1.05	1.53	0.58
	A share of samples not conforming to SER (sanitary-chemical parameters. Metals)	0.73	1.08	0.45
Parameters related to food products quality and safety	All food products. A share of samples not conforming to SER (sanitary-chemical parameters)	0.44	0.46	0.24
	Fish. A share of samples not conforming to SER (parasitological parameters)	0.24	0.21	0.37
	Vegetables. Greenery. A share of samples not conforming to SER (sanitary-chemical parameters)	0.22	0.06	0.09
	Butter. A share of samples not conforming to SER (sanitary-chemical parameters)	0.13	0.15	0.02
	Fish. A share of samples not conforming to SER (sanitary-chemical parameters)	0.08	0.12	0.06
	Bread and confectionary. A share of samples not conforming to SER (sanitary-chemical parameters)	0.07	0.07	0.05
	Culinary products. A share of samples not conforming to SER (sanitary-chemical parameters)	0.05	0.04	0.03

A group of parameters	Parameter	An increase in LEB (overall population), days	An increase in LEB (male population), days	An increase in LEB (female population), days
	Milk. A share of samples not conforming to SER (sanitary-chemical parameters)	0.04	0.04	0.99
	Meat. A share of samples not conforming to SER (sanitary-chemical parameters)	0.03	0.03	0.02
	Poultry. A share of samples not conforming to SER (sanitary-chemical parameters)	0.02	0.01	0.88
	Fish. A share of samples not conforming to SER (microbiological parameters)	0.003	–	–
	All food products. A share of samples not conforming to standards (calorie contents and chemical structure)	–	–	1.24
	Meat. A share of samples not conforming to SER (sanitary-chemical parameters)	–	–	0.02
Sanitary-hygienic properties of objects	A share of objects belonging to the 1st hazard category	12.28	2.59	20.20
	A share of objects belonging to the 3rd hazard category	2.88	1.46	4.17
Quality of air in working areas (taken in total at all economic entities)	A share of air samples with vapor and gases contents exceeding MPC	1.91	2.71	0.81
	A share of air samples with contents of substances belonging to the 1st and 2nd hazard category exceeding MPC (dusts and aerosols)	1.8	1.72	1.87
	A share of air samples with dusts and aerosols contents exceeding MPC	1.45	1.46	1.20
	A share of air samples with contents of substances belonging to the 1st and 2nd hazard category exceeding MPC (vapor and gases)	1.19	1.71	–
Physical factors at workplaces	A share of workplaces not conforming to sanitary standards (ILLUMINATION)	16.22	17.74	14.59
	A share of workplaces not conforming to sanitary standards (NOISE)	8.24	10.14	5.28
	A share of workplaces not conforming to sanitary standards (MICROCLIMATE)	7.09	7.16	6.49
	A share of workplaces not conforming to sanitary standards (EMF)	2.06	3.67	–
	A share of workplaces not conforming to sanitary standards (IR)	1.24	1.40	1.01
	A share of workplaces not conforming to sanitary standards (VIBRATION)	0.98	0.29	1.44
Total		140,39	146.9	117.62

Note: * – SER means sanitary-epidemiologic requirements.

Prediction assessment revealed that an increase in a share of population provided with qualitative drinking water would make the highest contribution into a growth in life expectancy at birth (61.65 days). Better conditions at workplaces as regards physical factors can result in LEB among overall population growing by 35.83 days (Table, Figure 2).

A decrease in a share of object belonging to the 3rd hazard category as per their sanitary-hygienic properties is substantial as an improvement in this parameter can lead to LEB among the overall population growing by 15.16 days. A decrease in a share of air samples with contaminants contents exceeding MPC in urban and rural settlements can result

in LEB among the overall population growing by 14.26 days. An increase in quality of air in working areas can lead to LEB among the overall population growing by 6.35 days. Should there be a 10.0 % decrease in a share of soil samples not conforming to standards as per sanitary-chemical parameters, LEB among the overall population would grow by 5.82 days. A small increase (1.32 days) could results from better quality and safety of food products.

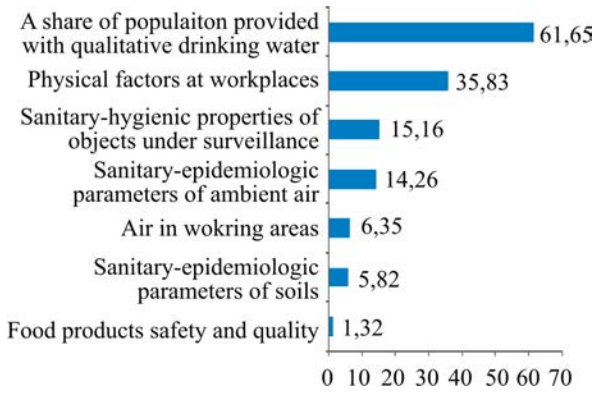

Figure 2. Predicted growth in life expectancy among the overall RF population determined by changes in sanitary-epidemiologic parameters in some groups (a scenario entails 10.0 % improvement in the given parameters), days

Results which we obtained in the present research work justify the hypothesis on sanitary-epidemiologic environmental factors exerting their impacts on LEB and don't contradict data available in foreign and domestic research works on negative effects produced on population health by environmental factors deviating from sanitary standards [38–41]. An increase in a share of population provided with qualitative drinking water makes the greatest contribution into LEB growth (61.65 days for the overall population) and it is fully in line with data taken from the state reports "On Sanitary-epideiologic welfare of the population in the Russian Federation" according to which poor quality of drinking water results in up to 1.8 million additional disease cases among the overall RF population (55–60 % of all the additional environmentally induced diseases).

The next significant parameter that can lead to additional 35.83 days in LEB for the overall population is physical factors at workplaces. Our data are consistent with the data taken from the state report[11] which indicate that physical environmental factors (noise, EMF, vibration, illumination, and IR) make the greatest contribution into death cases caused by circulatory system diseases and account for up to 52 housand additional death cases.

Improved sanitary-epidemiologic situation with ambient air in settlements can lead to 14.26 days increase in LEB for the overall population and it is also consistent with the data taken from the state report[11] which indicate that in 2018 up to 864,000 additional disease cases (circulatory system diseases, respiratory organs diseases etc.) were caused by ambient air being contaminated with hazardous chemicals.

It is necessary to implement a set of measures that are aimed at reducing impacts exerted by hazardous factors on the environment and providing safe living conditions for people; it will allow a substantial contribution into achieving national goals and solving strategic tasks of the RF development. Thus, activities performed by Rospotrebnadzor and aimed at reducing contamination of environmental objects annually prevent more than 150 additional death cases and more than 7 million disease cases among the RF population[11].

Conclusions. Our research results allowed us to make the following **conclusions**:

– should there be an improvement in analyzed sanitary-epidemiologic parameters, an overall increase in life expectancy at birth for the RF population can amount to 140.39 days;

– the most significant parameters that can potentially increase LEB for the overall RF population are the following: "A share of population provided with qualitative drinking water" (61.65 days); "Physical factors at workplaces" (35.83 days); "Sanitary-hygienic properties of surveillance objects" (15.16 days); and "Sanitary-epidemiologic parameters of ambient air" (14.26 days);

[11] 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 Well-being Publ., 2019, 254 p.

– LEB was also analyzed separately for both sexes, and it allowed revealing that improved sanitary-epidemiologic situation would results in greater growth in LEB among male population (by 146.9 days) than among female population (by 117.6 days);

– all the developed activities aimed at improving sanitary-epidemiologic parameters and, consequently, at increasing LEB should be differentiated according to a specific region where they are to be implemented. Thus, RF regions that are included into the 1st cluster should pay greater attention to sanitary-epidemiologic parameters of soils in settlements; RF regions that are included into the 2nd cluster, to quality and safety of certain food products (meat, milk, fish, and bread), and air in working areas at industrial enterprises. Sanitary-epidemiologic situation is not so favorable in RF regions included into the 3rd cluster. People living on these territories are potentially exposed to stronger effects produced by anthropogenic contamination; to improve the situation there, it is necessary to take complex inter-sectoral efforts, especially as regards providing people with qualitative drinking water and food products as well as creating favorable working conditions at workplaces. In order to preserve achieved levels of population health and LEB in all RF regions and increase them in future, it is advisable to continue implementing federal and regional programs aimed at providing sanitary-epidemiologic welfare and improving health of the population in the Russian Federation.

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EVALUATING THE CURRENT STATUS OF THE NATIONAL HEALTH, SAFETY AND ENVIRONMENT MANAGEMENT SYSTEM FOR INTEGRATION, HARMONIZATION, AND STANDARDIZATION OF ENVIRONMENTAL PROTECTION

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Our research focuses on a health and safety management system based on risk identification and analysis.

The research is vital due to its relation with GDP. GDP assessment performed in developing countries showed that losses caused by unmanaged HSE (Health, Safety, and Ecology) risks on average resulted in 4.2 % decline in GDP for those countries, with similar losses in various countries in this group. Hidden accidents and incidents caused by uncontrolled HSE result in damages that are about 12 times higher than the cost of direct damage.

Our research goal was to substantiate the necessity to create a national authority for managing HSE in one of the country's basic regulatory agencies. Its basic responsibilities will include developing national regulation in the sphere and increase awareness of various organizations that it is vital to identify risks and hazards within the HSE management system.

In order to solve the task, a think tank was organized that held regular meetings and included experts from three organizations, namely the Ministry of Health and Medical Education, Ministry of Labor and Social Welfare, and the Environmental Protection Agency located at the top three points of the "HSE triangle".

Another important part of our research was results and conclusions based on evaluating the existing situation with the national health, safety, and environment management system; it allowed introducing ten priority research projects. The research indicated that it was also very important to harmonize concepts, examples and methods of dealing with HSE, standardize and harmonize HSE systems at all levels in the country, finding possibility to transfer scientific and technical experience of foreign institutions and domestic institutions, as well as facilitating their maximum participation in sustainable development.

***Key words**: health, safety, ecology, GDP, hazard, risk, standardization, harmonization.*

The establishment of the HSE Management System (HSE-MS) in all sectors and national activities is considered an important factor in managing and meeting the health, safety, environmental and sustainability requirements. To date, HSE management system has been recognized as a vital one in any workplace as its implementation concerns economics, sustainable development, community and the environment. The important role of the HSE management system is to eliminate or reduce risks, anomalies, accidents, incidents and their consequences. In this study, we analyzed the current status of the national health, safety and environment management system taking into account vital

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tasks related to its integration, harmonization, and standardization of management means, tolls, and criteria. We should note that environmental protection is considered one of its main components.

In particular, nowadays volumes of products made by petrochemical plants as well as variety of them are growing rapidly. Both these facts are undeniable advantages of this economic activity. At the same time, petrochemical enterprises often face various accidents that occur due to equipment failures and it is often a natural phenomenon in national industries, and in particular in hydrocarbon refineries [1–2]. Workers employed at such enterprises are exposed to many dangers at workplaces and it required thorough inspection and management. Should sources of risk associated with it be neglected or underestimated, it can have an irreversible negative impact on sustainability, the environment, health and safety. One of the most important methods for preventing and / or reducing occupational accidents and their unintended consequences is risk diagnosis and subsequent risk assessment. Risk assessment can be effective in identifying risk sources and reasons (components) and developing an appropriate management strategy. The latter is aimed at reducing or even eliminating impacts exerted by hazardous factors in the areas of health, safety and the environment. In order to achieve targets this system is aimed at, an appropriate technique requires that you can assess the existing risks more accurately, more fully and reliably [3].

The Figure 1 shows a diagram based on Deming's Continual Improvement Cycle: the Health, Safety, and Environment Management Systems are interrelated. This interrelation provides not only a significant minimization of risks for the environment, infrastructure, and enterprise personnel, but also enhances their performance through continuous learning from past experiences as well as rigorous benchmarking of competitors.

In fact, management systems have become the main pillars of any organization and their most vital condition for survival [5]. Risk is defined as a combination of an incident probability and gravity of its potential negative consequences [6]. Risk analysis is a structural process that identifies both the incident's probabilities and the negative consequences imposed by a definite activity [5]. Risk assessment must comprise all phases and activities performed at a workplace and it should be performed before any activities start [7–8].

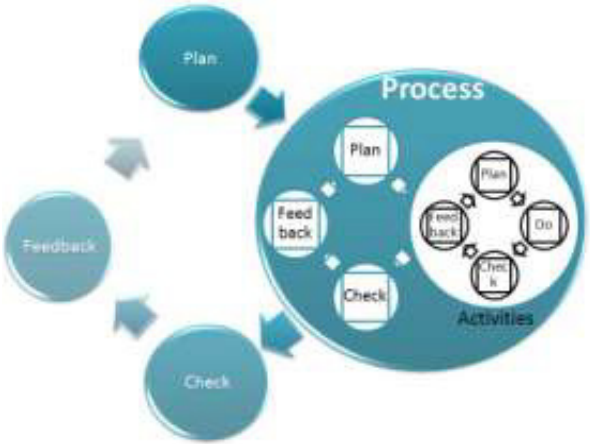

Figure 1. Deming Cycle
(planning – doing – checking – adjusting)
for HSE management system [4]

Data and methods. One of the key elements in HSE systems is risk management. Risk management and evaluation process start with identifying risks and their potential impacts. Risk assessment and management are critical processes here as they help to find relevant strategies for preventing accidents. Effects produced by environmental hazards are of great concern today. In recent decades, policymakers' attention has been paid to possible consequences their plans could have for the environment and risks of disasters.

The next important area in this field is environmental risk assessment, which can be used as an appropriate tool for assessing effects produced by human activities on the environment. Environmental risk assessment is quantitative and qualitative analysis of potential hazards and coefficients (indexes) related to a project taking into account how sensitive or vulnerable a surrounding environment is.

Different steps in the environmental risk assessment process include risk identification and analysis, exposure assessment, risk assessment, and risk management. So far most studies in our country and abroad have focused on safety aspects of projects (albeit they were often inadequately assessed) and less on environmental aspects. Environmental risk assessment in a HSE management system can be used as a complement to environmental impact assessment [9–10].

Environmental risk assessment includes identifying impacts on the environment, temporal modeling, determining a spatial location of emissions and leakage, and evaluating important ecological components. And here it is important to take into account environmental sensitivities and estimate risk levels compared to existing criteria. Qualitative analysis of potential risks and a coefficient of potential risks have many benefits, including a necessity to pay attention to the principles of prevention, environmental capability-based compliance, economic savings, and compensation for damages [9, 11, 12]. It provides a reliable basis for achieving sustainable development, environmental quality assurance and so on. With ever-expanding industries and scientific advances, new dimensions of environmental impacts and various health and safety implications are emerging each year. As a result, large industries are increasingly moving towards implementing integrated health, safety and environmental management systems and striving to achieve the goals fixed in these systems' vision documents. Environmental performance indicators are key ones applied to determine the effectiveness of health, safety and environmental management systems, as well as environmental impact assessments.

It is clear that development would not be possible without planning. Planning, in its turn, should be based on objective facts and natural potentials as it is only in this case that it will be possible to achieve its predetermined goals. If development fails to keep pace with natural resources, it will lead to human errors and loss of resources [13–14]. Therefore, in order to minimize the degradation of natural resources and the adverse effects produced by industrial development on the environment, it is best to utilize modern management systems in order to provide sustainable development. It should become an integral component in organizational culture in institutions, organizations and even training centers, as it will help to improve the performance of organizations. And this improvement should be persistent.

Results and discussion. To achieve the set goals, think tanks were organized and there were regular meetings of three organizations, namely the Ministry of Health and Medical Education, Ministry of Labor and Social Welfare, and the Environmental Protection Agency located at the top three points of the HSE triangle**.** The above mentioned allowed evaluating the current status of the National Health Safety (NHS) and environment management system; as a result, ten priority research projects were introduced (as listed below in Table 1).

Other goals were to harmonize concepts, examples and methods of dealing with HSE, standardize and harmonize HSE systems at all levels of the country, determine whether it was possible to transfer scientific and technical experience of foreign institutions and domestic institutions, and to facilitate their maximum participation in sustainable development.

Priority projects were aimed at solving the following basic tasks:

– enhancing a role of commitment and leadership in achieving efficiency of HSE Management Systems deployed at Ministries and Organizations;

– analyzing efficiency of individual HSE management systems in organizational excellence and development;

– providing improvement in an internal situation regarding safety, health, and environment including reduction of costs as well as risks and consequent growth in Gross Domestic Product;

Table 1

Ten priority research projects in National Health Safety and Environment

Number	Priority research projects	Organizations involved
1	The Role of Commitment and Leadership	All ministries and organizations
2	Efficiency of HSE Management Systems Deployed at Ministries and Organizations	All ministries and organizations
3	Efficiency of individual HSE	Organizational excellence
4	Internal Safety, Health, and Environment	All ministries and organizations
5	Deviations caused by environmental non-prioritization in global HSE standards	All ministries and organizations
6	Bayesian model on HSE risks	Ministry of health and medical education and ministry of co-operation, labor and social welfare
7	Impact of HSE management system in the oil industry	Oil and gas industry
8	HSE management system evaluation indicators	All ministries and organizations
9	Life Cycle Analysis and Measuring Green Productivity	All ministries and organizations
10	Cross-cutting thinking or Nexus thinking	Council for Health and Food Safety

– analyzing LCA (Process Life Cycle) in environmental protection within large national projects in HSE management system;

– assessing deviations from global HSE standards caused by environmental non-prioritization from the perspective of regulatory rules and the role of the media;

– Bayesian model analysis on HSE risks in three main HSE trustee organizations (Ministry of Health and Medical Education and Ministry of Co-operation, Labor and Social Welfare, and the Environmental Protection Agency) applied due to significant interaction between health risks, safety, and the environment;

– investigating influence produced by HSE management system on the oil industry and its direct and indirect impacts on environmental protection;

– developing HSE management system evaluation indicators in the country for standardization, integration, and protection of key components of health, safety and the environment;

– analyzing process life cycle and measuring green productivity in the National Department of Environmental Protection within the framework of examining zinc melting industry;

– promoting and adopting "cross-cutting thinking" or Nexus thinking in National Health, Safety and Environment system.

"Nexus thinking" means considering and understanding water, food and energy and their interactions taking into account their interaction, as they should not be isolated from each other. It is a holistic, strategic thinking style that considers long-term consequences throughout the relationship, balancing social, economic and environmental goals. Nexus thinking allows looking at a bigger picture: considering the whole catchment or river basin, boundary issues, multiple uses (present and future), and cumulative effects. It also includes thinking in agencies that have responsibility for water, food and energy.

One of the tasks this High Council has to perform is to "coordinate executive agencies" and, since health and food security are intertwined with occupational safety and environmental issues [15–21], they can be placed on the agenda of this council (Figure 2).

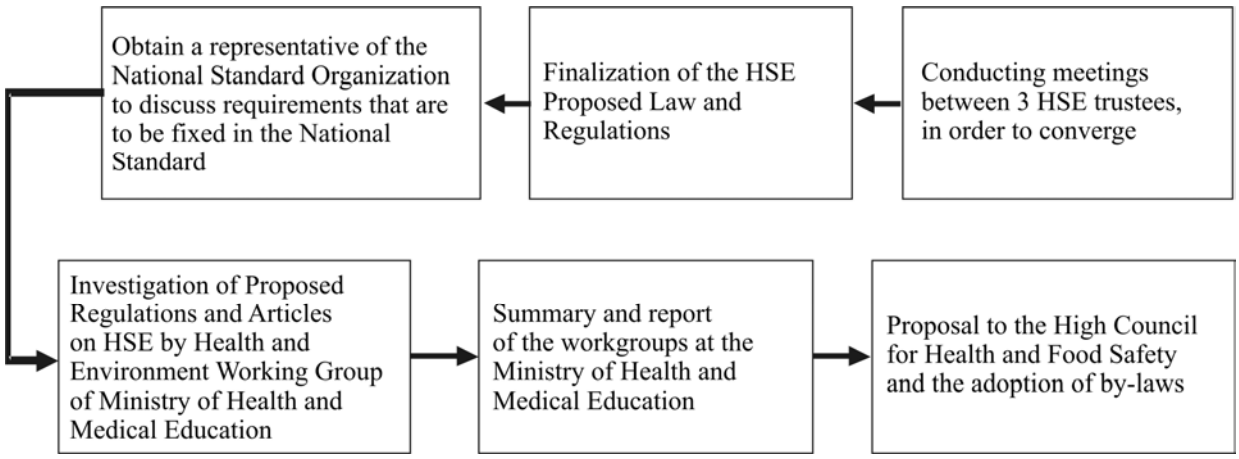

Figure 2. Flowchart procedures for approval by the Supreme Council for Health and Food Safety

Conclusion. As the industry is advancing, related risks will increase with its progress and many adverse effects will occur. If these risks are not controlled, it may result in numerous negative consequences. This study examines the position of risk and risk analysis in various standards and how risk assessment and risk analysis are performed. In industries and organizations accidents are always likely to occur due to risks that are inherent for an industry and lack of managing them. ISO in its global standards for organizations have set out guidelines and standards for risk identification and risk management. By adhering to these standards, we can identify and manage risks in any industry and it can help reduce disasters. Some research suggestions for domestic HSE-system are as follows:

– enhancing organizations knowledge about management of change, asset integration and PFM safety management system. Should there be any changes in an organization, including work processes, materials, structures, equipment, or labor, an organization should have the knowledge how to manage such changes;

– providing organizations with more comprehensive data regarding the above mentioned ISO standards;

– implementing ISO systems in industries and organizations;

– using specific knowledge related to ISO systems implementation;

– conducting training courses on risk management and standardization.

The current status of the National Health Safety (NHS) and environment management system was evaluated, and ten priorities in research projects were introduced. Other goals were to understand how to harmonize concepts, examples and methods of dealing with HSE, standardize and harmonize HSE systems at all levels of the country, determine whether it was possible to transfer scientific and technical experience of foreign institutions and domestic institutions, and to facilitate their maximum participation in sustainable development.

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EFFICIENCY OF HEALTH RISK MITIGATION: COMPLEX ASSESSMENT BASED ON FUZZY SETS THEORY AND APPLIED IN PLANNING ACTIVITIES AIMED AT AMBIENT AIR PROTECTION

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When industrial objects emitting substantial masses of dust and gas mixtures are located within a settlement or close to its borders, it often results in poorer quality of the environment and damages to population health. Such a situation is typical for many cities in the country; primarily, for those that are included into "Pure air" Federal project, a part of the "Ecology" National project. Negative effects are produced by a set of various substances emitted from various industries. And it is quite often that great numbers of people are exposed to such emissions and as a result multiple and variable responses from their health are registered. Assessment of share contributions made by different emissions sources and each particular substance into aggregated negative responses from human health is a fundamental stage in assessing damages to health that occurred due to them; it is significant for working out an action plan aimed at hazardous impacts mitigation.

Given that, we proposed an approach based on fuzzy sets theory as a relevant methodological basis for assessing efficiency of risk mitigation and damage to health when planning and implementing activities aimed at ambient air protection. Application of this methodology allows assessing conditions of multi-component negative impacts producing multiple negative effects including direct damage done to human health. And here key parameters are assessed not as per point values but as per interval ones that are characterized with their belonging to a range of scaled parameters. Our research goal was to suggest methodical approaches to assessing efficiency of risk mitigation and damage to health when planning and implementing activities aimed at ambient air protection; the approaches were based on fuzzy sets theory. Results obtained via hygienic (field or calculated examinations of ambient air quality in settlements under exposure and beyond it) and epidemiologic (controlled medical and biological) research are taken as initial data for fuzzy modeling of multiple parameters ratios within "damage to health – mitigation efficiency" system. Principles applied for research design take into account key postulates of exposure assessment, "dose – effect" relationship for an influencing substance, a concept of exposure risk acceptability, peculiarities related to body reactions under combined aerogenic burdens, and plans for ambient air protection activities (including complex ones).

Comparing a list of substances that do actual damage to exposed population's health with a list of substances included into plans on aggregated emissions reduction allows assessing adequacy; determining to what extent damage to health is mitigated allows assessing whether activities aimed at ambient air protection are sufficient and effective.

Key words: *damage to health, exposed population, ambient air contamination, mitigation, adverse effects, fuzzy sets theory, ambient air protection, adequacy, sufficiency, effectiveness.*

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Issues related to preserving demographic potential of our country as the basis for structural modernization of its economy[1] are truly vital; existing substantial negative impacts on ambient air call for more precise assessments of actual damage to population health caused by violation of obligatory requirements fixed in the sanitary legislation[2].

Industrial objects that emit huge amounts of dust and gas mixtures are often located within settlements or in close proximity to them; it often results in poorer living conditions and damage to population health. Such a situation is typical for many cities in the country, especially for those that are included into "Pure air" Federal project, an integral part of "Ecology" National project.

Several research works have revealed that negative impacts are often exerted by a set (an aggregate) of substances emitted by variable sources that differ in their power. A lot of people frequently have to live in a zone exposed to such emissions and there are multiple and various responses in their health caused by such exposure [1–6]. In such a situation it is crucial to assess a share contribution made by each source and each substance in aggregated negative effects produced on health taking into account hazards related to them. It is a substantial stage in assessing potential health risks and damage to health that is necessary for substantiating efficient programs for ambient air protection and activities aimed at mitigating such risks and damage [7, 8].

When assessing actual damage to health, it is advisable to preliminary assign activities performed by juridical persons and private entrepreneurs into different categories; the focus should be on those economic entities that are located close to settlements and as their activities result in people living in such settlements being exposed to their emissions. This approach makes all the performed risk management activities more focused and targeted[3] [9–11]. A lot of researchers consider assessment of health risks caused by exposure to chemicals that pollute the environment a vital and integral stage in assessing damage to health. Such preliminary assessments allow accomplishing targeted medical and biological studies taking into account expected scientifically proven effects on organs and systems that are critical in terms of specific exposure[4] [12–14].

Overall, a lot of research works are concentrating on searching and testing new approaches to assessing hazardous effects produced by ambient air contamination on health in order to provide more efficient management decision-making. Thus, Kliucininkas L., Velykiene D. (2009) examined abandoned industrial territories which had been previously exploited and suggested a procedure for calculating "emdavector', a complex index that comprised parameters of damage done to health and the environment due to economic activities [15].

Fabisiak et al. (2020) described a model based on risk assessment and clusterization of territories; the model showed how a burden of cardiovascular diseases occurred under exposure to certain environmental factors [16]. The model incorporated regression analysis and assessment of spatially distributed data on morbidity among population, ambient air contamination, and quantitative parameters of ex-

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[1] The concept of the demographic policy in the Russian Federation up to 2025 / approved by the RF President Order on October 9, 2007 No. 1351 with alterations and supplements made on April 14, 2016 No. 669-r. *Garant: portal with reference and legal information*. Available at: https://base.garant.ru/191961/ 53f89421bbdaf741eb2d1ecc4ddb4c33/ (03.03.2020).

[2] On sanitary-epidemiologic welfare of the population: The federal Law issued on March 30, 1999 No. 52-FZ (last edited on July 26, 2019). Clause 57. Civil responsibility for causing damage due to violation of sanitary legislation. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_22481/9fba0cf13c7f6e7ee38079c2317f39d2a09220d0/ (03.03.2020).

[3] MG 5.1.0116-17 Methodical guidelines. Risk-oriented model for control and surveillance activities for providing sanitary-epidemiologic welfare. Classification of economic entities, types of activities and object under surveillance as per potential health risks for organizing scheduled control and surveillance activities / approved by the Federal Service for Surveillance over Consumer Rights Protection and Human Well-being on August 11, 2017, 16 p.

[4] G 2.1.10.1920–04 Guide for assessing health risks under exposure to chemicals that pollute the environment. Moscow, The Federal Center for Sanitary and Epidemiologic Surveillance of the RF Public Healthcare Ministry, 2004, 143 p.

posure available in literature. It was developed for making timely and well-substantiated political decisions. The authors stressed it was important to objectively assess burdens caused by specific contaminants and to determine population groups that were the most prone to risks; they also noted that analysis procedures required further development.

Substantial uncertainties that occur in analyzing and, even more so, in quantitative assessment of relations within the "contamination sources – environmental factors – population health – management" system are well-known to practically all researchers in the sphere [17–19]. A lot of researchers state it is quite a complicated task to assess potential risks and actual damage given multiplicity and uncertainty of influencing factors and negative effects produced by them. Experts also understand that obtained results are not always consistent with an actual situation and they can lead to wrong managerial decisions and to additional inefficient costs [20–25]. Therefore, it is still vital to try and increase adequacy of assessments as an information base for decision-making; to achieve that, we require new approaches and analysis techniques.

Fuzzy sets theory (fuzzy logic) seems to have a capability to become a methodological basis for assessing efficiency of risks and damage mitigation when planning and implementing activities aimed at ambient air protection. The theory in its contemporary interpretation was developed by L. Zadeh [26–28]. Its main advantage, with relation to optimization and identification, is a formalized mathematical apparatus that allows working in uncertainty when there are no available data for applying theoretical-probabilistic techniques. The procedure allows [28]:

– working with fuzzy initial data, for example, values that constantly change over time (dynamic data series) as well as values which can't be set unambiguously;

– applying assessment criteria and comparisons that are not clearly formalized, such as "average", "high", "the greatest", "probable" etc.;

– performing qualitative assessments of initial data and output results and working with not only values but also reliability of data;

– quickly modeling complicated dynamic processes and comparing them with preset precision;

– assessing key parameters not with point values but with interval ones that are characterized with membership function (membership) within a range of scaled parameters.

All the above-mentioned advantages of the procedure sufficiently correspond to complicated tasks related to analyzing existing sanitary-epidemiologic situations in which damage is done to population health under exposure to multi-component ambient air contamination [29, 30].

Given all that, we suggest an approach based on fuzzy sets theory as a relevant methodological base for assessing efficiency of risks and damage mitigation when planning and implementing activities aimed at ambient air protection. Application of the theory will allow assessing multi-component negative influences that produce multiple hazardous effects including damage to health. And key parameters here are assessed not with point values but with interval ones that are characterized with membership function within a range of scaled parameters. There isn't sufficient experience in applying fuzzy sets procedures for solving such tasks. Given that, it seemed appropriate to develop and test scientific-methodical approaches based on fuzzy sets; those approaches enabled assessing efficiency of preventing and eliminating actual damage when economic entities planned and implemented activities aimed at ambient air protection under multiplicity and uncertainty.

Our research goal was to suggest methodical approaches to assessing efficiency of health damage mitigation based on fuzzy sets theory when planning and implementing activities aimed at ambient air protection.

Data and methods. Our research object was a large industrial city with ambient air being heavily contaminated. To assess aerogenic exposure of the city population we took results of field observations over ambient air quality as our initial data. The observations were accomplished by certified laboratories belonging to Rosgidromet and Rospotrebnadzor's Center for Hygiene and Epidemiology.

Instrumental data were supplemented with the results obtained via aggregated calculations of admixtures dispersion[5]. We specified zones in the city via creating a regular grid with its pitch being equal to 200 x 200 m. The regular grid covered the whole examined territory. We calculated ground concentrations at each node in the grid with linear inter- and extrapolation of data taken on the nearest posts for field observations.

We spotted out zones with different exposure as per criteria showing whether quality of ambient air in the city corresponded to the existing hygienic standards (average daily or average annual MPC) and/or reference concentrations (RfC chronic for chronic inhalation exposure)[6]. To perform comparative assessments, we took a territory where chemicals contents in ambient air corresponded to the hygienic standards (or didn't exceed reference concentrations); the zone was conditionally denominated as "a zone beyond exposure". All the zones were comparable as per their socio-economic, natural-geographic, and climatic parameters, as well as quality and availability of medical services rendered to population (types of medical services and procedures for rendering them in accordance with the existing standards for medical aid provision).

We quantitatively assessed aerogenic exposure of population taking into account ground concentrations of admixtures in each square of the regular grid. Exposure was given with average annual daily dose (ADDch, mg/(kg*day) calculated in accordance with the section 6.4.8 of the Guide 2.1.10.1920.04.

To establish whether there was damage done to health of people living in an exposed zone, we selected a representative sampling of people who were to have an individual profound medical examination. There was a comparative plan created for each person from exposed and non-exposed groups according to approaches and criteria given in details in methodical guidelines[7]; we performed diagnostics for each person including chemical and analytical research on biological media in order to determine xenobiotics and/or other technogenic admixtures; there were also general, biochemical, immunologic, and other laboratory examinations, and functional tests; people were examined by a medical expert, and a diagnosis was put in each particular case. We confirmed (or didn't confirm) any relation between detected health disorders and aerogenic exposure basing on the analysis of cause-and-effect relations between exposure markers and markers of negative effects.

The next stage was aimed at determining a number of people with established diseases caused by aerogenic exposure to contaminants and involved creating an information database containing consistent (including a period of observation) data as per a disease category according to a chronic diseases that was revealed in a person for the 1st time. And data on each person were correlated with a chronic average daily dose of each substance.

We analyzed our data array step by step applying fuzzy sets technique; the same approaches were applied to assess efficiency of activities aimed at ambient air protection (target reductions in emissions into the atmosphere from emission sources (tons per year), volumes and sources of funding, etc.) as per a criterion that described mitigation of actual damage to health of exposed population.

Results and discussion. We assessed efficiency of health damage mitigation when

[5] The Order by the RF Ministry of Natural Resources and The Environment issued on June 06, 2017 No. 273 "On Approving on the techniques for calculating dispersion of hazardous substances (contaminants) in ambient air". *Garant: portal with reference and legal information*. Available at: https://www.garant.ru/products/ipo/prime/doc/71642906/ (03.03.2020); The Order by the RF Ministry of Natural Resources and The Environment issued on November 29, 2019 No. 813 "On Approving on the rules for performing aggregated calculations of ambient air contamination including their actualization" (registered in the RF Ministry of Justice on December 24, 2019 No. 56955). *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_341489/ (03.03.2020).

[6] G 2.1.10.1920.04 Guide for assessing health risks under exposure to chemicals that pollute the environment. Moscow, The Federal Center for Sanitary and Epidemiologic Surveillance of the RF Public Healthcare Ministry, 2004, 143 p.

[7] MG 2.1.10.3165-14 The procedure for using results of medical and biological research in order to prove damage done to health due to negative impacts exerted by chemical environmental factors. Available at: https://files.stroyinf.ru/Data2/1/4293766/4293766706 (04.03.2020).

planning ambient air protection and took it this assessment as an example to suggest and test an algorithm that included sequential phases.

The 1st stage involves estimating a total number of people with the same chronic disease that was first diagnosed in them over a period of observation separately as per each category ($\sum N^Z$) and overall as per the whole aggregate of revealed diseases ($\sum N^K$) correlated with chronic average daily dose of each substance that determined both each separate disease as per each revealed disease category (ADV^Z) and the whole range of diseases over a 1-year observation period (ADV_i^K). And here we took into account negative responses not only in accordance with critical organs and systems but also with comorbid states revealed via profound medical examinations.

The 2nd phase involved several steps. First of all, we ranked chronic average daily doses of contaminants under which, over a relevant observation period, experts revealed people with first diagnosed chronic diseases correlated with aerogenic exposure; then we determined a list of contaminants that were potentially hazardous in terms of damage to health. Doses were ranked as per potential hazards of damage to health according to a hazard scale that showed correlations between a chronic average daily dose of each substances (in fractions) and a number of people who could potentially suffer a damage to their health. There should be a permissibility creation for an actual chronic average daily dose of a substance (*PDD,* mg/(kg*day) and a number of people correlated with it who could suffer a damage to their health. It is advisable to apply a limit value of "significant exposure" corresponding to an average daily (annual) dose of a substance under aerogenic exposure calculated from 0.5MPC ($0.5\,MPC_{a.a.} \equiv 0.5\,PDD_{a.a.} \equiv 0.5\,PDD_{a.d.}$). Daily long-term exposure to such a dose is correlated with one additional case of damage to health in a form of diagnosed grave chronic disease per 1 million of exposed people during any lifespan of the present and the following generations (1×10^{-6}) (Table 1).

A substance is considered to be potentially hazardous as per damage to health in case its potential hazard ranks 2 or higher; such substances are recommended to be included into plans for air protection activities.

The 3rd phase involves applying fuzzy sets theory in order to prove there was damage to health of exposed people in a form of additional chronic diseases that were first diagnosed over the analyzed period and were determined by long-term combined exposure to substances. Actual damage is scaled within a value range from 0 to 1. A basic instrument required for implementing the procedure is a membership function $\mu(x)$ of a trapezoidal fuzzy number $x=(a_1,a_2,a_3,a_4)$; overall, the function is given as follows:

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

Table 1

A hazard scale showing potential damage to health under aerogenic exposure to contaminants

Scale parameter	Potential hazard of damage to health: ranks				
	1	2	3	4	5
	Potential hazard of damage to health				
	Negligible	Low	Average	High	Extremely high
Fractions in PDDa.d. (or PDDa.a.) for a year averaging	(0; 0.25]	(0.25; 0,5]	(0,5; 1]	(1; 5],	(5; +∞)
Number of people who can suffer a damage to health correlated with a certain number of population	(0; $1\cdot10^{-8}$]	($1\cdot10^{-8}$; $1\cdot10^{-6}$]	($1\cdot10^{-6}$; $1\cdot10^{-4}$]	($1\cdot10^{-4}$; $1\cdot10^{-3}$],	($1\cdot10^{-3}$; +∞)

Actual damage that corresponds to a revealed disease (disease category) for each person from an exposed group determined by a combined aerogenic exposure to chemicals is taken as a variable *r;* its value corresponds to a variable *x* in the general formula (2) and a range of values *a* that corresponds to a value of the variable *r*. The value of the variable *r* is determined as per complex analysis performed on a system of parameters; these parameters are multiple chronic average daily doses of substances creating aggregated aerogenic exposure that is potentially hazardous as regards damage to health in a form of the whole aggregate of first diagnosed chronic diseases under long-term combined exposure.

To analyze a system of parameters, a chronic average daily dose of each substance included into the list is taken as a variable (b_i) and its quantitative value given as B_i, is taken as a range of values. We should determine a membership of a chronic average daily dose of each substance (the variable b_i) within a certain range of values comprising chronic daily average doses (B_{ik}). A value of membership function (μ_{ki}) for a chronic average daily dose of each substance within a range of values for chronic average daily doses that create aerogenic exposure and related damage to health of a certain number of people is determined as per the formula (2). Ranges of values for chronic average daily doses correspond to ranges on a hazard scale showing potential damage to health caused by aerogenic exposure (Table 1) with their boundaries being "fuzzy" ($\pm 20\,\%$) and probable overlapping between values belonging to neighboring ranges. Actual damage to health is differentiated as per 5 categories (Table 2).

It is obligatory to adjust plans for activities aimed at ambient air protection regarding contaminants that cause damage to health belonging to the 2nd category ("low") and higher in order to provide efficient mitigation of damage to health of exposed population.

To quantitatively assess actual damage to health of exposed people, we gave a rank to a negative response (a disease category from C00 to R99 according to ICD10) (*l*) taking its gravity into account; responses were ranked within a range from 0 to 1. A weight (frequency) of each disease category ranked as per its gravity in an aggregated negative response (P_l) is determined as per Fishburn's rule (3) [27]:

$$P_l = \frac{2(n-l+1)}{(n+1)n}, \qquad (3)$$

where P_l is a weight of a ranked disease category in an aggregated negative response; n is an overall number of disease categories determined in an aggregated negative response caused by aerogenic exposure to all the substances; l is a rank of a negative response (a disease category).

An established weight of each disease category (P_l) applies to each substance that creates exposure and a related aggregated negative response (P_i). A weight of actual damage as per each disease category (an observed weight) is determined according to a rule for transition from values for a weight of a chronic average daily dose of a substance to weights of actual damage to health determined by aerogenic exposure as per the following formula:

$$P_k = \sum_i G_i \cdot \mu_{ki}, k = 1,2,3,4,5, \qquad (4)$$

where P_k is actual damage as per each disease category determined in an aggregated negative response caused by aggregated aerogenic exposure; G_i is a weight of each substance that creates aerogenic exposure and a related aggregated negative response; μ_{ki} is a membership function for a chronic average daily dose of each substance within a relevant range on the scale showing values of chronic average daily doses of substances that do damage to health; k is a category of actual damage to health.

Aggregated actual damage to health (r) is calculated basing on an established weight of actual damage as per each disease category revealed in an aggregated negative response and related to aggregated aerogenic exposure as per the following formula:

Table 2

The scale showing ranges of values for chronic average daily doses of substances that determine damage to health of a certain number of people

A range of values on the scale	A value of membership function for chronic average daily doses of substances that cause damage to health (ADV_i^Z), within ranges of values on the scale, mg/(kg*day)	Actual damage	
		degree (k)	rank (Rg)
$B_{i1} \in [0;0.3\text{PDD}]$	$\mu_1(\text{ADV}_i^z) = \begin{cases} 1, & \text{if } 0 \le \text{ADV}_i^z \le 0.2\text{PDD} \\ \frac{10}{\text{PDD}}(0.2\text{PDD} - \text{ADV}_i^z), & \text{if } 0.2 \le \text{ADV}_i^z \le 0.3 \end{cases}$	Negligible	1
$B_{i2} \in (0.2\text{PDD};0.6\text{PDD}]$	$\mu_2(\text{ADV}_i^Z) = \begin{cases} 1-\frac{10}{\text{PDD}}(0.3\text{PDD}-\text{ADV}_i^Z), & \text{if } 0.2 \le \text{ADV}_i^Z \le 0.3 \\ 1, & \text{if } 0.3 \le \text{ADV}_i^Z \le 0.4 \\ \frac{10}{\text{PDD}}(0.6\text{PDD}-\text{ADV}_i^Z), & \text{if } 0.4 \le \text{ADV}_i^Z \le 0.6 \end{cases}$	Low	2
$B_{i3} \in (0.4\text{PDD};1.2\text{PDD}]$	$\mu_3(\text{ADV}_i^Z) = \begin{cases} 1-\frac{10}{\text{PDD}}(0.67\text{PDD} - \text{ADV}_i^Z), & \text{if } 0.4 \le \text{ADV}_i^Z \le 0.67 \\ 1, & \text{if } 0.67 \le \text{ADV}_i^Z \le 0.94 \\ \frac{10}{\text{PDD}}(1.2\text{PDD} - \text{ADV}_i^Z), & \text{if } 0.94 \le \text{ADV}_i^Z \le 1.2 \end{cases}$	Average	3
$B_{i4} \in (0.8\text{PDD};6\text{PDD}]$	$\mu_4(\text{ADV}_i^Z) = \begin{cases} 1-\frac{10}{\text{PDD}}(2.53\text{PDD} - \text{ADV}_i^Z), & \text{if } 0.8\text{PDD} \le \text{ADV}_i^Z \le 2.53\text{PDD} \\ 1, & \text{if } 2.53\text{PDD} \le \text{ADV}_i^Z \le 4.26\text{PDD} \\ \frac{10}{\text{PDD}}(6\text{PDD} - \text{ADV}_i^Z), & \text{if } 4.26\text{PDD} \le \text{ADV}_i^Z \le 6\text{PDD} \end{cases}$	High	4
$B_{i5} \in (4\text{PDD};+\infty)$	$\mu_5(\text{ADV}_i^Z) = \begin{cases} \frac{10}{\text{PDD}}(6\text{PDD} - \text{ADV}_i^Z), & \text{if } 4\text{PDD} \le \text{ADV}_i^Z \le 6\text{PDD} \\ 1, & \text{if } \text{ADV}_i^Z \ge 6\text{PDD} \end{cases}$	Extremely high	5

Note: PDD stands for permissible daily dose.

$$r = \sum_{k=1}^{5} \overline{r}_k \cdot P_k \qquad (5)$$

where r is aggregated actual damage confirmed by an actual disease (a disease) determined by aerogenic chemical exposure to a set of substances; $\overline{r}_k$ is the middle of each range on the scale showing values of actual damage; P_k is a weight of actual damage as per each disease category determined in an aggregated negative response related to aggregated aerogenic exposure.

The scale showing ranges of values for actual damage (R) is given in Table 3, and is graphically shown in Figure 2.

Table 3

The scale showing ranges of values for actual damage to health

Actual damage (k)	A range of values for actual damage (R)	The middle of a range of values for actual damage
Negligible	$R_1 \in [0;0.25]$	0.125
Low	$R_2 \in (0.15;0.45]$	0.3
Average	$R_3 \in (0.35;0.65]$	0.5
High	$R_4 \in (0.55;0.85]$	0.7
Extremely high	$R_5 \in [0.75;1]$	0.875

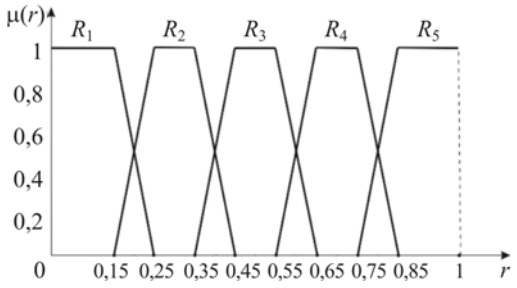

Figure 2. Ranges on the scale showing values of actual damage to health (r)

Actual damage to health (R) is assessed basing on a determined value of membership function for actual damage ($\mu_k(r)$) within ranges on the scale (Table 4).

Damage is considered to be proven provided that an established value of actual damage is within a range on the scale of values estimated as "low" and higher (rank 2 or higher). In order to achieve efficient mitigation of damage to population health in an exposed zone, it is necessary to draw up a list of contaminants that are subject to obligatory regulation as their rank of potential damage to health is 2 or higher.

A contribution made by each substance into actual damage to health is estimated as per the following formula:

$$Q_i = \overline{r_k} \cdot G_i \cdot \mu_{ki} \cdot 100\,\% \qquad (6)$$

where Q_i is a contribution made by each substance into actual damage to health; $\overline{r_k}$ is the middle of each range on the scale showing actual damage; G_i is a weight of each substance creating aerogenic exposure and a related aggregated negative response; μ_{ki} is a membership function for a chronic average daily dose of each substance within a relevant range on the scale showing values of average daily doses of substance that cause damage to health.

Actual damage as a negative effect as per a specific disease (Δr) is determined for an exposed age group against the analogue non-exposed group.

Table 4

The scale showing actual damage to health

A range of values on the scale	Membership function for actual damage within ranges on the scale	Actual damage	
		degree (k)	rank (Rg)
$R_1 \in [0;0.25]$	$\mu_1(r) = \begin{cases} 1, & \text{если } 0 \le r \le 0.15 \\ 10\,(0.25 - r), & \text{если } 0.15 \le r \le 0.25 \end{cases}$	Negligible	1
$R_2 \in (0.15;0.45]$	$\mu_2(r) = \begin{cases} 1-10(0.25-r), & \text{если } 0.15 \le r \le 0.25 \\ 1, & \text{если } 0.25 \le r \le 0.35 \\ 10\,(0.45-r), & \text{если } 0.35 \le r \le 0.45 \end{cases}$	Low	2
$R_3 \in (0.35;0.65]$	$\mu_3(r) = \begin{cases} 1-10(0.45-r), & \text{если } 0.35 \le r \le 0.45 \\ 1, & \text{если } 0.45 \le r \le 0.55 \\ 10\,(0.65-r), & \text{если } 0.55 \le r \le 0.65 \end{cases}$	Average	3
$R_4 \in (0.55;0.85]$	$\mu_4(r) = \begin{cases} 1-10(0.65-r), & \text{если } 0.55 \le r \le 0.65 \\ 1, & \text{если } 0.65 \le r \le 0.75 \\ 10\,(0.85-r), & \text{если } 0.75 \le r \le 0.85 \end{cases}$	High	4
$R_5 \in [0.75;1]$	$\mu_5(r) = \begin{cases} 1-10(0.85-r), & \text{если } 0.75 \le r \le 0.85 \\ 1, & \text{если } 0.85 \le r \le 1 \end{cases}$	Extremely high	5

Note: "если" = if.

Table 5

Criteria for assessing adequacy and sufficiency of air protection activities

Criterion denomination	Assessment criterion	Assessment
Activities are adequate as regards the list of contaminants	The planned list of substances fully coincides with the adjusted list of substances that are potentially hazardous in terms of damage to health and recommended to be included into programs for ambient air protection	Adequate
	The planned list of substances partially (not sufficient or excessive) coincides with the adjusted list of substances that are potentially hazardous in terms of damage to health and recommended to be included into programs for ambient air protection	Partially adequate
	The planned list of substances doesn't coincide at all with the adjusted list of substances that are potentially hazardous in terms of damage to health and recommended to be included into programs for ambient air protection	Not adequate
Activities aimed at achieving a planned reduction in contaminants emissions are sufficient	Hazard of damage to health has reached its target level $(Rg < 2)$ after activities aimed at ambient air protection have been accomplished	Sufficient
	Hazard of damage to health has decreased but hasn't reached its target level $(Rg < 2)$ after activities aimed at ambient air protection have been accomplished (they are not sufficient)	Partially sufficient
	Hazard of damage to health hasn't decreased after activities aimed at ambient air protection have been accomplished	Not sufficient

An adjusted list of substances with their emissions being subject to obligatory regulations is drawn up on the basis of proven actual damage to health (a diagnosed disease) of a certain number of exposed people and an estimated contribution made by each substance into actual damage to health.

The 4th phase involves assessing adequacy of air protection activities aimed at reducing actual emissions of contaminants into ambient air. The assessment is based on comparative analysis of the list of substances included into activity plans and a recommended list of substances that actually contribute into damage to health and are subject to obligatory regulation. Sufficiency of implemented activities is assessed basing on criterion comparative analysis of actual damage prior to and after those activities have been implemented (Table 5).

The list and emission volumes are to be adjusted only for those contaminants included into planned air protection activities for which efficiency of activities is estimated as being "partially adequate" and "not adequate" and/or as "partially sufficient" and "not sufficient".

In order to eliminate potential hazard of damage to health and achieve "sufficient" efficiency of planned ambient air protection, we should calculate a value of a recommended additional reduction in a chronic average daily dose for each substance included into the list of substances that are subject to obligatory regulation. The value is calculated as per the following formula (7):

$$\Delta ADD_{iN}^{K} = \frac{\left(ADV_{iN}^{K} - 0.5PDD\right)}{ADD_{iN}^{K}} \cdot 100\,\%, \quad (7)$$

where ΔADD_{iN}^{K} is an additional fraction of a chronic average daily dose of a substance that is recommended for eliminating potential hazard of damage to population health, %; ADV_{iN}^{K} is a chronic average daily dose of a substance that doesn't create any potential hazards of damage to health (as per any diseases out of the overall detected range) under combined exposure in unfavorable meteorological conditions, mg/ (kg*day).

A necessary volume of emission into the atmosphere that provides an additional reduction in the aerogenic burden is calculated for each substance; such a reduction should be sufficient for mitigation of actual damage. This volume is calculated taking into account a contribution made by each economic entity into aggregated volume of contaminants emissions on the basis of finding a solution to an inverse task or calculations of dispersion.

Efficiency of damage mitigation is estimated on the basis of the re-estimation of actual damage as a negative effect as pre a specific disease category (Δr_N) after activities aimed at ambient air protection have been implemented. Average value of actual damage as a negative effect determined by aerogenic exposure, as per aggregate of all the detected diseases, before $(cp\Delta r)$ and after $(cp\Delta r_N)$ activities aimed at ambient air protection have been implemented, is calculated as per the following formula:

$$cp\Delta r = \frac{\sum_K \Delta r_K}{\mathrm{K}}, \qquad (8)$$

where $cp\Delta r$ is an average value of actual damage as a negative effect determined by aerogenic exposure, as per aggregate of all the detected diseases prior to (or after) activities aimed at ambient air protection have been implemented; Δr_K – is a value of actual damage as a negative effect determined by aerogenic exposure as per aggregate of all the detected diseases prior to (or after) activities aimed at ambient air protection have been implemented; K is the total quantity of all the detected disease categories.

Efficiency of activities aimed at reducing actual damage determined by aerogenic exposure after they have been implemented is estimated as per the following formula (9):

$$Э = \left(\frac{\mathrm{cp}\Delta\mathrm{r} - cp\Delta r_N}{\mathrm{cp}\Delta\mathrm{r}} \right) \cdot 100\,\%, \qquad (9)$$

where Э is efficiency of activities aimed at reducing actual damage determined by aerogenic exposure after they have been implemented (prevented damage), %; $cp\Delta r$ is an average value of actual damage as a negative effect (as per aggregate of all the detected nosologies) determined by aerogenic exposure before activities aimed at ambient air protection have been implemented; $cp\Delta r_N$ is an average value of actual damage as a negative effect (as per aggregate of all the detected diseases) determined by aerogenic exposure after activities aimed at ambient air protection have been implemented.

Efficiency of activities aimed at preventing damage determined by aerogenic exposure after they have been implemented is assessed as per a scale given in Table 6.

Table 6

A scale for assessing efficiency of planned (implemented) activities aimed at ambient air protection as per prevented damage to health

Efficiency (E), %	Degree of efficiency
0–20	unacceptable
20–40	low
40–60	acceptable
60–80	sufficient
80–100	high

Should the efficiency be absent or low, it is necessary to develop (plan) additional activities aimed at ambient air protection; should such activities turn out to be excessive, it is advisable to adjust them on order to make their efficiency “sufficient”.

Suggested methodic approaches were tested on a territory with susbtantial aerogenic burden; the tests included assessing adequacy, sufficiency, and efficiency of a set of activities aimed at ambient air protection and planned to be implemented by major economic entities operating on the given territory.

All the above stated allowed us to come to the following **conclusions:**

– suggested approaches to applying elements of fuzzy sets theory for solving tasks related to assessing whether mitigation of

damage to health is efficient allow assessing adequacy and sufficiency of planned or implemented activities aimed at ambient air protection under existing uncertainty. They can be treated as techniques that supplement and adjust results obtained via other research works within the "environmental factors – population health" system;

– it is vital to apply profound medical research on health disorders in order to prove there has been damage to health caused by combined aerogenic exposure as such research allows obtaining more precise estimations both at an individual level and a group one;

– when we compare a list of substances that actually contribute into damage to health of exposed population with a list of contaminants included into plans for reducing emissions into the atmosphere as per specific substances, it allows us to assess adequacy of ambient air protection; when we determine mitigation of damage to health when such activities are implemented, it helps assessing their efficiency and sufficiency.

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STUDYING THE CONTAMINATION OF TEA AND HERBAL INFUSIONS WITH MYCOTOXINS (MESSAGE 2)

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The authors performed screening of a wide range of mycotoxins by ultra-high-performance liquid chromatography combined with tandem mass spectrometry (UHPLC-MS/MS) in various tea products distributed on the RF market. Samples were selected in retail outlets and obtained from wholesalers. Seventy-seven tea samples were examined: 54 out of them were Camellia sinensis tea, not packed (semi-finished product) and packed; 23 were mono- and multi-component herbal tea. The analytes were 29 mycotoxins including regulated in food products (aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, T-2 toxin and zearalenone), their derivatives and structural analogues (A and B trichothecenes, structural analogues of zearalenone); emergent mycotoxins (sterigmatocystin, mycophenolic acid, moniliformin, enniatins, beauvericin and Alternaria toxins). C. sinensis tea samples, both green and black, were the least contaminated. In contrast, multi-component herbal tea samples tended to be simultaneously contaminated with several mycotoxins (over five) both regulated in food products and emergent ones. Beauvericin, mycophenolic acid and enniatin B were the most frequently detected. Toxigenic properties of mixed tea microflora were examined in vitro. Model experiments were carried out on a substrate consisting of C. sinensis green tea leaves in the absence of any growth factors. Mixed mycoflora from tea, which contained potentially toxigenic species of mold species proved to be capable to simultaneously produce substantial quantities of several mycotoxins including emergent ones. Mycotoxins accumulation amounted to 290 and 5,600 μg/kg of fumonisins B1 and B2 accordingly; 130 μg/kg of zearalenone; 14 μg/kg of sterigmatocystin; 160 μg/kg of alternariol methyl ester. The present survey indicates there is a potential health risk associated with mycotoxins in teas, especially herbal ones. The systematic study of contamination of tea products distributed in the RF with mycotoxins and their producers has been performed for the first time. Long-term monitoring over variety of mycotoxins in this kind of food products is essential for assessing its safety.

Keywords: *mycotoxins, emergent mycotoxins, C. sinensis tea, herbal tea, UHPLC-MS/MS, mycotoxins producers, mycotoxins occurrence in vitro.*

Mycotoxins (MTs) are secondary metabolites of mold fungi. They are globally recognized food contaminants, which affect its safety for the customers. According to the Food and Agricultural Organization (FAO), approximately 25 % of food and feed worldwide is contaminated with MTs [1]. Toxic fungal metabolites produce a wide range of adverse effects on human health, starting from immune suppression and up to carcinogenesis.

Reduction of risks associated with MTs in food is a vital task of health preservation. To solve it, leading world mycologists and toxicologists adopted the Mycotox Charter (chrter.mycokey.eu) calling for responsibility borne by the present generation for developing and implementing solutions aimed at minimizing MTs exposure worldwide and securing enhanced food safety for future generations [2]. Mold fungi are widely spread and are almost

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inevitable in plant commodities. Toxins can be produced both during vegetation and after harvesting, at any stage in a technological chain (storage, processing and transportation). Contents of the most hazardous MTs (deoxynivalenol (DON), T-2 toxin, zearalenone (ZEA), ochratoxin A (OTA), aflatoxin B1 (AFL B1), fumonisins (FBs)) in food are regulated in most countries. The Technical Regulations of the Customs Union CU TR 021/2011 "On food products safety"[1] and CU TR 015/2011 "On the safety of grains"[2] are the principal regulations in the Russian Federation (RF). There are also about forty state standards and guidelines describing analytical procedures based on up-to-date analytical technologies (ELISA (enzyme-linked immune-sorbent assay), HPLC and HPLC-MS) developed for mycotoxins determination in food.

HPLC-MS/MS provides opportunities for selective and sensitive determination of miltiple contaminants simultaneously. It is widely used for monitoring over broad spectrum of fungal metabolites [3–6]. Their list is being constantly enriched. There are analytical procedures validated for the determination of regulated MTs and their structural derivatives (3- and 15-acetyl deoxynivalenol, nivalenol, fusarenone X – DON group (trichothecenes B); HT-2 toxin, T-2 triol, diacetoxyscirpenol – T-2 toxin group (trichothecenes A); aflatoxins B2, G1, G2, sterigmatocystin (STC) – AFL B1 analogues; zeranol, taleranol, α- and β-zearalenol – ZEA analogues), *Alternaria* MTs (alternairol (AOH), its methyl ester (AME), altenuene, tentoxin (TEN)), as well as 'emergent' MTs (EMTs: enniatins A and B (Enn A and B), beauvericin (BEA)) [7–10]. The number of surveys concerning the occurrence of non-regulated MTs in plant commodities and food products is increasing. Meanwhile, the toxic effects produced by these MTs and their combinations are explored insufficiently. Data on combined chronic dietary exposure to small doses of MTs are insufficient. This determines the relevance of extensive research on plant products that belong to mass consumption segment including tea.

Traditional *C. sinensis* tea is a popular drink all over the world; it is usually associated with beneficial health effects. However, subtropics favorable for tea cultivating, are also suitable for mold fungi development and toxins production. Concentrations of FBs, OTA, AFLs, ZEA, trichothecenes and citrinin detected in tea were reported at dozens and even hundreds of μg/kg. Literature data on analytical methods used for the determination of MTs in tea, their occurrence and legislation were summarized in [11]. At present, only AFL B1 or total AFLs in tea are subject to regulation in some countries. Maximum levels (MLs) for these toxins were set in Argentina, India, Sri-Lanka, Japan and China. MLs vary from 5 to 30 μg/kg. Five μg/kg is set as ML for AFL B1 in dry tea at in the CU countries (Russia, Kazakhstan, Belorussia, Armenia, Kirgizia) (CU TR 021/2011[1]).

Herbal teas are becoming increasingly popular. They often contain medicinal herbs (mint, chamomile, nettle, liquorice, wild dog-rose etc.) and are specified by manufacturers as "health-related products". However, there are reports on the detection of MTs and their producers in foodstuff of herbal origin. AFLs, OTA and *Alternaria* toxins were detected in herbal food supplements of similar composition [12].

In previous studies, the authors obtained data on the levels and frequency of mold contamination of more than 50 tea samples (black and green *C. sinensis* tea, herbal tea) [13]. The maximum levels of contamination in all *Camellia* sp. tea samples were due to fungi of the *Aspergillus* genus, among which the *Aspergillus* species of the *Nigri* section dominated. These species were the major fungi found in herbal teas. Alongside *Penicillium sp., Alternaria sp., Fusarium sp.,* and *Cladospo-*

[1] CU TR 021/2011. On food products 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://docs.cntd.ru/document/902320560 (20.11.2019).

[2] CU TR 015/2011.On safety of grains (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: http://docs.cntd.ru/document/902320395 (20.11.2019).

rium sp. were also detected. Certain species from this group are able to produce toxins. Thus MTs synthesized by those mold fungi can be found in herbal infusions. An issue related to microbiological regulations concerning mold fungi established for tea and the possibility of changing them in order to harmonize national standards with international requirements is being discussed in the RF at present. So far, there has been no full-scale study on the occurrence of wide range of MTs in *C. sinensis* and herbal tea distributed on the RF market. The present research is aimed at filling this gap.

Our research goals were (1) to screen *C. sinensis* and herbal tea for a wide range of MTs: regulated (AFLB1, B2, G1, G2; OTA, DON, FB1, FB2, T-2, ZEA), their derivatives and structural analogues (DAS, T-2 triol, NeoS, HT-2 toxin; 3- and 15-AcDON, NIV, FusX; α–, β–ZEL,α–, β–ZAL), and emergent MTs (STC, MPA, MO, EnnA and B, BEA, AOH и AME, TEN). (2) To reveal possible correlations between MT contents and fungal contamination of studied tea samples (3) we also examined the toxigenic properties of tea microflora *in vitro* under conditions as close to real as it was only possible.

Samples: We examined 77 tea samples. 30 samples were semi-finished (not packed) traditional black and green teas (*C. sinensis)* from six tea-producing regions (Vietnam, India, Indonesia, Kenya, China and Sri-Lanka). They were obtained from wholesalers. 22 samples were traditional packed green and black teas (*C. sinensis*) and teas with additives. Two samples were Pu'er tea. 23 samples were herbal tea: monocomponent ones: Sudan rose *(Hibiscus sabdariffa),* fermented Ivan-tea (*Epilobium angustifolium*), thyme *(Thymus serpyllum),* mint *(Menthae piperita)* and chamomile *(Chamomilae vulgaris)* and multi-component ones. The composition of the latter is presented in Table 1.

Methods: *29 MTs were detected* by UHPLC-MS/MS consisting of UHPLC Vanquish system (equipped with a binary pump, autosampler, and thermostat) combined to triple quadrupole mass-spectrometer with heated electrospray source TSQ Endura conducted by Xcalibur 4.0 QF2 Software (all Thermo Scientific, the USA).

Table 1

Composition of multi-component herbal tea samples

Sample No.	Components
2	Ivan-tea (*E. angustifolium*), currant (*Ríbes sp.*)
8	Hawthorn berries (*Crataegus sp.),* white mistletoe leaves *(Víscum álbum*), melilot (*Melilótus officinális*), motherwort (*Leonurus cardiaca*), valerian root (*Valeriana officinalis*)
9	Echinacea **(***Echinácea purpúrea*), origanum (*Oríganum vulgáre*), brandy mint (*Méntha piperíta*), nettle (*Urtíca sp.*), thyme *(Thymus serpyllum),* chamomile *(Chamomilae vulgaris),* wild rose (*Rōsa sp.*), sage (*Sālvia officinālis*), violet (*Víola sp.*), licorice (*Glycyrrhíza sp.*)
10	Thyme *(Thymus serpyllum),* St. John's wort (*Hypericum perforatum*)
11	Chamomile *(C.vulgaris),* brandy mint (*M.piperíta*), everlasting leaves (*Helichrysi arenarii*), tansy flowers (*Tanacetum sp.*), coriander berries (*Coriándrum sátivum*), holy-thistle (*Sílybum mariánum*), mint (*Méntha sp.),* melilot (*M.officinális*), wild rose berries (*Rōsa sp.*), inula root (*Inula sp.*), hawthorn berries (*Crataegus sp.)*
12	Marigold (*Bídens tripartíta*), sarcarolla (*Caléndula sp.*), everlasting (*Agrimónia eupatória*), chamomile *(C.vulgaris),* St. John's wort (*H.perforatum*), birch leaves (*Betula pendula*)
14	Ivan-tea (*E. angustifolium*), cowberry (*Vaccínium vítis-idaéa*)
19	Ground lemon (*Cymbopogon citratus*), lemon myrtle (*Backhousia citriodora)*, ginger root (*Zīngiber officināle),* licorice root (*G.glábra*), dried lemon peel (*Cítrus límon)*
20	Chamomile *(C.vulgaris),* mint (*Méntha sp.)*
36	Ivan-tea (*E. angustifolium*), sea-buckthorn (*Hippóphaë sp.)*
38	Ivan-tea (*E. angustifolium*), linden (*Tília sp.*)

Analytes were separated on a reversed-phase column Titan C18, 2.1*100 mm, 1.9 μm (Supelco, PA, USA), under gradient elution. The mobile phases were constituted of: (A) methanol-water (10/90 % vol.); (B) methanol-water-acetonitrile (10/10/80 % vol.). Both

phases were modified with 1mM ammonium formate and 0.1 % formic acid. The gradient scheme was as follows: from the start to 1 min. - 0 % B; from 1 to 2 min. - a linear growth to 15 % B; from 2 to 5 min. - to 30 % B; from 5 to 13 min.- up to 70 % B; from 13 to 14 min. - 90 % B; from 14 to 16.5 min. - 95 % B; up to 17 min. - growth to 100 % B and then retention for 3 minutes; from 20 to 20.5 min. – a decrease down to 0 % B; equilibration – until 22 minutes. Mobile phase flow: 0.4 ml/min. The column temperature was set to 30 ºC. Injection volume: 2–4 µl. Run time: 22 minutes. Analytes were detected in the MRM mode. Limit of quantification (LOQ) was determined by 10σ criterion. Recovery was determined for spiked green tea. Summarized method parameters are provided in Table 2.

Neat standards of AFL B1, AFL B2, AFL G1, AFL G2, STC, T-2 and NT-2 toxins, DAS, NIV, DON, 3- and 15-AcDON, FusX, FB1, FB2, ZEA, α- and β-ZEL, α-ZAL, OTA were supplied by Sigma-Aldrich (Russia, Moscow). AOH, AME, BEA, EnnA, EnnB, MPA, MO, NeoS, T-2 triol, TE were obtained from Fermentek (Jerusalem, Israel). Stock standards solutions were prepared in acetonitrile (AFLs, STC, trichothecenes, ZEA and its analogues, OTA, MPA), methanol (*Alternaria* MTs, Enns, BEA, MPA, MO) or water-acetonitrile

Table 2

HPLC-MS/MS method characteristics summary

No.	MT	t_R, min	Parent ion, m/z		RF, V	Daughter ions, m/z (collision energy, V)	LOQ, µg/kg	Recovery, %
1	MO	0.7	$[M-H]^-$	97	54	**41* (12),** 80 (23)	800	76
2	NIV	1.8	$[M+H]^+$	313	100	**125 (10),** 177 (10)	1,000	81
3	DON	2.9	$[M+H]^+$	297	100	**203 (18),** 231 (12), 249 (11)	1,250	63
4	FusX	3.6	$[M+H]^+$	355	103	**175 (20),** 229 (16), 247 (12)	100	80
5	NeoS	3.9	$[M+NH_4]^+$	400	79	**185 (17),** 203 (17), 215 (18), 305 (10)	<10	75
6	AcDON	4.6	$[M+H]^+$	339	97	**203 (17),** 213 (18), 231 (13)	250	94
7	T-2 triol	4.8	$[M+NH_4]^+$	400	76	**215 (10),** 263 (13)	250	75
8	AFLG2	6.2	$[M+H]^+$	331	170	**189 (41),** 245 (30), 313 (24)	4	79
9	AFLG1	6.6	$[M+H]^+$	329	150	**200 (41),** 243 (26), 311 (21)	4	85
10	AFLB2	6.8	$[M+H]^+$	315	170	**243 (39),** 259 (29), 287 (26)	4	85
11	DAS	6.9	$[M+NH_4]^+$	384	89	**247 (14),** 307 (10), 349 (10)	20	95
12	AFLB1	7.3	$[M+H]^+$	313	166	**213 (45),** 241 (37), 285 (22)	4	85
13	AOH	8.0	$[M+H]^+$	259	100	**128 (44),** 184 (30), 213 (27)	1,000	87
14	HT-2	8.0	$[M+NH_4]^+$	442	91	**215 (10),** 263 (10)	500	77
15	FB1	8.0	$[M+H]^+$	772	217	334 (40), **352 (36)**	400	44
16	α-ZAL	8.3	$[M+H]^+$	323	66	**189 (22),** 305 (10)	125	76
17	TE	8.3	$[M+H]^+$	415	130	**302 (13),** 312 (19)	4	78
18	β-ZEL	8.5	$[M+H]^+$	321	88	**189 (20),** 303 (10)	1,000	88
19	MPA	8.9	$[M+H]^+$	321	113	**207 (22),** 275 (16)	50	101
20	α-ZEL	9.5	$[M+H]^+$	321	65	**189 (22),** 303 (11)	1,000	84
21	T-2	9.7	$[M+NH_4]^+$	484	137	**215 (17),** 305 (13)	10	100
22	FB2	9.7	$[M+H]^+$	706	150	**318 (36),** 336 (36)	100	73
23	OTA	10.4	$[M+H]^+$	404	123	**221 (35),** 239 (24), 358 (14)	2.5	78
24	AME	10.5	$[M+H]^+$	273	150	**185 (40),** 199 (40), 258 (30)	750	87
25	ZEA	10.5	$[M+H]^+$	319	90	185 (20), **283 (10),**301 (10)	150	86
26	STC	10.9	$[M+H]^+$	325	152	**253 (44),** 281 (36), 310 (24)	4	78
27	EnnB	15.1	$[M+NH_4]^+$	657	142	196 (30), 214 (31), 527 (27), **640 (17)**	2.5	73
28	BEA	15.5	$[M+NH_4]^+$	801	215	**244 (32),** 262 (30), 784 (17)	2.5	80
29	EnnA	16.2	$[M+NH_4]^+$	699	255	**210 (24),** 228 (24)	6	92

Note: daughter ions selected for quantitative analysis are given **in bold**. Method was verified for *C. sinensis* black tea.

(50/50 % vol.) (FB1 and FB2). Stock individual standard solutions were used for the construction of the multi-analyte standard solution. All the standards solutions were stored at -18 °C.

Dry tea sample preparation. A representative portion (10–20 g) of dried tea sample was ground. 1 g of tea powder was extracted with 10 ml acetonitrile-water-formic acid (80/20/0.5 % vol.) by shaking and ultra sonicating for 30 minutes total; centrifuged for 10 minutes at 10,000 rpm. 1 ml of supernatant was diluted with 1 ml of the mobile phase A. After mixing, the diluted sample was centrifuged. 1.5 ml of supernatant was transferred into a chromatographic vial for analysis.

Screening toxins production in vitro. The nutrient medium constituted of agar with streptomycin (200 mg/l) and 6 % of ground dried tea as an only substrate. We used microbiologically clean green tea (<10 CFU/g of mold fungi and bacteria), tested negative for MTs. The substrate was ground in a mill with sterile disposable grinding chambers and then aseptically added to melted hungry agar at 40±1 ºC. Washouts of dried tea samples (10 g of tea in 90 ml of a sterile phosphate buffer) were used as inoculates. 1 ml of inoculates was placed into Petri dishes and imbedded with a nutrient medium. Cultivation lasted for ten days at 24 ºC in the dark. Next Petri dish contents (substrate mycelium) were thoroughly homogenized. 1 g of substrate mycelium obtained via this procedure was used for the extraction of mycotoxins.

Preparation of substrate mycelium samples for MTs analysis. 1 g of substrate mycelium was thoroughly mixed with 5 ml of water-acetonitrile-formic acid (refer to 'Dry tea sample preparation'). MTs were extracted in an ultrasound bath for 30 minutes and then centrifuged for 10 minutes at 4,000 rpm. After that 1 ml of supernatant was diluted with 1 ml of the mobile phase A, properly mixed and centrifuged for 10 minutes at 10,000 rpm. 1.5 ml of supernatant was transferred into a chromatographic vial for analysis.

Results results. Screening of mycotoxins in tea samples.

Screening of mycotoxins in tea samples. We examined MTs in samples of traditional unpacked loose *C. sinensis* tea from six tea-producing regions: China, India, Indonesia, Sri-Lanka, Vietnam, and Kenya. All but four samples from China were black teas. Eight of 29 analytes were detected, almost all below LOQ: AFLs group (AFL G2 and STC); trichothecenes (FusX, NeoS, T-2); *Fusarium* toxins (EnnB and BEA); MPA - a widely spread plant products contaminant (Table 3). 20 of 30 samples (66.7 %) were MTs positive. BEA was found in 18 samples out of 30 (60 %), occurrence of other MTs was much lower. Black tea samples from Vietnam and Indonesia were the most contaminated with MTs traces; then, in the descending order, tea samples from India, China, Kenya, and Sri-Lanka. Only one green tea sample from China was found to be positive for MTs. We did not detect any samples contaminated with regulated in tea AFL B1.

The results correlated with mycological studies of these samples [13]. Samples from Sri Lanka, Kenya and China characterized by low levels of mold contamination (<10^3 CFU/g) revealed low diversity of detected MTs. On the contrary, the higher diversity of detected MTs was noted for samples from Indonesia and India containing higher levels of mold ((1.5–2.3) 10^3 CFU/g). Samples from Vietnam were the exception: they proved to be microbiologically pure (<10^2 CFU/g), while number of detected MTs was comparable with that found for tea samples from Indonesia. The direct dependence of the content of MT and their producers was more characteristic of fresh samples. As storage proceeds, the viable forms of mold gradually die out. They are not detected within mycological analyses, while the MTs synthesized by them remain in tea.

Screening of MTs in samples of packed (loose and bagged) green and black *C. sinensis* tea and tea with additives revealed the occurrence of 12 MTs. Emergent MTs were detected in 20 of 24 (83 %) examined samples. BEA and MPA were the most frequent ones; they were detected in 13 out of 24 samples (56 %). AcDON and FusX were revealed in six and five samples correspondingly. NeoS, STC, Enn B, AME and TE were sporadic (Table 4). Contamination levels of BEA, MPA and TE in black and Pu'er tea were comparable with quantities occurring in other plant

Table 3

MTs in unpacked loose *C. sinensis* tea samples from six tea-producing regions

Mycotoxin	Vietnam, *n* = 5					Indonesia, *n* = 5					India, *n* = 5					China, *n* = 5					Sri-Lanka, *n* = 5					Kenya, *n* = 5				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2*	3*	4*	5*	1	2	3	4	5	1	2	3	4	5
MTs regulated in other food products																														
T-2	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Structure analogues of regulated MTs and emergent MTs:																														
AFL G2	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
STC	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FusX	–	–	–	+	+	–	–	–	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
NeoS	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MPA	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	200	+	–	–	–	–	–	–	–	–	–	–	–	–	–
EnnB	–	–	+	+	–	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BEA	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	–	+	+	–	–	–	+	–	–	–	–
Absolute occur-rence of MTs, cases	12					11					8					5					2					2				

Note: * – green tea samples are marked with;

"+" – MTs concentration is over LOD, but below LOQ;

"–" – MTs concentration is below LOD. The concentration of MPA in sample No1 from China is equal to 200 μg/kg.

Table 4

MTs in packed (loose and bagged) *C. sinensis* tea samples

Mycotoxins	Packed tea samples (*n* – number of samples)					
	Green loose, *n* = 1	Green bagged, |*n* = 3	Black loose, *n* = 10	Black bagged, *n* = 6	Pu'er, *n* = 2	Black with thyme, bagged, |*n* = 2
	Number of positive (>LOD) samples					
Total positive	1	3	7	6	2	1
MTs regulated in food products						
AFL B1	–	–	1	–	–	–
T-2	1	–	–	1	–	–
Structure analogues of regulated MTs						
AFL G2	–	–	–	1	–	
STC	–	–	1	–	–	1 (4.4 μg/kg)*
AcDON	–	2	3	–	1	–
FusX	–	–	2	3	–	–
NeoS	–	–	2	–	–	–
Emergent MTs						
MPA	–	3	4	3 (≤200 μg/kg)	2 (≤3040 μg/kg)	1
BEA	–	2	4 (≤6.0 μg/kg)	5	1	1
EnnB	–	–	–	–	–	1
AME	–	1	–	–	–	–
TE	–	–	–	1 (10)	–	–

Note: "–" – MTs were not detected (below LOD); * – concentration over LOQ is specified in brackets.

commodities [7, 14]. Traces of AFLs were detected in two samples of black tea. STC was detected in two black tea samples (loose tea and bagged black tea with thyme). Its concentration in the latter sample was 4.4 μg/kg. It should be noted, that STC is a biogenic precursor of hazardous AFL B1, and IARC lists it as a potential carcinogen [15].

Analysis of Pu'er tea samples did not reveal the expected variety of MTs; however, we should note that there were not enough analyzed samples to consider the obtained data applicable to the whole group of such products. Traditionally Pu'er is produced via extended fermentation of green tea that can last over 10 years; during this period, an initial microbiome is transformed, and it can create favorable conditions for accumulation of such MTs as patulin [16], AFL, DON [17], and OTA [18]. Hence it is not a coincidence that the first Pu'er brewing is not recommended for consumption. However, a traditional procedure is replaced with pile fermentation at present. It is less time consuming and lasts about 48 days [18]. Within the present study, one, about 3,040 μg/kg of MPA, was detected in one sample of Pu'er tea. MPA is produced by *Penicillium* spp. [19] and can accumulate in a product after harvesting in case storage conditions are improper [20]. Safety requirements for tea subjected to long fermentation, like Pu'er, are not defined clearly to-date. The microbiological standards recommended for teas by Tea & Herbal Infusions Europe (THIE, 2018) do not cover Pu'er [21].

Thus, according to the microbiological survey reported in [13] and screening of MTs, all studied *C. sinensis* tea samples, including those with additives, meet the established MLs concerning fungal and MTs contamination.

Twelve samples of mono-component and 11 samples of and multi-component herbal tea samples were studied. Eight out of 12 (66.6 %) mono-component herbal teas (thyme, mint, Ivan-tea, and hibiscus tea) proved to be positive for ten out of 29 examined MTs (Table 5). Emergent MTs prevailed. The highest occurrence was noted for BEA and MPA; then, in descending order: EnnB and TE, AME, Enn A, DAS, FuzX and STC. As for MTs regulated in food products, two Ivan-tea samples were positive for DON (<LOQ). Thyme and Ivan-tea turned out to be the most contaminated. Thyme samples contained nine MTs that are not subjected to control. It was the highest diversity in mono-component herbal tea samples. Moreover, five of them were in quantities exceeding LOQs. Supposedly, thyme was the source of STC detected in bagged black tea with the addition of this herb (Table 4).

Table 5

MTs in mono-component herbal tea samples

Mycotoxin	Mono-component herbal tea			
	Thyme, *n* = 4	Ivan-tea, *n* = 5	Mint, *n* = 2	Hibiscus, *n* = 1
	Number of MT-positive (>LOD) samples			
Total positive	4	3	1	–
MTs regulated in food products				
DON	–	2	–	–
Structure analogues of regulated MTs				
STC	1 (24 μg/kg) *	–	–	–
FusX	1	–	–	–
DAS	1	–	–	–
Emergent MTs				
MPA	2 (≤100 μg/kg)	2	–	–
BEA	2 (≤4 μg/kg)	1	1	–
EnnA	1	–	–	–
EnnB	2 (≤26 μg/kg)	1	–	–
AME	2	–	1	–
TE	2 (≤13 μg/kg)	–	1	–

Note: "–" – MTs were not detected (below LOD); * – concentration over LOQ is specified in brackets.

Microbiological testing of mono-component herbal tea samples [13] revealed that thyme and mint samples were the most contaminated with mold fungi. Two samples of thyme and two samples of mint herbal tea did not meet existing standards as mold fungi concentration reached 10^5 and 10^6 CFU/g correspondingly. All other samples proved to be safe. Ivan-tea samples provided interesting cases of fungi-bacteria competition. Low fungal contamination (about 50 CFU/g) was coupled with high spore-forming bacteria concentration (up $10^{6–8}$ CFU/g). Some spore-forming bacteria are known to be antagonistic to mold fungi. Their co-occurrence results in mutual competition for the substrate [22]. Thus, MTs found in Ivan-tea samples indicate that fungal contamination preceded bacterial. Therefore, low quantities or even absence of any viable mold fungi detected via mycological analysis do not necessarily mean that samples are MTs-negative. On the other hand, pronounced fungal contamination usually implies MTs occurrence.

The group of multi-component herbal teas included 11 samples (Table 1). They proved to be highly contaminated with MTs: 10 (91 %) out of 11 samples were positive for 18 out of 29 examined analytes (Table 6). Six (54 %) samples contained seven or eight MTs simultaneously. MTs content was considerably higher in these samples than in any other examined in the present study. The most frequently detected emergent MTs were MPA and *Alternaria* metabolite TE. They were found in 7 out of 11 samples; BEA, EnnB and STC were detected in six samples; AME - in five; EnnA - in three; β-ZEL - in two samples. AFL G1, T-2-triol, DAS, ZEA, and FusX were detected sporadically. As opposed to other examined tea products, multi-component herbal tea samples contained a variety of regulated MTs such as AFLB1, OTA (2 μg/kg), DON,

Table 6

MTs in multi-component herbal tea samples

Mycotoxin	Multi-component herbal tea										
	No.14	No.2	No.36	No.38	No.20	No.19	No.8	No.9	No.10	No.11	No.12
MTs regulated in food products											
AFLB1	–	–	–	–	–	+	–	–	–	–	–
OTA	–	–	–	–	–	2.0*	–	–	–	–	–
DON	–	–	–	–	–	+	–	–	–	–	–
T–2	–	–	+	–	–	–	–	–	–	–	9.2
FB2	–	–	–	–	–	–	–	–	100	–	–
ZEA	–	–	–	–	–	–	190	–		–	–
Structure analogues of regulated MTs											
AFLG1	–	–	–	–	–	3.2	–	–	–	–	–
STC	–	–	–	–	–	+	8.0	+	10.0	9.2	9.6
FusX	–	–	–	+	–	–	–	–	–	–	–
T-2 triol	–	–	–	–	–	–	–	–	–	–	+
DAS	–	–	–	–	–	–	–	–	–	+	–
β-ZEL	–	–	–	–	+	–	–	+	–	–	–
Emergent MTs											
MPA	–	–	+	+	+	+	770	690	1760	440	2240
BEA	–	–	–	–	–	20.4	6.0	5.6	5.6	8.0	8.0
EnnA	–	2.8	–	–	–	–	–	+	–	+	–
EnnB	–	–	–	–	–	13.6	22.4	52.0	34.0	55.0	36.0
TE	–	+	–	–	5.6	–	6.0	5.2	5.2	9.2	6.4
AME	–	–	–	–	–	–	+	+	+	+	+

Note: "+" – MTs concentration is over LOD, but below LOQ; "–" – MTs concentration is below LOD. * – concentration exceeding LOQ is in μg/kg.

T-2 (9.2 μg/kg), FB2 (100 μg/kg), ZEA (190 μg/kg). They occurred in quantities close to maximum levels (ML) set by regulations for food products in CU. Thus, five μg/kg of OTA, 200 μg/kg of FBs and 50 μg/kg of T-2 toxin are MLs for these MTs in some cereals for children (CU TR 021/2011). Five μg/kg of OTA is ML for coffee beans set in the EU.

Combined contamination of herbal tea samples with multiple MTs indicates that toxigenic fungi pounced on herbs both during vegetation, processing and storage. "Field fungi", such as *Fusarium* spp. are responsible for DON, T-2, T-2 triol, DAS, ZEN, b-ZEL, EnnA and B, BEA, FBs accumulation. *Alternaria* spp. also invade herbs "in fields" and produce AOH, AME, TE. "Storage" fungi are *Aspergillus* spp. (AFB1, AFG1, STC, OTA) and *Penicillium* spp. (MPA, OTA). This is following the results of the mycological study of these herbal tea samples [13].

Five samples of multi-component herbal teas (No. 8–12) turned out to contain from seven to eight different MTs together with high mold fungi contents (10^4–10^5 CFU/g). Eight MTs were detected in sample No. 19, meanwhile, fungal contamination was low (50 CFU/g). At the same time, the concentration of spore-forming bacteria was high ($8 \cdot 10^5$ CFU/g) in this sample. Similar results were obtained for Ivan-tea samples. They were discussed above in the paragraph, devoted to mono-component herbal tea. Sample No. 19 case also supports the idea that original mold fungal contamination was later suppressed by bacteria development. Therefore, even if a tea sample complies with the microbiological safety requirements fixed in the CU TR 021/2011, this does not mean that they do not contain MTs.

The increase in the number of detected MTs corresponded well with the growing number of components (see Tables 1 and 6). MTs variety in herbal tea was much higher as compared to *C. sinensis* tea samples. This is in accordance with literature data. For example, 12 MTs were discovered in 60 herbal tea samples marketed in Latvia. Co-occurrence of up to eight MTs was noted in 90 % of the samples. EnnB, DON, AFB1, OTA, and ZEA were detected the most frequently [23]. Survey carried out in in Spain revealed 99 % of 84 medical and aroma herbs samples were contaminated. OTA, FBs, AFLs, ZEA, T-2 toxin, DON and citrinin prevailed [24]. A wide variety of MTs was detected in meadow herbs and hay from European Russia regions: 16 MTs were detected including T-2, DAS, DON, ZEA, FBs, AOH, roridine A, AFL B1, STC, cyclopiazonic acid, emodin, OTA, citrinin, MPA, PR-toxin and ergot alkaloids [25].

Table 7 summarizes the occurrence of MTs in the examined *C. sinensis* and herbal tea samples. We detected emergent MTs in all kinds of studied tea. BEA, MPA, EnnB, TE, and FusX were detected the most frequently. We should note that structurally similar BEA and Enns are widely spread and occur in almost all types of plant raw materials and foods. For

Table 7

The occurrence of MTs in *C. sinensis* and herbal tea samples

Tea	MTs-positive samples, %	Quantity of detected MTs	Mycotoxins in decreasing order of occurrence
C. sinensis unpacked (semi-finished)	70	8	BEA>FusX>EnnB> STC>MPA>**T-2**> (AFL G2, NeoS)*
C. sinensis packed	83	12	(BEA, MPA) >AcDON>FusX> (T-2, NEOS) > (EnnB, AME, TE, STC, **AFL B1** and G2)
Mono-component herbal tea	66.7	10	(BEA, MPA) > (EnnB, AME, TE) >**DON**> (EnnA, DAS, FusX, STC)
Multi-component herbal tea	91	18	MPA>TE> (BEA, EnnB, STC) >AME>EnnA> (**T-2**, β-ZEL) >**AFL B1**>AFL G1> (**DON, FB2, OTA**, T-2 triol, DAS, **ZEA,** FusX)

Note: MTs with equal occurrence are put in brackets.

example, BEA was detected in 80 % of tested food, while Enns – in 63 %. A risk assessment carried out by EFSA in 2014 demonstrated that there might be a concern with respect human health effects and chronic dietary exposure to BEA and Enns [9].

Although MTs are detected in low quantities, there is a potential risk that cumulative effects might appear. Toxic impacts that occur due to multiple mycotoxins being consumed simultaneously can become obvious both via additive effects and synergetic ones and in the latter case, overall toxicity can be higher than a simple sum of individual toxicities [26]. Additive effects are described for structurally similar compounds. Results obtained via research on individual and combined toxic effects produced by B-trichothecenes (DON, NIV, 3- and 15-AcDON, DON-3-glucoside and Fus-X) on epithelial cells in a human stomach (GES-1) allowed assuming that their simultaneous occurrence in food products even in low doses can be more or less toxic than a prediction based on data obtained for individual MTs [27]. The same goes for other structural MT analogues from the same species or family when their effects and toxicity profile are similar, for example, FBs or Enns. Synergic effects are described for OTA and AFLs; thus, low mortality due to mycotoxicosis caused by OTA grows considerably when it is combined with AFLs [28]. BEA, DON, and T-2 produce high toxic effects and therefore, their combined exposure can induce certain diseases in people, especially in case exposure is long-term [29]. Overall, in most cases, a combined consumption of MTs results in additive or synergetic effects, and it causes more significant health risks for people and animals [30].

Nominally all the examined tea samples corresponded to hygienic standards. The concentration of AFLB1 did not exceed ML of 5 μg/kg. Still, the co-occurrence of several MTs, especially highly toxic ones, in low doses can cause health risks for people in case of long-term exposure. The obtained data indicate the necessity to assess health risks associated with combined MTs contamination of food, in particular, plant raw materials used in manufacturing specialized food products for babies, dietary products, herbal food supplements, *C. sinensis* teas with additives, herbal tea, spices, etc.

Examination of toxin production by tea microflora *in vitro*. Contamination of traditional *C. sinensis* teas with mold fungi tends to be neglected, by regulatory authorities as well. This is often due to the fact that the risks caused by tea and tea raw materials being contaminated wuth MTs are rather low if production, transportation, and storage conditions correspond to the fixed humidity and temperature [31]. Another opinion is that the lack of growth factors and the content of polyphenolic compounds in tea prevent MTs production even in case there is high mold contamination [32]. Nevertheless, the results of studies of tea samples from various regions confirm that hazardous MTs such as FB, OTA, AFL, T-2, ZEA can be detected in such products in large quantities [11]. For example, 82 % black and green tea samples obtained from retail outlets in Italy were contained OTA, and in 50 % cases its quantity amounted to 7–21 μg/kg (with predominant *A. niger* and *A. tubingensis*). These concentrations exceeded the MLs set for other food products, the consumption volumes of which are comparable to teas, in particular, coffee (5 μg/kg) [33]. Research performed in Switzerland revealed that black mold fungi were one of the most widely-spread ones in 22 samples of herbal teas and isolated strains of *A. niger* and *A. awamori* produced FBs *in vitro* [34]. Several studies have reported the production of toxins *in vitro* on several types of model culture media by certain strains of *Aspergillus* sp. and *Fusarium* sp. which were isolated from plant raw materials including tea made of medicinal herbs [34–36]. The results of these studies show that the types and levels of MTs accumulation by producing fungi in model nutrient media are substrate-specific and do not always reflect toxin production in natural conditions adequately. In nature, different mold fungi compete with each other and exometabolites (MTs) are their weapon used in fighting for a substrate.

We examined a possibility of MTs producing directly in tea substrate *in vitro* under

conditions closest to reality with excess humidity. Green tea was the only substrate, and MTs producers were a consortium of mold fungi that naturally present in certain tea samples. To do that, we selected tea samples with the highest contamination from those previously examined ones; they were multi- and mono-component teas contaminated with mold fungi in quantities equal to 10^3–$7 \cdot 10^4$ CFU/g which was higher than MLs. We made wash-outs out of them (a part of tea and 9 parts of water); wash-outs were used to inoculate an agarized nutrient medium with green *C. sinensis* tea. We used sterile water as an inoculate in a reference sample. Incubation lasted for ten days; then MT were extracted from the substrate and analyzed.

As a result, we revealed that MTs, emergent alongside with regulated ones, which had not been detected in initial dry tea samples, accumulated in extracts from a nutrient medium. Their production was up to: **FB**1 – 294 μg/g; **FB**2 – 4.8–5,694 μg/g; **ZEA** – 128 μg/g; STC – 14.4 μg/g; EnnB – 1.8 μg/g; BEA – 1.36–9.0 μg/g; MPA – 23–303 μg/g; AME – 158 μg/g of a nutrient medium (Table 8).

Obtained results confirmed that toxigenic species of molds from tea samples are capable of accumulating different types of MTs, including emergent ones, simultaneously. It is possible under favourable conditions (humidity - temperature) in a plant substrate with tea leaves as the only nutrient component. This supports the idea that fungal contamination of *C. sinensis* can result in contamination of teas with MTs.

Conclusions. *C. sinensis* and herbal tea samples were screened for 29 MTs. The results revealed that black and green *C.sinensis* tea samples, both bought in retail outlets and obtained from wholesalers, were contaminated with mycotoxins only at low (trace) quantities. A much wider spectrum of MTs, including regulated in other kind of food and emergent,

Table 8

MT production by mold fungi contaminants of selected tea samples *in vitro*

Sample No.	Species of viable mold fungi in initial dry tea samples [13]	Mycotoxins detected in a nutrient medium *in vitro*	
		Content, μg/kg	<LOQ (traces)
2	*Aspergillus sections Nigri, Mucor sp., Fusarium sp., Alternaria sp.*	EnnB-1.8	TE
3	*Aspergillus sp., Penicillium sp., Mucor sp., Fusarium sp., Alternaria sp.*	BEA-9.0	AME
4	*Aspergillus sections Nigri,Mucor sp., Fusarium sp., Alternaria sp.*	**FB1-294**; **FB2-218**; **ZEA-128**	BEA
5	*Aspergillus sections Nigri, Mucor sp., Penicillium sp.,Fusarium sp., Alternaria sp.*	**FB2-952**	STC, **T-2**, BEA, **DAS**
6	*Aspergillus sections Nigri, Mucor sp., Alternaria sp.*	STC-14.4; **FB2**-4.8	β-ZEL, AME
7	*Penicillium sp., Aspergillus sp., Aspergillus sections Nigri, Mucor sp., Epicoccus sp., Fusarium sp., Alternaria sp.*	MPA-23	AFLB1, BEA, **T-2**
8	*Aspergillus sections Nigri, Mucor sp., Fusarium sp., Penicillium sp.,Alternaria sp.*	BEA-1.36	–
9	*Penicillium sp.,Aspergillus sections Nigri, Aspergillus sp., Mucor sp., Fusarium sp. Alternaria sp.*	**FB2-5,624;** MPA-303	EnnA and B, BEA
10	*Penicillium sp.,Aspergillus sections Nigri, Aspergillus sp., Fusarium sp.*	MPA-45	–
11	*Penicillium sp.,Aspergillussp., Aspergillus sections Nigri, Mucor sp.,Fusarium sp. Epicoccussp.,Alternaria sp.*	**AME-158**	BEA
12	*Aspergillus sections Nigri, Aspergillus sp., Penicillium sp.,Mucor sp., Fusarium sp.*	–	**AFLB1**, BEA
Substrate	Not detected	–	–

Note: Substrate: “clean” *C. sinensis* green tea; “–“ – MTs were not detected (<LOD).

was detected in herbal tea samples. Twelve MTs were detected in quantities over LOQs. Neither of 77 samples contained AFLB1 in quantities higher than fixed standards (<5 μg/kg).

Co-occurrence of regulated and emergent MTs in *C. sinensis* and herbal teas can be a potential health hazard under long-term exposure taking into account cumulative effects even at low levels. More representative monitoring and data accumulation is essential for assessing health risks associated with MTs in such products.

Comparison of fungal and mycotoxin contamination in the studied tea samples revealed that low fungal contamination doesn't necessarily mean the absence of mycotoxins. Conversely, an increase in the number and variety of fungal species leads to a greater variety of metabolites.

Toxigenic properties of microflora that occurred in teas were examined *in vitro* in conditions that were as close to real ones as it was only possible; we applied green *C.sinensis* tea leaves as a substrate. Our experiment confirmed the ability of toxigenic mold fungi to accumulate different types of MTs and EMTs simultaneously in significant amounts comparable to MLs established for foods of plant origin (μg/kg): FB1-294 μg/kg; FB2-5,624 μg/kg; ZEN-128 μg/kg; STC-14.4 μg/kg; AME-158 μg/kg.

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Conflict of interests. The authors declare no conflicts of interests.

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Abbreviations: alternariol (AOH); aflatoxins B1, B2, G1 and G2 (AFLB1, B2, G1 and G2); 3- and 15-acetyldeoxynivalenol (3- and 15-AcDON); beauvericin (BEA); deoxynivalenol (DON); diacetoxyscirpenol (DAS); α-zearalanol (zeranol, α-ZAL); β-zearalanol (taleranol, β-ZAL); α- and β-zearalenol (α- and β-ZEL); zearalenone (ZEA); mycophenolic acid (MPA); alternariol methyl ether (AME); moniliformin (MO); neosolaniol (NeoS); nivalenol (NIV); sterigmatocystin (STC); tentoxin (TE); T-2 (T-2 toxin); HT-2 (HT-2 toxin); T-2 triol (T-2 triol); fusarenone X (4-acetyl nivalenol, FusX); fumonisins B1 and B2 (FB1 and FB2); enniatins A and B (Enn A and B).

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ANALYZING NUTRITION RATIONS AT PRE-SCHOOL CHILDREN FACILITIES IN A LARGE INDUSTRIAL CITY IN RUSSIA

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Our research goal was to assess nutrition rations (menus) offered to children at pre-school children facilities after they had raised the costs of their services.

We performed hygienic assessment of cycle menus in order to establish whether they conformed to the existing standards; the assessment was performed in 28 pre-school children facilities in different districts of a large Russian city. All the calculations were made with our own software program called "Menu" that contained a database on a chemical structure of food products and product charts for dishes and culinary products.

Model menus offered to children at pre-school children facilities were able to satisfy their needs in macro-nutrients (by 102–127 %), vitamins (by 102–176 %), and minerals (by 102–162%). However, vegetable fats were not provided in sufficient quantities (20 %). We revealed that there was a deficiency of certain products in a ration and it amounted to 10 % regarding vegetables and wheat flour; more than 20 %, potatoes; more than 30%, sour milk drinks and vegetable oil. We also revealed that there were discrepancies between a stated quantity of a product and its actual provision in a ration or dishes and culinary products were not provided in a quantity stated in an official menu; sometimes the same dishes were offered to children for two days. Greater payments made by parents to a pre-school facility for taking care of their children didn't result in better nutrition provided by such facilities.

Our research results revealed that any sanitary-epidemiologic surveillance aimed at preventing risks of alimentary dependent diseases should concentrate on eliminating deficiency of certain products (sources of essential nutrients such as animal proteins, vegetable fats, food fiber, and vitamins). Optimizing nutrition in pre-school facilities will require certain social and preventive activities.

Key words: *nutrition, pre-school children facilities, menu, chemical structure, a set of products, nutrient charts for dishes, age-related needs, elimination of products deficiency.*

The RF President Order No. 240 issued on May 29, 2017 fixes that the decade from 2018 to 2027 is The Decade for Childhood. The order stipulates activities that should be implemented in this decade and make for the next generation in Russia to be healthy [1]. Providing children and teenagers with healthy nutrition is a vital component in the process.

At the same time, in many regions in the country there have been persistent violations detected at pre-school children facilities (PSCFs) and related to nutrition provided for children who attend such facilities. These violations generally involve deficiency of certain products in daily rations such as sources of full-fledged proteins or, on the contrary, ex-

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cessive consumption of macaroni, cereals, confectionary, and sugar. These violations can probably result from food products being not affordable due to insufficient budgetary allocations provided for pre-school children faculties as inflation processes and growing prices for food products are not taken into accounts when budgets are drawn up [2].

In 2018 municipal authorities fixed new differentiated charges for services provided by pre-school children facilities in a large industrial city in Russia (Perm); to be exact, the charges doubled[1, 2]. Given that, it seems interesting to assess daily menus and establish whether they conform to existing hygienic requirements and are sufficient for providing children with rational nutrition

Our research goal was to assess daily rations (menus) provided for children at pre-school children facilities after charges for their services had been increased in 2018.

Data and methods. We assessed nutrition provided for children at PSCFs using cyclic menus (drawn up for 10–20 days); menus were in free access on PSCFs official web-sites. The first stage in our research involved preliminary assessment of existing nutrition rations in 28 PSCFs located in seven administrative districts in the city; overall, 10,202 children aged 3–7 attended those facilities; 28 examined PSCFs accounted for 20 % of the total municipal PSCFs located in the city.

At the second stage we selected nutrition rations provided for children who attended PCFs and daily spent 10–12 hours there. We applied calculation procedures to examine nutrition rations. Cyclic menus (their nutrient and biological value, to be exact) offered at PSCFs were analyzed as per basic parameters fixed in the existing regulatory documents, SER 2.4.1.3049-13[3] and Methodical Guidelines 2.3.1.2432-08 MR[4]. We calculated product sets, chemical structure and energy value for each ration with "Menu" software package which we had developed ourselves. The package contained a database on chemical structures of food products based on the reference book called "Chemical structure of food products made in Russia" [3] as well as on technological charts and nutrient-grams for dishes and culinary products that took into account losses involved in cooking processes and were created on the basis of "A collection of technological standards, precipices, and culinary products for pre-school children facilities" [4], a manual which was to be obligatory applied at PSCFs.

The third stage involved processing all the obtained data and developing recommendations on adjusting nutrition rations provided for pre-school children. Obtained results were drawn up to form a database with Excel; they were statistically processed with Statistica 6.0 applied software package; we calculated certain parameters used in descriptive statistics.

Results and discussion. Cyclic (draft) menus were approved only by a head of a pre-school children facility at all 28 examined PSCFs as it is not obligatory to submit them for approval to Rospotrebnadzor authorities (SER 2.4.1.3049-13, item 15.3). Children are provided with 4 or 5 meals a day at a PSCF, a breakfast, brunch (not always), lunch, mid-afternoon snack, and dinner; such scheme is optimal when children spend 12 hours a day in a PSCF; each meal conforms to hygienic stan-

[1] On Approval on differentiated charges to be paid by parents (legal representatives) for educational and nursing services provided by municipal pre-school children facilities dealing with childcare and education in Perm for 2015: The Regulation by Perm City Administration dated October 31, 2014 No. 801. *KODEKS: the electronic fund for legal and reference information*. Available at: http://docs.cntd.ru/document/432445827 (20.11.2019).

[2] On Approval on differentiated charges to be paid by parents (legal representatives) for educational and nursing services provided by municipal pre-school children facilities dealing with childcare and education in Perm for 2019: The Regulation by Perm City Administration dated October 25, 2018 No. 829. *KODEKS: the electronic fund for legal and reference information*. Available at: http://docs.cntd.ru/document/550218824 (20.11.2019).

[3] SER 2.4.1.3049-13. Sanitary-epidemiologic requirements to organizing and maintaining proper functioning of pre-school children facilities. *KODEKS: the electronic fund for legal and reference information*. Available at: http://docs.cntd.ru/document/499023522 (20.11.2019).

[4] MR 2.3.1.2432-08. Standards fixed for physiological needs in nutrients and energy for various population groups. Available at: https://rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=4583 (20.11.2019).

dards as per nutrition structure. However, as we assessed a total volume of dishes included into all meals, we revealed that helpings and culinary products were somewhat smaller in their mass in 10 pre-school children faculties (36 % of the examined PSCFs) and it more frequently happened with evening meals.

Dishes included into a ration should be variable to provide children with all the necessary nutrients in maximum quantities. Nevertheless, we revealed that some dishes were repeatedly offered to children during two subsequent days (for example, semolina and wheat cereal, shchi, mashed potatoes, boiled macaroni, omelet, stewed cabbage, cutlets made of fish and poultry) and it violated requirements fixed in SER (item 15.5).

We analyzed nutrient structures of rations and revealed that calorie contents as well as contents of proteins, fats, and carbohydrates corresponded to age-related standards at all the examined PSCFs (Table 1).

Thus, calculated protein quantity in nutrition rations amounted to 65–73 g (median value was equal to 68 g); fats, 66–71 g (median value, 68 g); carbohydrates, 256–276 g (median value, 266 g); energy value, 1,883–2,025 kcal (median value, 1,972 kcal). Macronutrients ratio was close to a recommended standard, 1:1:3.9. Taking into account additional meals consumed by children at home, we can assume that theoretically their meals contain necessary nutrients in more than sufficient quantities.

Contributions made by specific nutrients into overall energy value (12–15 % by proteins; 30–32 %, by fats; 55–58 %, by carbohydrates) of examined menus corresponded to a basic principle of rational nutrition, notably, achieving proper balance between all the components (the 2nd level).

But still, we should note that we detected vegetable fats deficiency (74 % of SPN or not more than 20 % of the overall fats, the standards being not less than 30 %), that is the 3rd level of proper balance was not achieved.

Besides, there was another problem related to vitamin and mineral contents of meals provided for children; although they were sufficient for completely satisfy age-related physiological needs, they were still imbalanced (Table 2).

Table 1

Calorie contents and chemical structure of rations provided for children at pre-school children facilities

Nutrients	Absolute value (n = 28)	SPN*	% of SPN (n = 28)
Calorie contents, kcal	1,972 (1,883; 2,025)	1,800	110 (105; 113)
Proteins, g	68 (65; 73)	54	127 (121; 135)
Animal proteins, g	40 (38; 43)	35	114 (109; 122)
Fats, g	68 (66; 71)	60	114 (110; 118)
Vegetable fats, g	13 (12; 14)	18	74 (68; 78)
Carbohydrates, g	266 (256; 276)	261	102 (98; 106)

Note: SPN* means standard physiological need.

Table 2

Contents of certain vitamins and minerals in rations provided at pre-school children facilities

Nutrients	Absolute value (n = 28)	SPN	% of SPN (n = 28)
Thiamin (B_1), mg	0.9 (0.9; 1.0)	0.9	102 (98; 108)
Riboflavin (B_2), mg	1.5 (1.4; 1.5)	1.0	147 (140; 153)
Vitamin C, mg	64 (58; 79)	50	129 (116; 157)
Vitamin A, μg RE	0.9 (0.4; 1.2)	0.5	176 (87; 237)
Vitamin E, mg	8.7 (8.3; 9.3)	7.0	124 (118; 133)
Calcium, mg	911 (889; 938)	900	101 (99; 104)
Phosphor, mg	1,284 (1,223; 1,318)	800	160 (153; 165)
Magnesium, mg	287 (272; 295)	200	143 (136; 147)
Iron, mg	16 (15; 17)	10	161 (153; 169)

Table 3

A set of products used to make up rations at pre-school children facilities

Products	Absolute value in grams (n = 28)	RC*	% of RC (n = 28)
Wheat bread	85 (77; 97)	80	106 (97; 122)
Rye bread	50 (45; 50)	50	100 (90; 100)
Wheat flour	24 (20; 27)	29	82 (70; 92)
Potato flour	3 (2; 5)	3	100 (73; 153)
Macaroni	12 (11; 13)	12	98 (89; 108)
Cereals and bans	51 (47; 58)	43	118 (109; 135)
Potatoes	189 (177; 207)	234	80 (76; 88)
Vegetables	295 (275; 317)	325	90 (85; 98)
Fresh fruits and berries	120 (107; 135)	114	105 (94; 118)
Dried fruits and berries	15 (11; 19)	11	132 (103; 173)
Fruit, vegetable, and berry juices	100 (93; 100)	100	100 (93; 100)
Meat	65 (58; 75)	60.5	107 (96; 123)
Poultry	32 (27; 38)	27	119 (98; 141)
Sausage	7 (6; 7)	7	102 (81; 103)
Fish fillet	41 (35; 49)	39	106 (89; 125)
Milk	346 (309; 372)	270	128 (114; 138)
Sour milk products	103 (74; 144)	180	69 (49; 96)
Curds	40 (38; 43)	40	101 (95; 108)
Cheese	6 (5; 7)	6,4	95 (77; 103)
Cream	9 (7; 12)	11	80 (67; 113)
Eggs	24 (20; 27)	24	98 (85; 112)
Butter	32 (29; 33)	21	151 (138; 159)
Vegetable oil	7 (6; 8)	11	64 (53; 69)
Confectionary and bakery	21 (19; 23)	20	106 (96; 115)
Sugar	53 (48; 55)	47	112 (103; 117)

Note: RC* means recommended consumption as per SER 2.4.1.3049-13.

A child's body should be provided with calcium and phosphor in proper quantities. Insufficient consumption of calcium causes higher rachitis risks and can aggravate the disease; excessive calcium quantities can result in pathologic calcification of the kidneys and other internal organs. Consequently, when assessing nutrition rations provided for children, we should take into account adhering to proper ratios between calcium and phosphor contents as it is a significant preventive factor. We established that calcium to phosphor ratio was 1: 1.4 in the examined ratios and it didn't conform to hygienic recommendations (1: 0.88). Median value for phosphor quantity in rations was equal to 1,284.0 and it was substantially higher than the recommended one (800 mg).

Table 3 contains data on a daily set of products offered by PSCFs. Cyclic menus offered by PSCFs where children daily spend more than 8 hours should contain all the necessary products according to the requirements fixed in the sanitary legislation. However, our quantitative assessment of food products consumption (as components in cooked dishes and recalculated as per raw products) revealed that there was deficiency of certain products whereas others were present in excessive quantities

Regardless of a city district, children were offered the same products in quantities higher than recommended: cereals, beans (19 % excess), milk (28 %), dried fruits (32 %), butter (51 %), and poultry (19 %). Unnecessary surplus could reach 100 % in some PSCFs. On the contrary, vegetables and potatoes were provided to children in quantities lower than recommended consumption, deficiency reaching from 10 to 20 % accordingly. Vegetables were provided in insufficient quantities in only two PSCFs whereas potatoes were provided in low

quantities in half of all the examined PSCFs. We should also note that most menus contained certain products in rather low quantities such as sour milk products (31 % deficit), sunflower oil (36 %), cream (20 %), and wheat flour (18 %) which were used as ingredients to cook dishes.

Taking into account a consideration that documentary data can differ from actual consumption due to children refusing to eat some dishes and culinary products (it is confirmed by substantial quantities of food wastes accumulated at PSCFs) [5], we can assume that it is allowable to have some products provided in quantities higher than recommended consumption. But on the other hand, excessive quantities of certain products displace other products from rations resulting in their deficit and it undoubtedly violates hygienic requirements.

We should also note that optimal macronutrients contents are provided both due to some products offered to children in quantities within recommended consumption limits and due to above-mentioned products offered in excessive quantities. But quantitative nutrient structure of food can't be adequate as there is deficit of its significant components. Thus, for example, in case flour, potatoes, and vegetables are not provided in proper quantities, carbohydrates can be obtained from excessive quantities of cereals and sugar. When we examined a set of products in order to determine animal proteins sources, we established that sour milk products were not provided in proper quantities; it is rather bad as such products are, among other things, a significant factor that creates microbiocenosis in the intestines. Detected low quantities of vegetable fats result from insufficient use of sunflower oil whereas butter is provided in significantly excessive quantities and it makes overall fat consumption conform to hygienic requirements.

Excess vitamin quantities detected as per calculations should be considered conforming to the standards. Leading experts think that actual macronutrient quantities can be lower due to losses occurring when products and first stored and then processed while being cooked [6]. Moreover, such quantities don't mean it is not necessary to perform additional vitamin fortification of dishes. As it is known from literature, even if rations have vitamins in sufficient quantities, their concentrations are usually low in blood of children who are chronically exposed to adverse environmental factors which is typical for large industrial cities, Perm being one of them [7].

Our research revealed that a draft menu didn't always meet the requirements fixed for food variety. And we should take into account that there are additional factors that prevent assessing actual repeatability, for example, an actual ration can deviate from data stated in a cyclic menu as per a list of offered dishes and culinary products as it was detected via social control procedures performed at PSCHs in the city and aimed at assessing how nutrition was organized[5].

Therefore, despite each PSCF has a draft menu approved by its head and placed on its web-site for free access, we still managed to detect certain violations that contradicted sanitary legislation and principles of rational (healthy) nutrition provided for children.

A probable reason for such a situation can be absence of obligatory procedures requiring all draft menus to be approved or inspected by Rospotrebnadzor bodies.

Another, and the most significant, reason is absence of qualified staff; usually staff who are involved in developing and adjusting draft menus are rather low-skilled. We can't completely exclude a consideration that existing standards for food products consumption might be imperfect at the moment [8].

Given the fact, that nutrition quality is closely connected with economic factors as it was proven in a research work that focused on analyzing nutrition in families [9] another reason for improper draft menus can be insufficient financing.

Another question which we would like to find an answer to in our research is whether an increase in charges for PSCFs services had any positive influence on quality of nutrition provided for children in them. To do that, we

[5] The Report on activities performed by Perm Regional Civic Chamber in 2016. *Perm Regional Civic Chamber*. Available at: http://oppk.permkrai.ru/docs (28.11.2019).

should return to our previous research that was accomplished prior to the increase in charges during a period when they remained steady (2014). Data taken from menus indicated that children were similarly provided with all the necessary nutrients and energy according to their physiological needs, excluding vegetable oils, and they were offered all basic products in quantities equal to 90–118 % of recommended consumption [5]. However, apart from other deficit, present day rations contain significantly lower quantities of sour milk drinks (by 1.7 times, t-test = 2.78, $p<0.05$), curds (2.5 times, t-test = 3.1, $p<0.01$), and macaroni (by 3 times, t-test = 18.8, $p<0.01$). As prices are constantly growing, even higher charges that parents have to pay for PSCFs services can't provide sufficient funding for such organizations and it influences nutrition quality and structures of product sets used to cook dishes.

There are unified requirements to organizing nutrition in pre-school children facilities in Russia; still, authors of various research works prove that products sets do not conform to hygienic standards in various regions in the country and in some cases even macro-and micronutrients contents are imbalanced. Insufficient quantities of vegetables, fruit, fish, milk, and milk products are a prevailing violation in structures of product sets [10–13]. Taking into account persistent violations detected in rations provided for pre-school children at home [14–16] optimization of nutrition at pre-school children facilities should become a top priority. Imbalanced and irrational nutrition provided for children is a pressing issue in other countries [17–20]. The Russian Federation adheres to its decisiveness to achieve stable welfare as regards food consumed by population, including nutrition provided for children, as our country signed the Rome Declaration on Global Food Security in 1992 among 159 world countries.

Conclusions. We analyzed nutrition rations (menus) provided for children who attended pre-school children facilities in a large industrial city in Russia. Our analysis revealed that draft menus offered at PSCFs can satisfy age-related needs in macronutrients (by 102–127 %), vitamins (102–176 %), and minerals (by 102–126 %), but vegetable oils are provided in insufficient quantities (20 % of recommended consumption), and calcium and phosphor consumption is not balanced well. Vegetables and wheat flour were provided in quantities that were by 10 % lower than recommended; potatoes and cream, by 20 %; sour milk drinks and sunflower oil, by 30 %. We also detected situations when dishes and culinary products were provided with their weight being lower than it was stated in a menu; sometimes the same dishes were offered to children during two subsequent days. Consequently, higher charges that parents had to pay for education and childcare provided by pre-school children facilities are not a key factor that can result in improved quality of nutrition at PSCFs. At present, in order to prevent risks of alimentary-dependent diseases, sanitary and epidemiologic surveillance should be oriented at detecting and eliminating any food product deficiency (especially regarding food products that provide people with essential nutrients such as animal proteins, vegetable fats, dietary fibers, and vitamins). It is also necessary to update regulatory and methodical documents that stipulate how to organize nutrition; to develop and implement up-to-date cyclic menus with the help of experts; to train medical personnel employed at PSCFs how to adjust rations; it is also very important to provide state support for priority projects focused on eliminating vitamin and mineral deficiency in nutrition.

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ASSESSMENT OF OCCUPATIONAL HEALTH RISK FOR WORKERS EMPLOYED AT CONTEMPORARY RUBBER PRODUCTION

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The paper dwells on the results obtained in assessing occupational health risk for workers employed at rubber production; the assessment was based on examining both a priori and a posteriori parameters. It was shown that workers were exposed to a set of factors related to working environment and labor process including hazardous chemicals, in-plant noise, adverse microclimate, as well as physical overloads. Chemical factor played the leading role as there were various chemicals in working area air such as benzene in concentrations equal to 1.2–3.0 MPC (maximum single concentration amounted to 4 MPC); dichloromethane in concentrations equal to 0.2–1.5 MPC average monthly (maximum single ones equal to 2 MPC). Overall working conditions assessment for workers with different occupations employed at rubber production belonged to 3.2 hazard category. Workers who glued parts of articles together ran high risks of occupational diseases; risks were average for spreading machine operators and vulcanizers. Total occupational diseases index amounted to 0.83 for workers who glued articles and to 0.80 for spreading machine operators and vulcanizers and it meant that occupational risk was high. We also revealed that certain diseases were occupationally induced and the dependence was significant; it was true for segmental disorders in the vegetative nervous system with sensitivity failure in hands for gluing workers (RR – 10.3, CI = 95 %, EF – 90 %), musculoskeletal system diseases (RR – 2.5, CI = 95 %, EF – 55 %), and skin and subcutaneous tissue diseases for spreading machine operators and vulcanizers (RR – 2.6, CI = 95 %, EF – 61 %). We ranked occupations as per related occupational health risks and revealed that workers who glued articles had the highest integral occupational risk assessment.

Occupational risk assessment gave grounds for developing a risk-oriented program that included priorities and ways to prevent damage to health of workers employed at rubber production such as managerial and medical and prevention activities as well as social support and protection.

***Key words**: occupational risk, health, workers, hazardous occupational factors, working conditions, rubber production, occupational diseases, occupationally induced diseases, prevention.*

Three are certain branches in the economy of the Russian Federation that determine its scientific, technical, and economic development; chemical industry is a leading one among such brunches. Among other productions, it includes rubber and plastic products manufacturing. Chemical productions usually involve emitting multi-component chemical admixtures into working area air, as it is well-known in occupational hygiene, and it creates risks for workers' health [1–4].

Rubber products (module pneumatic constructions or constructions used in rescue work, for example) are usually made of rubber cloths and such manufacturing requires formed materials, engineering constructions, tanks for storing oil, glues, and sealants. Cloth rubberizing is a basic process in rubber production and it involves applying glues that are a solution of rubber mixtures in solvents (petrol, dichloromethane, benzine, etc.).

Workers that glue parts of engineering constructions together (hereinafter called gluers) and spreading machine operators (hereinafter called spreading operators) are two basic

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occupational groups in workshops where rubber cloths and glues are manufactured. Gluers are mostly women; spreading operators, men. Overall assessment of working conditions reveals that working conditions for gluers correspond to 3.1 hazard category (the chemical factor being the leading one); working conditions for spreading operators, 3.3 hazard category (the leading factor is labor hardness).

All technical equipment applied in rubber production is placed in enclosed space. A whole set of hazardous occupational factors influences workers' health; these factors are related to industrial environment and labor processes and are potentially harmful. They occur due to high temperatures in workshops and open processes; use of toxic multi-component rubber mixtures and glues; production equipment that generates a lot of noise. According to official statistics, occupational morbidity among workers employed in chemical industry has been higher than on average in the country over recent years (1.17 cases per 10,000 workers in the RF; 2.16 cases per 10,000 workers in manufacturing); it calls for assessing working conditions and predicting damage to workers' health basing on procedures for occupational risks assessment.

Prevention of occupational and work-related diseases as well as workers' health preservation is a vital task the occupational medicine is to solve [5–9].

Our research goal was to assess occupational risks of health disorders among workers employed at rubber production via examining a priori and a posteriori parameters in order to give grounds for a risk-oriented prevention program.

Data and methods. We analyzed and assessed occupational risks according to Guide 2.2.1766-03[1] using hygienic and medical and biological parameters [10, 11].

Violation of hygienic standards (concentrations exceeding MPC) was assessed in accordance with the Guide 2.2.2006-05[2]. To assess a priori health risks, we took data obtained via the Specific assessment of working conditions (SAWC)[3], industrial control procedures accomplished according to SR 1.1.1058-01[4], and targeted scientific research as well [12]. We assessed occupational risks for specific occupational groups with the same working conditions and used it a basis for assessing group occupational risks.

We examined health of 370 workers employed at rubber production; 210 out of them were female gluers (84.5 %); and the rest were male spreading operators (15.5 %).

Workers were distributed as per their age and working experience length; the results were as follows: 17.7 % had 5–10 years of working experience; 59.6 %, 11–15 years; 22.7 %, 15 years or longer. 46.0 % workers were 39–49 years old; 24.0 %, 30–38 years old; 21.0 %, 50 years and older; 9.0 %, 20–29 years old. Our reference group was made up of 130 inspectors from the quality control department; they were comparable with workers from the examined occupational groups in terms of their age and working experience.

A posteriori risk assessment was performed as per medical and biological parameters, notably occupational and work-related diseases.

We calculated intensive parameters of occupational morbidity for a specific occupational group taking into account a number of first detected occupational diseases per 10,000 workers with the same occupation. Rubber production involves combined exposure to

[1] P 2.2.1766-03. The Guide on assessment of occupational risks for workers' health. Organizational and methodical grounds, principles, and assessment criteria. *KonsultantPlus*. Available at: https://consultant.ru/document/cons_doc_LAW_130907/ (02.10.2018).

[2] P 2.2.2006-05. The Guide on hygienic assessment of factors related to working environment and labor process. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_85537/ (15.02.2018).

[3] On Approval of Procedure for conducting a special assessment of working conditions, Classifier of adverse and (or) hazardous production factors, reporting form on a specific assessment of working conditions and instructions how to fill it in: The Order issued by the RF Ministry for labor and Social Protection on January 24, 2014 No. 33n. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_158398/ (10.04.2018).

[4] SR 1.1.1058-01. How to organize and perform industrial control over adhering to sanitary rules and accomplishing sanitary and anti-epidemic (preventive) activities. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_33872/ (10.04.2018).

hazardous occupational factors; given that, it is necessary to assess not only each occupational disease separately but their combination as well. In such cases, to assess occupational risks, we took an integral parameter for occupational diseases frequency and gravity, a so called occupational diseases index (Iocc) that was a an inverse value of risk category (Cr) and hardness category (Ch); it was an one-number index that combined Cr and Ch parameters as a probable risk level [13, 14].

Besides, we assessed how strong a cause-and-effect relation was for health disorders and occupational factors.

We assessed an extent to what diseases were occupational or work-related depending on relative risk (RR), 95 % confidence interval (CI), and etiological fraction (EF).

Ultimate assessment of occupational risks that could cause health disorders in workers employed at rubber production was performed taking into account hygienic and medical and biological parameters basing on occupational risk categories; those categories took into account classes of working conditions, occupational morbidity, and urgency of activities aimed at reducing risks. Urgency of such activities was determined on the basis of occupational risk categories. Assessment results were communicated to workers, employers, and other concerned parties.

Our research focused on assessing occupational risks for health of workers employed at rubber production and dealing with either materials rubberizing or products assembling.

Results and discussion. We analyzed our research results and revealed that workers employed at contemporary chemical productions were exposed to a set of occupational factors and labor process; it included toxicants (predominantly solvents such as benzine or dichloromethane), intense in-plant noise, adverse microclimate, and physical overloads as well.

Workers from basic occupational groups (gluers, spreading operators, and vulcanizers) were exposed to chemical factors, namely benzine in concentrations equal to 1.2–4.0 MPC and dichloromethane, 0.2–1.5 MPC taken as per average monthly values; maximum single exposure to benzine could reach 4 MPC; dichloromethane, 2 MPC. Both these substances produce acute targeted neuro-, hepato-, and gematotoxic effects as well as irritant ones[2, 5] [15–19].

Besides, maximum permissible noise levels and microclimate parameters (so called (HIS or heat stress index) at workplaces of spreading operators and vulcanizers corresponded to hazard category 3.1–3.2.

Workers with basic occupations had to lift and move weights during their work shifts; they were exposed to local physical loads (weights lifted and moved manually (kg); (single) lifting and moving a weight followed by other work tasks (up to 2 times per hour, hazard category 3.1); body bending (forced posture at an angle exceeding 30º), (times per a shift)), hazard category 3.2); all the above substantiated assessing such working conditions as hazardous (3.1–3.2) according to Guide 2.2.2006-05[2].

Ultimate assessment of working conditions for workers with basic occupations employed at the examined production allowed ranking them as hazard category 3.2 (Table 1).

Exposure to hazardous occupational factors and factors related to labor process caused occupational and work-related diseases.

Chronic intoxications were mostly detected among gluers (30 %) who dealt with making large engineering constructions and were exposed to combined effects produced by a mix of solvents in concentrations which were 2–4 times higher than MPC; they also occurred among spreading operators (7.5 %). Clinical signs of solvent-induced intoxication included disorders in the vegetative nervous system (VNS) as per segmental type with initially occurring vegetative-sensory disorders in hands.

[5] HS 2.2.5.3532-18 Maximum permissible concentrations (MPC) of hazardous substances in working area air. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_296440/ (24.01.2018).

Table 1

Occupational factors and occupational risks occurring at rubber production

Occupational factor		Spreading operator	Vulcanizer	Gluer
Chemical, benzine, higher than MPC	C_{sm}	3.6	2.6	4,0
	$C_{av.s.}$	1.6	1.2	1,2–3,0
Dichloromethane, $C_{av.s.}$		0,2	0.4	1.2–1.5
$C_{s.m.}$		0,3	0.5	2
Working conditions category		3.1	3.1	3.2
Noise, Higher than MPL, dB		By 5	By 5	Not detected
Working conditions category		3.1	3.1	2.0
Microclimate (increased HSI, °C)		By 0.4	By 1.2	Not detected
Working conditions category		3.1	3.2	2.0
Labor process hardness		3.2	3.2	3.1
Overall working conditions assessment		3.2.	3.2.	3.2.
Risk category		Average	Average	Average

Note:
Cav.s. means average shift concentration;
Cms maximum single concentration.

Occupational diseases caused by physical overloads occurred as a rule under working conditions belonging to hazard category 3.2 as per labor process hardness. Occupational diseases with predominant damage to the vegetative nervous system and musculoskeletal system were diagnosed in 28.6 % cases; they were hand polyneuropathy, facioscapulohumeral periathrosis, epicondylitis, and radiculopathy. Occupational sensorineural hearing loss was diagnosed in 2.2 % cases.

Occupational risk parameters, depending on risk category and occupational diseases gravity, indicate that gluers run high risks of occupational diseases; spreading operators and vulcanizers, average risks. Overall occupational diseases index that took into account each occupational disease amounted to 0.83 for gluers and to 0.8 for spreading operators and vulcanizers and it allowed ranking occupational risks as high (Table 2). We detected that occupational diseases frequency and gravity depended on working conditions category.

Table 2

Occupational risk categories for workers employed at rubber production depending on occupational morbidity index (OMI)

Occupation	Nosology	C_r*	D_g*	OM index*	Risk category
Spreading operator, vulcanizer	facioscapulohumeral periathrosis without functional disorders	2	3	0.16	average
	radiculopathy without functional disorders	2	3	0.16	average
	initial epicondylitis	2	3	0.16	average
	hand polyneuropathy	2	3	0.16	average
	sensorineural hearing loss	2	3	0.16	average
Overall OM index				**0.80**	
Gluer	mild chronic intoxication with a mix of organic solvents (benzene, dichloromethane)	1	2	0.52	extremely high
	hand polyneuropathy	1	3	0.33	high
Overall OM index				**0.85**	

Note:
* – C_r and D_g mean risk category and (occupational) disease gravity;
OMI means occupational morbidity index.

Chronic non-infectious diseases that were the most frequently diagnosed in workers employed at rubber production included disorders in the vegetative nervous system as per segmental type; musculoskeletal system diseases; skin and subcutaneous tissue inflammations occurring due to skin micro-injuries; as well as biliary dyskinesia, and primary hypertension.

Having assessed an extent to which the diagnosed diseases were occupationally induced, we revealed that it was high for disorders in the vegetative nervous system as per segmental type with sensory disorders in hands among gluers (RR–10.3, 95 % CI, EF–90 %); musculoskeletal system diseases (lumbodynia) (RR –2.5, 95 % CI, EF–55 %), and skin and subcutaneous tissue diseases among spreading operators and vulcanizers (RR –2.6, 95 % CI, EF– 61 %) (Table 3).

Complex clinical and hygienic research gave grounds for ranking occupations as per occupational risks of health disorders taking into account for basic criteria: working conditions; occupational morbidity; a share of people who had signs of occupational disorders; an extent to which chronic non-communicable diseases were occupationally induced.

The highest integral assessment of occupational health risks was determined for gluers. Results obtained in assessing health of workers employed at rubber production as per medical and biological parameters were adjusted with overall assessment of working conditions (Table 4).

Table 3

An extent to which diseases occurring in workers employed at rubber production are occupationally induced

Occupation	Leading hazardous factor, working conditions category	Nosology	RR	EF, %	Extent
Spreading operator	labor hardness (3.2)	musculoskeletal system diseases	2.2	55	high
Vulcanizer		skin and subcutaneous tissue diseases	2.6	61	high
Gluer	chemical (3.2)	disorders in the vegetative nervous system as per segmental type	10.3	90	almost completely
		biliary dyskinesia	1.8	44	average

Note:
RR means relative risk;
EF means etiological fraction.

Table 4

Integral assessment of occupational risks for workers employed at rubber production

Occupation	A priori risk		A posteriori risk					Workers with signs of occupational diseases, %	Total occupational risk assessment
	Working conditions category	Risk category	Risk level	OM index	Risk category	Occupational conditionality of diseases			
						RR	Extent		
Gluer	3.2	average	above average	0.5	extremely high	4.9-7.6	extremely high	24.3	extremely high
Spreading operator, vulcanizer	3.2	average	above average	0.33	high	2.5-3.9	high		high

Note:
RR means relative risk;
‰ is given per 10,000 workers.

High occupational risks made it necessary to develop a risk-oriented program that included priority strategies and procedures for preventing damage to workers' health (managerial medical and preventive activities and social protection) (Figure 1). The program was then tested at a plant that manufactured rubber engineering products and implemented at corporate and individual levels. The corporate level covered providing safe working conditions based on applying technological charts for uninterrupted flow-production, wide use of automated lines, supply-and-exhaust mechanic ventilation with rational air inflow that allowed reducing benzine and dichloromethane concentrations in working area air. The individual level involved preventing complex exposure to solvents via using efficient individual protection means including specific clothing and footwear, benzine-resistant gloves, and skin protective creams.

Medical examinations play a significant role within prevention activities aimed at preventing occupational and work-related diseases [20]. When workers are hired, they are to have a preliminary medical examination; when they perform their work functions, they should have periodical medical examinations (PME). Medical examinations are performed according to requirements fixed by the RF Ministry for Public Healthcare and Social Development issued on April 12, 2011 No. 302n[6]. An obligatory condition for performing periodical medical examinations is that workers with long work experience are to be examined at a center for occupational pathology every five years; should initial signs of any occupational pathology be detected in a worker, he or she should have annual periodical examinations at such centers.

It seems rational to divide workers employed at rubber production into three groups for periodical examinations with determining necessary prevention activities for each group.

The 1st group includes healthy workers. They are to have periodical medical examinations.

The 2nd group includes those workers who ran risks of occupational diseases and are to have annual medical examinations and improve their health at sanatoriums once a year.

The 3rd group includes:

a) workers suffering from occupational diseases. They are to visit medical establishments more frequently and if a repeated examination is required, they are to visit a center for occupational pathologies and have an out-patient or in-patient hospital treatment;

b) people with work-related diseases. They are to visit medical establishments and improve their health in sanatoriums; in case of necessity they are to have an out-patient or in-patient hospital treatment.

It is very important to provide timely and rational employment for workers suffering form occupational and chronic non-communicable diseases who can't perform their work tasks under hazardous working conditions; it is a key component in a system of medical and preventive, social, and rehabilitation activities. Medical examinations and recommended treatment as well as health-improving procedures are to be regulated according to a health group a worker belongs to; these health groups are determined as per results obtained via PME according to the Order by the RF Public Healthcare Ministry issued on October 26, 2017 No. 869n[7].

[6] On Approval of the list of adverse and (or) hazardous occupational factors and work tasks that require obligatory preliminary and periodical medical examinations of workers and the procedure for performing obligatory preliminary and periodical medical examinations of workers employed at workplaces with hard work and adverse and (or) hazardous working conditions: The Order by the RF Ministry for Public Healthcare and Social Development issued on April 12, 2011 No. 302. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_120902/ (24.01.2018).

[7] On Approval of the procedure for medical examinations of specific adult population groups: The Order by the RF Ministry for Public Healthcare and Social Development issued on October 26, 2017 No. 869n. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_284986/ (24.01.2018).

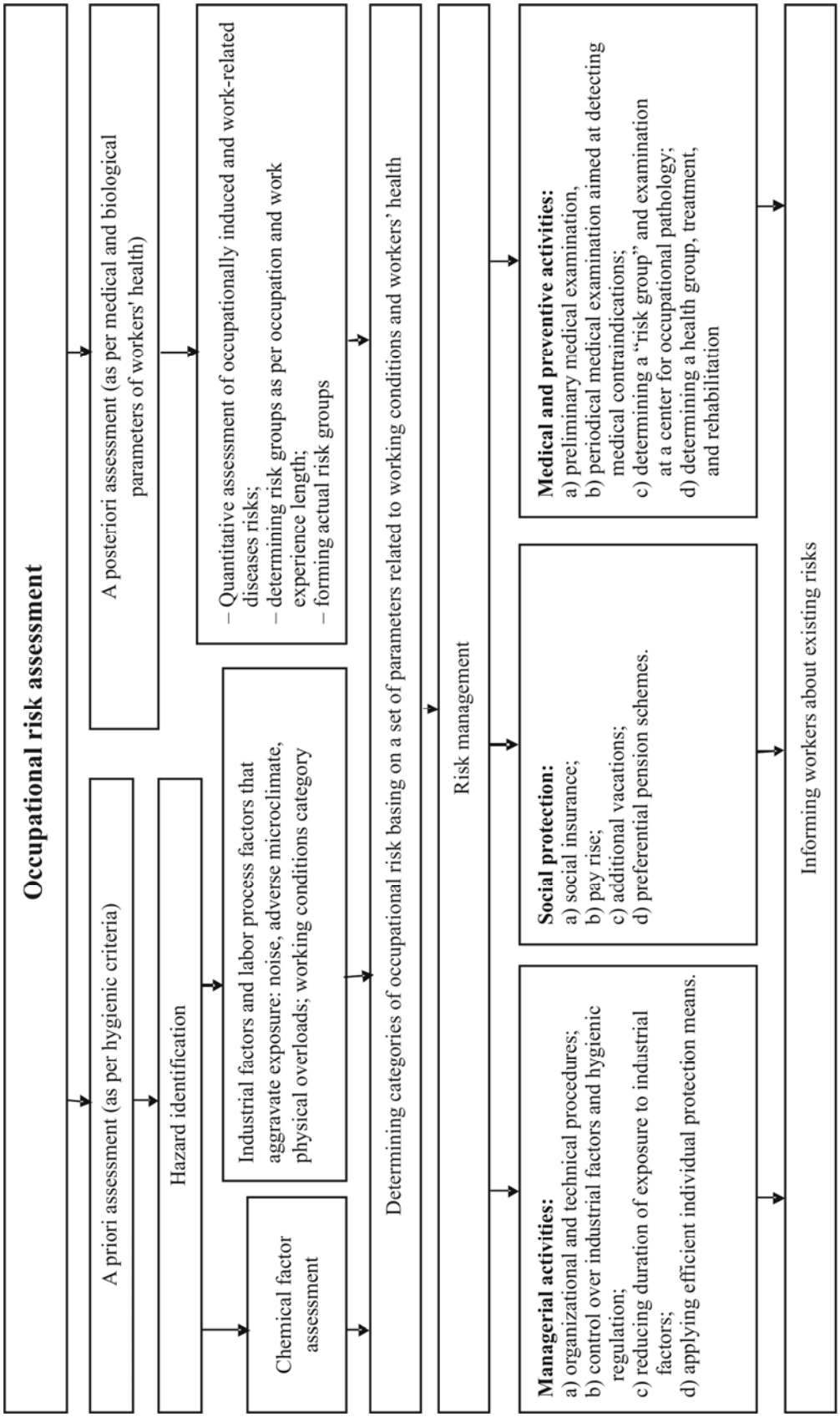

Figure. A scheme for assessing and managing health risks for workers employed at rubber production

Conclusions.

1. We determined that there were several hazardous industrial factors and labor process factors at rubber production; they were hazardous chemicals, in-plant noise, adverse microclimate, and physical overloads. Overall assessment of working conditions for workers employed at rubber production ranked them as belonging to 3.2 hazard category.

2. Working conditions existing at up-to-date rubber productions directly induce occupational diseases and are potentially hazardous for workers' health.

Gluers ran high risks of occupational diseases; spreading operators and vulcanizers, average risk. Total occupational morbidity index corresponded to high occupational risk.

3. We detected cause-and-effect relations between working conditions and frequency of specific chronic non-communicable diseases: there was significant occupational conditionality of disorders in the vegetative nervous system as per segmental type with sensory disorders in hands among gluers; hand polyneuropathy, facioscapulohumeral periathrosis, epicondylitis, and radiculopathy of skin and subcutaneous tissues among spreading operators.

4. A program for managing risks for workers' health was developed and implemented basing on occupational risk categories; the program included managerial and medical and preventive activities as well as social support and protection.

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ASSESSING OCCUPATIONAL RISKS FOR WORKERS EMPLOYED AT HEAT-POWER ENGINEERING ENTERPRISES

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Our research goal was to assess occupational health risks and analyze whether it was possible to assess occupational risks for workers employed at heat-power engineering enterprises applying the same procedures as those used in the our research.

Our research involved basic occupational groups of workers employed at a heat-power engineering enterprise. They were power generating unit drivers, repairmen, and electricians responsible for electric machinery repair and maintenance. Hygienic assessment of working conditions was performed according to the valid regulatory and methodical documents. Occupational morbidity was analyzed on the basis of data arrays that contained data taken from Reports on occupational diseases (intoxications). Occupational risks were assessed as per Fine Kinney risk assessment method, matrix method, and Guide R 2.2.1766-2003; we also performed a semi-quantitative risk assessment.

Labor performed by power generating unit drivers belonged to 3.2 hazard category as per related hazards, its hardness, and intensity; repairmen, 3.3 hazard category; electricians responsible for electrical machinery repair and maintenance, 3.3 hazard category. Occupational morbidity among workers employed at enterprises dealing with energy production and distribution in Irkutsk region was analyzed over 2000–2018; the analysis revealed there was a decrease in first diagnosed occupational diseases and the most frequent nosology was sensorineural hearing loss. Occupational risk assessment performed as per different procedures indicates that obtained results are rather ambiguous. Semi-quantitative risk assessment, Finn Kinney method, and the "Finnish" model turned out to be subjective but it is still possible to apply them provided that it is done by highly qualified experts in the sphere of labor safety and occupational risks management. The most objective risk assessment procedure allows assessing probability of damage to health applying parameters that describe deviations in adverse or hazardous occupational factors from maximum permissible concentrations and levels and a cause-and effect relation with risk (Guide G 2.2.1766-03).

Key words: workers employed at heat-power engineering enterprises, working conditions, occupational morbidity, sensorineural hearing loss, occupationally morbidity, occupational risk, risk assessment procedures, occupational risk factors.

Any labor process involves a worker being exposed to industrial (occupational) factors that can cause occupational accidents and work-related diseases [1, 2]. As per data provided by the International Labor Organization, work-related diseases and occupational accidents annually result in more than 2.2 deaths [3]. More than 300 million occupational accidents and more than 150 million occupational disease cases are registered annually all over the world. At present great efforts are being taken to make working conditions safer for workers and a promising trend here is implementation of prevention activities based on occupational risks (OR) analysis and assessment.

At present in the RF different types of OR are controlled and accounted; they are risks caused by injuries with various degrees of severity including those with lethal outcomes; risks of damage to health caused by a work-related disease; risks caused by industrial accidents and emergencies at hazardous industrial objects [4–6]. Experts employed by federal institutions use a procedure that ranks economic activities as per categories of occupational risks[1]; the proce-

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dure is based on calculation of expenses caused by all the detected work-related disease cases and industrial injuries in a branch [7]. This calculated parameter is an economic one in its essence and is applied to determine volumes of insurance payments; therefore, it can't be used to communicate work-related risks of health damage to workers.

There are multiple publications on managing OR; however, in order to manage risk properly, it is necessary to assess health risks for workers.

At present there is no unified and conventional procedure for OR assessment. Given that, various schemes and procedures for OR assessment are applied; they all have certain advantages and drawbacks and are used depending on specific tasks to be solved and available information[2]. However, it is obvious, that efficient OR management, in an organization, region, or a country as a whole, requires cohort, group, and population parameters of occupational risk that will allow ranking occupations, organizations, or branches as per risk levels [4, 8].

As it is well known, OR assessment usually involves a five-stage procedure that includes identification of hazards that result in risks; risk assessment and risk "ranking"; working out preventive activities; implementation of protection and preventive activities; monitoring [9]. At the stage when hazards are identifies, one can use legal and normative acts, instructions, standards, reports on inspections aimed at checking adherence to labor protection requirements, statistical data on injuries, reviews of past accidents and injuries, data on occupational and qualification properties and health of workers, monitoring results, and other reliable information sources [4, 5, 8–11]. OR assessment can be performed with variable techniques and procedures, and sometimes a combination of those is applied. Basically, there are three groups of techniques and procedures:

– statistical procedures based on determining how probable specific risks are or on examining statistical data on risks;

– expert techniques based on collecting, examining, and generalizing risk assessment results;

– calculation and analytical procedures that allow assessing a risk in case there are no statistical data on it and obtaining a quantitative assessment [5].

Use of statistical procedures allows obtaining the most reliable risk assessment provided that there is thorough monitoring and reliable data. Expert techniques can be applied practically in any activity sphere; however, their universality can sometimes be extremely low, especially when a risk is analyzed in a specific situation. Calculation and analytical procedures are the most widely spread and simple in use.

When assessing risks, one can apply both direct and indirect techniques; choice on them depends on available statistical data, purposes, and qualifications and professional skills of personnel responsible for labor protection. Direct quantitative risk assessment is possible only in a case when there are accumulated data on occupational injuries and their gravity. However, in real life conditions data on health damage are frequently either insufficient or unavailable. Should there be not enough data for statistical analysis, we should use direct qualitative assessment based on analysis that takes into account scores given to working conditions by experts or assessment teams. Some direct techniques for risk assessment are Fine-Kinney method; a technique applying weighing coefficients; a matrix method when ranking parameters related to gravity and probability are given in a matrix; Monte-Carlo modeling and Bayesian technique that apply mathematical tools of probability theory. Indirect techniques for health risk assessment apply parameters that characterize deviations in existing parameters from their standard values

[2] GOST R 12.0.010-2009. The system of occupational safety standards (SOSS). Systems for labor protection management. Hazard determination and risk assessment. *KODEKS: the electronic fund for legal and reference information*. Available at: http://docs.cntd.ru/document/1200080860 (30.11.2019).

and have a cause-and-effect relation with risks. Indirect techniques for risk assessment are a technique involving use of control "checklists" that contain a list of hazards or risks; interviewing; assigning risk into categories as per categories of working conditions; analyzing true causes of accidents; ELMERI method.

There aren't sufficient data in literature on how to calculate OR for workers in multiple brunches of industry and transport [12–14], including workers employed at heat power-stations [15, 16].

Our research goal was to assess occupational health risks and analyze whether it was probable to apply certain risk assessment techniques to assess actual risks for workers from basic occupational groups existing at a heat-and-power enterprise.

Data and methods. We accomplished our research on basic occupational groups existing in heat-and-power engineering; chosen occupations included power generating unit drivers; repairmen; electricians responsible for electric machinery repair and maintenance.

Working conditions were hygienically assessed in conformity with the existing standards and procedures accepted in the RF (sanitary rules and standards; State Standards; methodical guidelines). We examined working conditions for workers employed at a heat-and-power enterprise and analyzed the obtained results.

Occupational morbidity was analyzed using a database containing data from Occupational diseases (intoxications) charts drawn up over 2000–2018 in Irkutsk region[3].

OR was assessed as per Fine-Kinney method [17, 18]; matrix method [19, 20]; Guide R 2.2.1766-2003 "Assessment of occupational health risks for workers"[4]; we also performed a semi-quantitative risk assessment [20]. We also analyzed workers' health as per data obtained via periodical medical check-ups (n = 63, average age was 39.6±1.5; average working experience was 13.6±1.5 years) and calculated to what extent health disorders were work-related. Our reference group was made up of people who were not exposed to hazardous occupational factors and factors related to labor process; they were comparable with the test group in terms of their age and length of working experience ($n = 50$). Authenticity of relative risk parameters was assessed with χ^2 criterion.

Results and discussion. Occupational factors were examined at workplaces of power generating unit drivers; repairmen; electricians responsible for electric machinery repair and maintenance. Hygienic assessment of working conditions revealed that noise reached 88–100 dBA and it was 7–13 dBA higher than the hygienic standard in average- and high-frequency ranges. As workers from these occupational groups didn't have a permanent workplace, we calculated equivalent noise levels for a work shift. They amounted to 83 dBA for power generating unit drivers; 93 dBA, for repairmen; 92 dBA, for electricians responsible for electric machinery repair and maintenance.

Equipment applied at a heat-and-power enterprise (transporters, mills, turbogenerators, and grinders) produces not only noise, but also overall vibration that is equal to 81–83 dB.

Microclimate at workplaces (temperature, relative humidity, and air speed) conformed to hygienic standards during warm seasons. As for cold seasons, temperature deviated from hygienic standards during them and was 8.0–14.0 °C; relative humidity and air speed were within acceptable limits. Illumination mostly conformed to hygienic standards and amounted to 60–310 luxmeter-candela.

Ash and coal dust concentrations in working area air didn't exceed MPC at basic workplaces and amounted to 1.05–5.90 mg/m^3 and 7.1–14.3 mg/m^3 accordingly.

[3] On improving the system for investigating and accounting occupational diseases in the Russian Federation (with alterations and supplements): The Order by the RF Public Healthcare ministry dated May 28, 2001 No. 176. Appendix 5. An occupational disease (intoxication) chart. *Garant. Information and legal database*. Available at: http://base.garant.ru/4177627/c9c989f1e999992b41b30686f0032f7d/ (30.11.2019).

[4] P 2.2.1766-03.Occupational hygiene. Guide on assessing occupational health risks for workers. Organization and methodical basics, assessment principles and criteria: Guide. Moscow, 2003, 18 p.

Hardness of labor process for workers with the examined occupations was determined by dynamic physical loads with predominantly used hand, arm and shoulder muscles when a weight was moved from a place to place over 1 meter (up to 7,000 kg*min); static loads when a weight was held with two arms (more than 70,000 kg*sec); uncomfortable and/or fixed body pose (up to 50 % of a work shift); body bends (more than 100 per a shift); moving around due to necessities related to a technological process (up to 8 km horizontally and up to 2.5 km vertically); all these factors ranked working conditions into 3.1 and 3.2 hazard category (hard physical labor). Labor intensity (intellectual, sensory, and emotional loads; monotony and working regime) were acceptable (average labor intensity).

According to parameters characterizing hazards, labor hardness, and labor intensity, working conditions correspond to 3.2 hazard category for power generating unit drivers; 3.3 hazard category, for repairmen; 3.3 hazard category, for electricians responsible for electric machinery repair and maintenance; higher hazard category is due to workers being exposed to high noise levels.

Apart from occupational factors, working environment, and workplace ergonomics, risk factors can also occur due to moving machinery and mechanisms, moving parts of industrial equipment, surfaces of equipment and tools, and a workplace being located high above the ground or workshop floor.

We analyzed occupational morbidity in Irkutsk region in "Energy production, transfer, and distribution" over 2000–2018 and revealed that there was a decrease in detected occupational disease cases; over recent years they amounted to 1.7–6.9 cases per 10,000 workers employed in the branch. We also analyzed the structure of occupational morbidity and revealed that sensorineural hearing loss was a basic nosology detected in workers with the examined occupations (RR = 18.92, EF = 95 %, χ^2 = 16.71 for repairmen; RR = 13.16, EF = 92 %, χ^2 = 7.42 for electricians; RR = 28.57, EF = 97 %, χ^2 = 16.95 for power generating unit drivers).

We calculated OR as per Fine-Kinney method based on analyzing three factors: an extent to which a worker was exposed to impacts exerted by adverse factor at a workplace (P), a probability of a threat to health occurring at a workplace (V), and consequences for a worker's health and/or safety in case such a threat comes to life (C) [14, 17]. The calculation revealed that OR for the examined workers was rather serious (substantial) (R = 108) and it required planning and accomplishing activities aimed at reducing this risk (Table 1).

Semi-quantitative risk assessment calculated as per the formula R + Q x p where Q is a probability of a damaging event and p is consequences, severity, or a volume of losses [20] revealed that OR was acceptable for all three examined occupational groups (R < 40 scores) (Table 2).

Table 1

Occupational risk assessment as per Fine-Kinney method

Occupation	A stage in assessment				Comments
	A worker being exposed to adverse factors (P)	Probability of a threat at workplace (V)	Consequences for a worker's health (C)	Risk (R)	
Power generating unit drivers	Regular 6 scores	Very probable 6 scores	Injury (TD) 3 scores	108	Substantial risk
Repairmen	Regular 6 scores	Very probable 6 scores	Injury (TD) 3 scores	108	Substantial risk
Electricians responsible for electric machinery repair and maintenance	Regular 6 scores	Very probable 6 scores	Injury (TD) 3 scores	108	Substantial risk

Note: TD means temporary disability.

Table 2

Semi-quantitative occupational risk assessment

Occupation	Probability, Q	Severity, p	Risk (R), score	Risk level
Power generating unit drivers	Very probable 8 scores	Temporary disability for less than 4 weeks 4 scores	32	Acceptable
Repairmen	Very probable 8 scores	Temporary disability for less than 4 weeks 4 scores	32	Acceptable
Electricians responsible for electric machinery repair and maintenance	Very probable 8 scores	Temporary disability for less than 4 weeks 4 scores	32	Acceptable

We also applied a so called "Finnish" risk management model to analyzed OR [19]; the analysis revealed that risk index corresponded to unacceptable risk for all the examined occupations (Table 3).

We assessed a priori risk as per data obtained via special assessment of working conditions and sanitary-industrial control as well as occupational morbidity index (according to G 2.2.1766-03); the assessment revealed that OR taken as per working conditions were average (substantial) for power generating units drivers; and high (intolerable) for repairmen and electricians responsible for electric machinery repair and maintenance (Table 4).

We calculated occupational morbidity index and revealed that risks were extremely high for all three examined occupational groups (Table 5).

Table 3

Occupational risk assessment as per "Finnish" model

Occupation	Frequency of accidents	Hazard category	Risk index	Risk criterion
Power generating unit drivers	Probable B	Substantial 2	2B	Unacceptable
Repairmen	Probable B	Substantial 2	2B	Unacceptable
Electricians responsible for electric machinery repair and maintenance	Probable B	Substantial 2	2B	Unacceptable

Table 4

Working conditions and occupational risk categories

Occupation	Working conditions category as per G 2.2.2006-05	Occupational risk category	Urgency of activities aimed at reducing risks
Power generating unit drivers	3.2 (hazardous, the 2nd degree)	Average (substantial) risk	Activities aimed at reducing risks are required
Repairmen	3.3 (hazardous, the 3rd degree)	High (intolerable) risk	Urgent activities aimed at reducing risks are required
Electricians responsible for electric machinery repair and maintenance	3.3 (hazardous, the 3rd degree)	High (intolerable) risk	Urgent activities aimed at reducing risks are required

Table 5

Occupational morbidity index and occupational risk categories

Occupation	Occupational morbidity index	Occupational risk category	Urgency of activities aimed at reducing risks
Power generating unit drivers	0.5	Extremely high (intolerable) risk	It is forbidden to start or continue any work before risks are reduced
Repairmen	0.5	Extremely high (intolerable) risk	It is forbidden to start or continue any work before risks are reduced
Electricians responsible for electric machinery repair and maintenance	0.5	Extremely high (intolerable) risk	It is forbidden to start or continue any work before risks are reduced

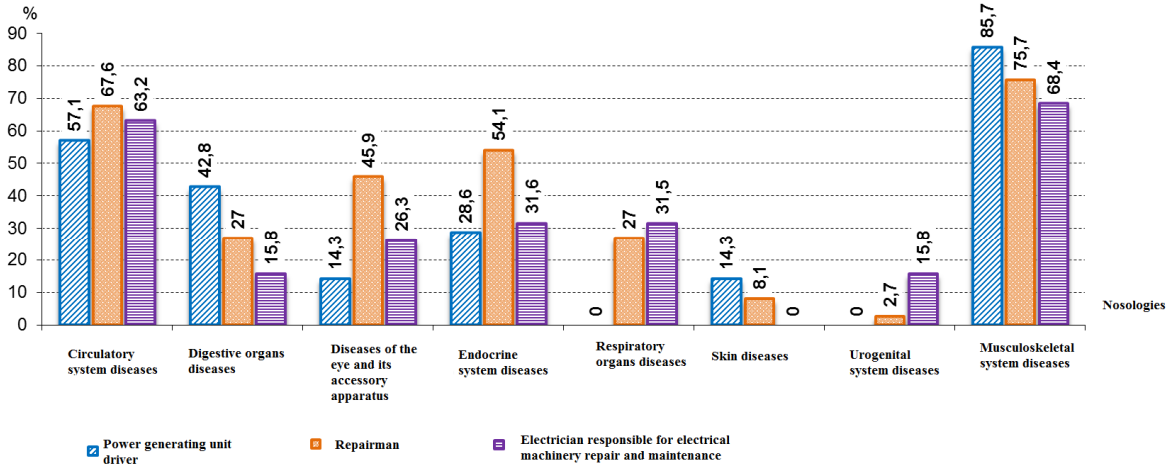

Figure 1. Frequency of chronic common diseases revealed in workers due to periodical medical examinations (per 100 workers)

In addition to a priori assessment of occupational risks as per working conditions, we analyzed frequency of common chronic diseases revealed in workers due to periodical medical examinations and assessed to what extent they were work-related (as per Guide 2.2.1766-03).

We established that power generating unit drivers, repairmen, and electricians responsible for electrical machinery repair and maintenance most frequently suffered from diseases of the musculoskeletal system and connective tissue; most common diseases were low-back pains, neck pains, and pains in the thoracic spine (75.7 cases, 68.4 cases, and 85.7 cases per 100 examined workers accordingly) (Figure 1).

We revealed almost complete occupational causality of these nosologies among workers with the examined occupations (RR = 7.57, EF = 87 %, $\chi^2 = 36.22$; RR = 6.84, EF = 85 %, $\chi^2 = 21.44$; RR = 8.57, EF = 88 %, $\chi^2 = 18.00$ accordingly). Besides, we established practically complete occupational causality of diseases of the eye and adnexa its accessory apparatus for repairmen and electricians (RR = 22.97, EF = 96 %, $\chi^2 = 22.42$; RR = 13.16, EF = 92 %, $\chi^2 = 7.42$ accordingly). We should note that there was also almost complete occupational causality for all three occupational groups regarding health disorders in the endocrine system, namely, obesity, diabetes mellitus, and diffuse thyroid gland enlargement (RR = 27.03, EF = 96 %, $\chi^2 = 28.69$ for repairmen; RR = 14.29, EF = 93 %, $\chi^2 = 4.18$ for power generating unit drivers; RR = 15.79, EF = 94 %, $\chi^2 = 10.17$ for electricians). Circulatory system diseases were also rather widely spread among workers from the examined occupational groups as per data obtained via periodical medical examinations; despite that,

comparative analysis didn't reveal any statistically significant discrepancies from the reference group as per these nosologies.

Conclusion. Therefore, our research revealed that applying different procedures for assessing OR for workers employed at a heat-and-power enterprise yielded somewhat contradictory results. OR assessment as per Fine-Kinney method and "Finnish" model doesn't involve either instrumental or laboratory measurements or clinical, physiological, medical-biological, and epidemiologic research; however, it does allow determining whether there is a risk to get injured. Besides, semi-quantitative OR assessment, Fine-Kinney method, and "Finnish" model have a serious drawback as they employ rather subjective approach to occupational risk assessment; at the same time, they should be given some attention, especially provided that there are skilled experts in labor protection and OR management who have relevant knowledge in specific industrial brunches. In our opinion, the most objective procedure for assessing occupational health risks is a procedure that allows assessing probability of damage to health and employs such parameters that characterize deviation of occupational and industrial factors (concentrations, doses, levels, etc.) from hygienic standards and have a cause-and-effect relation with risks. When OR are calculated as per this procedures, experts take into account factor values, duration of exposure to them, and workers' health; however, should there be no long-term occupational morbidity in an organization, it becomes rather difficult to qualitatively assess OR. Besides, OR assessment as per Guide 2.2.1766-03 doesn't allow assessing risks of injuries but at the same time it is quite possible to assess to what extent diseases are work-related or occupational and it provides much better insight into OR essence.

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DYNAMICS OF ACUTE MORBIDITY AND RISK FACTORS THAT CAUSE FREQUENT ACUTE RESPIRATORY TRACT INFECTIONS IN INFANT CHILDREN DURING THEIR FIRST YEAR IN A FOSTER FAMILY

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Our research goal was to study dynamics of acute morbidity and reveal risk factors that caused frequent acute respiratory tract infections (acute RTIs) in infant children during their first year in a foster family (FF).

We examined health of 100 infant children at the moment they were adopted and after a year spent in a foster family; we also examined psychological peculiarities their foster mothers had. Our control group included 90 infant children of the same age who lived with their blood parents and their mothers. We studied social and biological case histories and performed clinical examinations of children. Psychological examinations of foster mothers and parent-child relations in families were performed with mini MMPI tests, procedures developed by Yu.A. Alyoshina, L.Ya. Gozman and E.M. Dubovskaya; A.Ya. Varg; V.V. Stolin; E.G. Eidemiller and V.V. Yustitskis. We applied Wald's sequential analysis to draw up an expectancy table.

Our research allowed us to reveal that infant children who had spent a year in a foster family were more frequently assigned into "often ill" category than children who lived with their blood parents. Adopted children tended to suffer from complicated acute RTIs more frequently. We detected several risk factors that could cause frequent acute respiratory infections; they were a child being born in the second or more delivery; reduced or drastically reduced resistance at the moment a child was adopted; a foster family being a one-parent one; insufficient amount of time spent by a foster mother with a child; such personality traits in a foster mother as impulsivity, masculinity, eccentricity, emotional immaturity; parents being primarily oriented at autonomous activities; a significant psychological distance between foster parents and a child; parents not treating a baby as an infant and ignoring its needs. We developed a forecast for acute respiratory tract infections in children during their adaptation in a new family taking into account not only biological factors, but also social and psychological ones.

Key words: *risk factors, acute respiratory tract infections, children, infancy, foster families, social and biological case history, a psychological profile of a mother, parent-child relations.*

Abandoned children who are deprived of parent care need help; it is a key priority of any state to provide it. Activities performed in the sphere and aimed at supporting such children are vital components in social policies [1]. A number of children who are left without parent care in their infancy is growing. 75 % of all children who are annually placed in orphanages are infants younger than 12 months; such children are abandoned by their mothers

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in maternity hospitals in 96.4 % cases [2, 3]. Practically all such children initially have unfavorable conditions for formation of their health. Many authors stress that such children, as a rule, are unwanted and have burdened social and biological case history; they are often born by mothers who are too young; smoking mothers; mothers who drink alcohol and take drugs during pregnancy; mothers who pursue asocial lifestyle; there are also impacts exerted by disorders in their intrauterine development and diseases in the neonatal period [4–6].

Early and long-term separation from a mother produces grave negative effects on a child's health and development, causes neurotic and affective disorders; a child experiences fear and aggression and doesn't trust anyone [7–9]. Infant children tend to react on the somato-vegetative level and children who are left without parent care obligatorily have various signs of neuropathic syndrome (increased overall and vegetative excitability, proneness to digestive and sleep disorders, absence of any skills to keep oneself tidy, and lower resistance) [7, 10, 11].

Issues related to health of orphans who were raised in state orphanages have been given considerable attention in scientific literature. As per data provided by many authors, from 25 % to 47 % orphans raised in state orphanages belong to "often sick" category [4, 5, 12, 13].

It was proven that orphans and children left without parent care who were adopted by foster families (FF) had better health than children who were left in orphanages; however, even after being adopted, such children were still behind their counterparts who lived with their blood parents as per many parameters [3, 9, 14, 15].

Adaptation to a new family to a great extent depends on a child's age; parents' personal traits; relations existing in a foster family; somatic and mental health of a child and foster parents; parents' psychological competence [16, 17].

According to available data, children who had been adopted by a foster family developed rapidly during the first year after adoption; it was especially true for physical and neuropsychic development. However, there were great difficulties related to children adapting to foster families in 17.5 % cases [16, 18, 19].

Children still are not fully adapted to a foster family after the first year spent in it despite more favorable micro-social conditions there than in an orphanage. Adopted children tend to have lower resistance that results from negative impacts exerted by biological and social factors on infants (burdened heredity; unfavorable clinic course of pregnancy and birth; irrational nutrition and improper care; asocial parents being cruel or careless; a child being raised in an orphanage and it leading to social deprivation). An adopted child is placed in a new micro-social environment and it makes him or her feel afraid and diffident, dependent on external circumstances and unknown people; any affection to foster parents is to occur under such unfavorable conditions [18–20]. We revealed that infant children still had lower resistance during their first year spent in a foster family [21].

There are no sufficient data on health of infant children adopted by foster families in scientific literature; besides, available data are rather contradictory. It calls for a longitudinal study and search for new biological, social, and psychological markers that can be used as predictive criteria. Implementation of prediction technologies will allow performing prevention activities in due time.

Our research goal was to examine dynamics of acute morbidity and determine risk factors that cause frequency acute respiratory tract infections (RTIs) in small children during their first year in a foster family.

Research techniques. We examined health of 100 children aged 1–3 at the moment they were adopted by a foster family and after they spent 1 year in that family; we also examined psychological peculiarities of their foster mothers. Our reference group was made up of 90 children of the same age who lived with their blood parents and their mothers. To draw up our prediction, we divided children who

lived in foster families into two groups; the 1st one included children who suffered from acute RTIs 6 times per year or even more often, and the 2nd one was made up of children who didn't have RTIs or had them so often (less than 6 times per year).

We examined social and biological case histories via taking data from medical documents, questioning and interviewing parents. All the children had clinical check-ups and their medical documents were examined; we questioned foster parents using our own structured clinical-statistic chart.

All the examinations were performed with obligatory adherence to ethical standards fixed in Helsinki Declaration (1975, supplemented in 1983 and 1989). Parents (legal representatives) of all children gave their informed voluntary consent to medical intervention and personal data processing.

We accomplished psychological examination on foster mothers in order to reveal their personal traits and it was done with mini MMPI test; attitudes accepted by a married couple were revealed with a procedure developed by Yu.A. Alyoshina, L.Ya. Gozman and E.M. Dubovskaya; parent – child relations were studied with procedures developed by A.Ya. Varg and V.V. Stolin; family relations were examined with procedures developed by E.G. Eidemiller and V.V. Yustitskis.

All the data were statistically processed with MS Excel XP and Statistica 6.0.

We applied Wald's sequential analysis to draw up our expectancy table. First, we proved that there were authentic discrepancies in frequency of an examined factor among children who were often sick with acute RTIs, who were sick on rare occasions, and who didn't fall sick; having done that, we calculated predictive quotients (PQ) for each factor gradation. Predictive quotient was calculated as per the following formula: PQ = 10 lg (P1/P2) provided a factor was present; PQ = 10 lg (1-P1/1-P2) provided that a factor was absent, where P1 and P2 were frequencies of an examined factor in groups that were being compared. A positive value meant a forecast was unfavorable.

Results. Most small children (71 %) were ranked as "sick on rare occasions" at the moment they were adopted by a foster family; a number of "often sick" children was 6.3 times higher than a number of children who didn't fall sick (25 % against 4 %).There were almost no changes in a number of children in all three groups after they had spent a year in a foster family (71 % against 70 %; 25 % against 24 %; 4 % against 6 %). However, a number of children who were ranked as "often sick" was 2.7 times higher among children from foster families than among children who lived with their blood parents (8.8 % against 24 %, $p = 0{,}007$); a number of children who didn't fall sick was 2.8 times lower among children from foster families than among those who lived with their blood parents (16.6 % against 6 %, $p = 0{,}033$).

We analyzed peculiarities related to clinical course of acute RTIs among children from different groups and revealed that 87 % small children suffered from acute RTIs with complications at the moment they were adopted by a foster family. Complications were predominantly diseases in the lower respiratory tracts, such as acute bronchitis, acute obstructive bronchitis, tracheobronchitis (80 %), and pneumonia (19 %). Besides, there were such complications as acute conjunctivitis (37 %), acute otitis (33 %), obstructing laryngotracheitis (12 %), and pharyngitis (5 %).

After adopted children spent their first year in a foster family, there was a descending trend in a number of acute RTIs with complications (87 % against 77 %), and an ascending one for acute RTIs without any complications (13 % against 23 %). As for structure of occurring complications, there was an authentic decrease in frequency of acute bronchitis, acute obstructive bronchitis, tracheobronchitis (80 % against 58 %, $p = 0.007$), and pneumonia (19 % against 7 %, $p = 0.035$), whereas a share of pharyngitis increased by 4 times (5 % against 20 %, $p = 0.007$), and there was an ascending trend in frequency of acute otitis (33 % against 47 %).

Children who were adopted by foster families had acute RTIs without complications two times less frequently than children who lived with their blood parents (46 % against 23 %, $p = 0.001$); they suffered from acute RTIs with complications authentically more frequently (54 % and 77 %, $p = 0.001$). Children from foster families more frequently had such complications as acute bronchitis, acute obstructive bronchitis, tracheobronchitis (58 % against 39 %, $p = 0.034$), acute conjunctivitis (33 % against 24 %), and acute otitis (47 % against 34 %).

Therefore, after their first year in a foster family, children were more frequently ranked as "often sick" than children who lived with their blood parents; children from foster families more frequently suffered from acute RTIs with complications which were predominantly diseases in the lower respiratory tracts. So, it is important to predict frequent acute RTIs for children who are adopted by foster families.

Basing on analysis of social, psychological, and biological factors, we revealed the most significant risk factors; they were a child being born in the 2nd and the subsequent births; reduced and extremely reduced resistance at the moment a child is adopted; a foster family being one-parent; a foster mother not spending enough time with a child; a foster mother being emotionally unstable (she had such personality traits as impulsivity, masculinity, eccentricity, and emotional immaturity); parents being predominantly oriented at autonomous activities; a significant psychological distance between parents and a child; parents not treating a baby as an infant and ignoring its needs.

We created an expectancy table to predict frequent acute RTIs (Table).

An expectancy table to predict frequent acute RTIs in small children during their first year in a foster family

Risk factors	PQ
Biological factors	
A child being born in the 2nd or subsequent births	
- yes	+2.30
- no	-3.49
Reduced and extremely reduced resistance at the moment a child is adopted	
- yes	+3.65
- no	-3.17
Social factors	
One-parent foster family	
- yes	+3.36
- no	-1.87
A foster mother spending with a child not more than 6 hours a day	
- yes	+1.58
- no	-6.69
Psychological factors	
Personality traits of a foster mother (mini MMPI test):	
Self-conceit, impulsiveness	
- yes	+6.88
- no	-2.54
A desire to stress decisiveness and masculinity; difficulties in interpersonal communication	
- yes	+4.49
- no	-2.74
Eccentricity	
- yes	+10.80
- no	-1.48

Emotional immaturity, hypomania	
- yes	+4.45
- no	-2.15
Parents – child relations (procedure by A.Ya. Varg and V.V. Stolin):	
A significant psychological distance between parents and a child	
- yes	+8.75
- no	-3.39
Treating baby as an infant	
- yes	+1.10
- no	-13.63
Ignoring baby's needs (U scale in family relations analysis)	
- yes	+7.20
- no	-1.13
Parents being predominantly oriented at autonomous activities (test developed by Yu.A. Alyoshina, L.Ya. Gozman and E.M. Dubovskaya)	
- yes	+2.20
- no	-4.26

Note: PQ means predictive quotient.

To predict acute RTIs, a pediatrician from an orphanage takes data from a child's case history and applies an expectancy table to determine whether risk factors are present or absent. A psychologist at a support center for foster families should accomplish a psychological examination of a foster mother with specific test and reveal probable psychological risk factors.

Should a sum of all PQs be equal to +13 scores or higher, it means a child is going to be often sick with acute RTIs. Should this sum be equal to -13 scores or lower, it means absence of acute RTIs. Should the PQs sun be between -13 and +13 scores, it means there are not enough data in this case to make any prediction.

When a forecast for a child from a risk group is unfavorable, a pediatrician prescribes differentiated preventive activities; in case of necessity a consultation and examination by an immunologist is required; a pediatrician also develops and implements an individual program aimed at supporting a foster family as it will allow reducing risks of frequent acute RTIs; such programs require psychologists' participation.

Conclusions.

1. Small children, even after a year spent in a foster family, more frequently fall sick with acute RTIs than children who live with their blood parents. They also more frequently suffer from RTIs with complications which are predominantly diseases in the lower respiratory tracts. Therefore, when children are adopted by foster families, it is important to predict frequent acute RTIs.

2. Significant risk factors that cause frequent acute RTIs among children adopted by foster families are as follows: a child being born in the 2nd and the subsequent births; reduced and extremely reduced resistance at the moment a child is adopted; a foster family being one-parent; a foster mother not spending enough time with a child. Psychological risk factors are such personality traits of a foster mother as impulsivity, masculinity, eccentricity, and emotional immaturity; parents being predominantly oriented at autonomous activities; a significant psychological distance between parents and a child; parents not treating a baby as an infant and ignoring its needs.

3. We developed a procedure for predicting acute RTIs in small children during their first year in a foster family. Implementation of prediction technologies will allow accomplishing preventive activities in due time.

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ON EPIDEMIOLOGIC RISKS, THEIR CATEGORIES AND PREDICTORS IN SANITARY-EPIDEMIOLOGIC (BIOLOGICAL) EMERGENCY SITUATIONS

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Nowadays sanitary-epidemiologic (biological) emergency situations can be overlooked, especially at an initial stage in epidemiologic risks realization and the consequent development of epidemic process. A clear example here is how Ebola epidemics started in the Western Africa (2013–2016).

***Our research goal** was to obtain actual data on any existing crucial combinations of epidemiologic risk categories and predictors as emergency situations precursors.*

Our basic research procedure was a complex epidemiologic one. Our work was based on analyzing official data provided by the World Health Organization, WHO Regional Office for Africa, as well as taken from multiple research works and monographs.

We analyzed two epidemics caused by infectious agents belonging to the 1st pathogenicity group, Ebola epidemics in the Western Africa (2013–2016) and pneumonic plague epidemic in Madagascar (2017). Both those epidemics were characterized with such potential emergency situations properties fixed in the International Health Regulations (2005) as unexpectedness, unusualness, and gravity. We showed that each epidemic had its own crucial combination of epidemiologic risk categories and their functionality and predictors that were emergency situations precursors. Those combinations occurred just at the very beginning of the epidemics development, after epidemiologic risks had manifested and an epidemic process started to develop intensively and extensively. We assume that should a data base with data on such combinations be created, monitoring over them and targeted activities aimed at their elimination will allow enhancing a preventive potential of the International Health Regulations (2005) as regards emergency situations.

***Key words:** sanitary-epidemiological (biological) emergency situation, epidemiologic risk, epidemiologic risk categories, predictors, epidemic process, International Health Regulations (2005), Ebola virus disease epidemic in West Africa (2013–2016), pneumonic plague epidemic on Madagascar (2017), prevention and control of emergency situations.*

Eliminating Ebola epidemics in the Western Africa in 2013–2016 was a rather painful experience and lessons learnt from it were critically analyzed and assessed by the WHO experts; these assessments can be found in official reports issued after several sessions of the World Health Assembly, mostly the 68th (A68/22 – 2015) and the 69th (A69/21 – 2016). It became obvious that WHO activities aimed at managing emergency situations (ES) in public healthcare needed reforming; it was also necessary to adopt the WHO program on ES in public healthcare within streamlining and enhancing implementation of the International Health Regulations (IHR, 2005) in WHO member states. The above-mentioned lessons as well as results obtained via scientific research on the matter [1] revealed that an epidemic onset in Guinea Republic was actually missed; the epidemic was registered by public healthcare officials on the local, regional, national and international levels when it was already too late; its subsequent etiological verification was too protracted. All the above mentioned resulted in steady growth in the epidemic process spreading into large settlements, cities, and other countries as well; overdue announcement by the WHO that there was an interna-

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tionally significant ES in public healthcare; the epidemic becoming a priority threat to the national securities of countries in the grip of it and the security of the overall international society as well.

Bearing all this in mind, it calls for developing a set of activities aimed at early detection and, consequently, timely prevention of such ES. At present such a set can be most easily developed basing on creating a methodological base in addition to methodical instruments for ES verification outlined in the IHR (2005). This methodical base would allow detecting and monitoring over the earliest and the most dangerous combinations of epidemiologic risk categories and predictors that were "catalysts" for an epidemic process development.

Initial realization of an epidemiologic risk is known to underlie any epidemic event (a sporadic infectious disease case, an outbreak, an epidemic, a pandemic). An epidemiologic risk is a potential possibility that an epidemiologic situation will deteriorate. Russian epidemiologists V.D. Belyakov and B.L. Cherkasskiy differentiated an epidemiologic risk into 4 categories it was made up of; they were "risk territory", "risk factors", "risk period", and "risk groups" [2, 3].

"Risk territory" is a territory where an infectious agent occurs and persists; there are landscape-ecological and natural-biocoenotic conditions necessary for its circulation; people can get infected there, and an epidemic process can then develop. "Risk factors" are animated and non-animated nature objects that contain an infectious agent and a person gets infected when contacting them; it results in a threat that an epidemic process will develop. "Risk period" is a period during which there is a long-term or seasonal growth in a quantity of an infectious agent in risk factors and people are most likely to get infected and an epidemic process is most likely to develop. "Risk groups" are population who are (permanently or temporarily) on a risk territory including an epidemic focus and who are, due to various circumstances, most likely to contact risk factors, infected people, infection sources, and infectious agents.

Nowadays when epidemiologic diagnostics is accomplished in real life conditions, an epidemiologic risk is assessed taking into account all categories it is made of; any targeted (risk-oriented) sanitary-preventive (anti-epidemic) activities or activities within epidemiologic control, to be exact, should be aimed at minimizing these categories. Thus, a complex assessment of an epidemiologic risk is accomplished when it is necessary to provide sanitary-epidemiologic welfare of mass events with international participation and to prevent any ES; such assessment should be accomplished as per all the components of an epidemiologic risk without any differentiation of their functional significance and for any infectious disease that can be potentially dangerous in terms of ES occurrence. When calculating potential epidemic threats related to a mass event with international participation, one should take into account both external threats (actually existing and additionally imported) and internal ones[1].

Naturally, probability and fast realization of an epidemiologic risk as a whole or its separate categories to a great extent depend on biological properties of infectious agents and them belonging to I–II pathogenicity groups; their ability to induce an epidemic process with aggravated clinical course and high lethality; extensive and intensive manifestation; and their overall ability to disseminatingly circulate in nature and a human society accordingly on the ecosystem and socio-ecosystem levels in an epidemic process [4]. But still, bearing in mind the experience gained in eliminating Ebola epidemic in the Western Africa, we can assume that significant epidemic events, like epidemic or pandemics, that is, internationally significant sanitary-epidemiologic (biological) ES occur and develop provided that there are extraordinary combina-

[1] M.A. Patyashina Scientific grounds for providing sanitary-epidemiologic welfare of international mass events and their implementation exemplified by the XXVII Summer Universiade in Kazan: doctoral thesis. Saratov, 2015, 337 p.

tions of functional peculiarities related to separate categories included into an overall epidemiologic risk and specific predictors that are "catalysts" for an epidemic process development over time and space.

As for predictors that are able to result in faster ES occurrence, there are such analogues to them in epidemiology as social and natural factors that activate an epidemic process[2] and, consequently, increase a probability that it will grow significantly enough to become an ES. Among them there are geophysical phenomena or natural disasters (earthquakes, floods, or droughts); biological events (mass spread of small rodents on vast territories and those rodents being carriers of dangerous infectious agents). There are also social factors (predictors) such as ethnical military conflicts, high migration activity of population, intense traffic flows between territories, etc. A principally significant predictor is relevant development and structural and functional organization of epidemiologic monitoring, epidemiologic surveillance, and epidemiologic control. It means that there are efficient organizational, epidemiologic and diagnostic (clinical-epidemiologic diagnostics and etiological verification), medical, preventive, and anti-epidemic activities and measures accomplished and taken in case of well-known infectious diseases. It is also necessary to have an algorithm for fast creation of such a set of activities and measures to control new emerging nosology forms as they constantly appear all over the world (predominantly in Asia and Africa) and to meet the requirements fixed in the IHR (2005) and developed basing on accomplishing response activities in case of such epidemic events. In particular, the List No.1 in the Appendix 2 of the IHR (2005) contains a model that describes a new infectious disease with maximum possible destructive potential that threatens the whole international society. This new infectious disease is flu of a new sub-type which is unknown at the moment but predicted by the WHO to cause pandemic as it is expected to have high lethality typical for A (H5NI) bird flu and to be easily transferred from a person to person as it is the case with seasonal flu.

All the hypotheses regarding occurrence of infectious agents with such combinations of biological properties that make them a potential threat of an ES, relevant clinical and epidemiological signs of infectious diseases, epidemiologic risk categories and predictors (ES precursors) are to be initially examined on examples of epidemics that have occurred over recent years. It is advisable to choose epidemics that were caused by infectious agents belonging to I pathogenicity group[3] and had such properties as unexpectedness, unusualness, and gravity; these properties were, according to the IHR (2005), typical ES-preceding signs and epidemics that had them could cause a potential threat of an ES on national and international levels. Examples of such epidemics are an Ebola epidemic in the Western Africa (2013–2016) and a wide-scale pneumonic plague epidemic in Madagascar in 2017.

Our research goal was to obtain actual data on critical combinations of epidemiologic risk categories and predictors as precursors of epidemic process development and occurrence of a sanitary-epidemiologic (biological) ES.

Data and methods. We applied a complex epidemiologic procedure in the present work. Our information resources to obtain data for analysis were official reports by the WHO, WHO Regional Office for Africa, scientific papers and monographs. The text was designed on a PC with installed Microsoft Windows 7 Professional and Microsoft Office 2017.

Results and discussion. The Ebola epidemic in the Western Africa is of special importance for assessing combinations of epidemiologic risk categories and predictors. It occurred in 2013–2016 and predominantly spread in three countries, namely Guinea, Liberia, and Sierra Leone. The epidemic was defined by the WHO as a grave large-scale internationally sig-

[2] Guide on infectious diseases epidemiology. In: N.I. Briko, G.G. Onishchenko, V.I. Pokrovskiy eds. Moscow, "Medical Information Agency" Publ., 2019, vol. 1, 2019, 880 p.

[3] SR 1.3.3118-13. Safety of work with microorganisms belonging to the I–II pathogenicity (hazard) groups issued on November 28, 2013 No, 64. Moscow: The Rospotrebnadzor's Federal Center for Hygiene and Epidemiology Publ., 2014, 195 p.

nificant ES in public healthcare. Scientific works treated it as an internationally significant ES in the sphere of biological safety. 28,616 people got infected and fell sick, 11,310 of them died (lethality was equal to 39.5 %) [5]. Such a great number of sick people registered in such a short period of time (mostly in 2014–2015) is incommensurable with much smaller numbers of infected and sick people detected in Africa over comparably long-term periods of time.

Prior to the epidemic in the Western Africa, Ebola outbreaks were registered mostly in the Central Africa (the Democratic Republic of Congo, Gabon, and Congo), and the Eastern Africa (Southern Sudan and Uganda) [6]. Since Ebola was first described in 1976 and up to 2012 there were 24 outbreaks and single disease cases, the overall number of sick people amounted to 2,433, 1,581 out of them died (lethality was equal to 65 %). In 2014, 2017, 2018–2019 there were 4 Ebola outbreaks in the Democratic Republic of Congo, overall number of sick people amounted to 3,049 and 2,050 out of them died (WHO data on September 03, 2019).

Ebola epidemic process in the Western Africa has already been analyzed with the focus on step-by-step development of an ES in the sphere of biological safety and efficiency of methodical tools provided in the IHR (2005) for early detection, timely communication, and taking relevant measures aimed at eliminating the said epidemic, especially at initial stages in its development. It seems logical to examine combinations of epidemiologic risk categories and predictors and their dynamics during the said epidemic as an ES as per 4 stages in its development that were previously identified.

The 1st stage was characterized with local outbreaks in a zone where initial contagion occurred and epidemiologic risk was realized in Guinea. As per its phenomenological characteristics, it was a typical natural-focus infection starting with the first infected person (it was a two-year old boy who fell sick on December 02, 2013 and died on December 06, 2013 in Meliandu village in Gekedu prefecture in south-eastern Guinea). Ebola virus is thought to circulate in nature in Guinea thanks to bats, and small predator rodents belonging to viverrids family can be an interlink in a mechanism of infection transfer to people.

An initial outbreak was limited to rural population and forest zones [7]. It was characterized with grave clinical course of the disease, high lethality, and high frequency of anthropogenic transfer of the infectious agent, medical personnel being involved into the epidemic process, and the infection being a hospital-acquired one. Such features are a sufficient reason for verifying this epidemiologic situation with IHR (2005) tools as unexpected, unusual, grave, and potentially hazardous in terms of ES occurrence. But any signs hinting at a probable future ES were not verified due to the outbreak being local and occurring in remote places; therefore, its social and economic significance was practically imperceptible at that stage. Partially it was due to national and international public healthcare experts both in the Western Africa and the African continent as a whole traditionally paying the greatest attention to the most significant and gravest issues related to diseases with serious social and economic consequences, such as malaria, HIV/AIDS, tuberculosis, B type hepatitis, etc.

Only on March 10, 2014 hospitals and medical institutions located in Gekedu and Macenta settlements passed data on the outbreak to the Guinea Ministry for Public Healthcare and Hygiene; on March 12 the information was given to the local office of “Doctors without Borders” international nongovernmental organization that had been operating in Guinea since 2010 within a project aimed at fighting against malaria [8]. Practically at the 1st stage in the outbreak there were certain predictors that allowed forecasting it would most likely develop into an epidemic and then ES. They were as follows:

– a moment an outbreak started in forest zones and rural areas was totally missed and it made for the epidemic process spread into large settlements and cities;

– changes occurring in Ebola syndromes such as a substantial decrease in frequency of

hemorrhagic fever syndrome and an increase in frequency of diarrhea symptoms in the clinical picture of the diseases in the Western Africa in comparison to the Central Africa;

– too much time required for clinical and epidemiological diagnostics and etiological verification of the disease (first cases were communicated in January 2014) due to changes in syndromes;

– public healthcare experts not being ready to verify ES signs fixed in the IHR (2005);

– national and international public healthcare institutions being predominantly preoccupied with finding solutions to epidemiological problems existing in Africa that were urgent, large-scale and resource-consuming and not being ready for a probable Ebola outbreak in the Western Africa;

– underestimation of landscape-ecological and natural biocoenotic complexes existing in Guinea that were favorable for Ebola spread among animals and occurrence of all epidemiologic risk categories taking into account results of seroconversion among people which indicated Ebola infectious agent might have circulated in Guinea and other Western African countries in the past [9–11].

The second stage was characterized with the disease spreading from its initial epidemic focus into large cities and Conakry, the capital of Guinea, where Guinea international airport was located and, consequently, there was a risk that the disease could spread internationally. The basic predictor at the stage was a delay by the WHO in declaring there was an ES; the declaration was made 4 months after the outbreak started even though there were practically all signs fixed in the IHR (2005) and, therefore, such a delay is hardly explainable. It made it impossible to adequately mobilize all sources and efforts to fight against progressive development of the epidemic process in cities in Guinea and first disease cases in Liberia.

At the 3rd stage the disease spread into neighboring countries (Liberia and Sierra Leone) and there was an actual large-scale ES in public healthcare which remained undeclared by the WHO but already had international significance due to integral effects produced by predictors at the 1st and 2nd stages. "Risk groups" category became more and more significant at this stage, due to ethnic peculiarities, rumors, and conjectures regarding Ebola outbreak that had never previously occurred in cities and it made for faster infection spread, and to negative attitudes towards anti-epidemic activities, especially when deceased patients were buried [12].

At the 4th stage morbidity grew explosively in Guinea, Liberia, and Sierra Leone, spread into other countries on the continent (Nigeria, Senegal, and Mali) and beyond it (European countries and the USA). The predictor at the stage was late declaration by the WHO that the Ebola epidemic in the Western Africa was an internationally significant ES in public healthcare (8 months after the epidemic started), when the epidemiological situation reached a critical point next to actual spread of the disease beyond any control. We should also mention such a background predictor as insufficient range and efficiency of medications for treating and preventing Ebola. At this stage "risk groups" category remained the most significant one; it was sue to population who were exposed to the greatest risk to be infected and fall sick not willing to come to terms with the epidemic occurrence; people didn't want to cooperate when anti-epidemic activities were performed and actually tried to prevent them and fight against medical personnel who took enormous efforts to eliminate Ebola and prevent its spread [13–14].

Therefore, at the 4th stage in the Ebola epidemic in the Western Africa that was an internationally significant sanitary-epidemiological (biological) ES there occurred the most critical complex of biological properties that the infectious agent had (viral nature and I pathogenicity group), epidemiologic risk categories, and predictors; it is hardly possible to theoretically imagine a more critical complex in terms of threats to global security and to see how these threats find their realization in real life conditions.

We analyzed one more epidemic event; it was the pneumonic plague outbreak in Madagascar in 2017. It started on August 23, 2017

and by December 12, 2017 there were 2,529 registered disease cases, most of them (1,945 or 77 %) being pneumonic plague cases. Pneumonic plague was detected in 57 districts in the country out of total 114 (50 %), including non-endemic [15]. All the above data indicate there was a pneumonic plague epidemic in 2017 in Madagascar and it occurred when the overall epidemiologic situation in the world in terms of plague was more than favorable (3 cases detected in the USA).

The epidemic situation in 2017 in Madagascar had certain unusual features; the most significant one was that an annual number of people who got infected with pneumonic plague and a period of its growth (practically 3.5 months) turned out to be the highest in the world over the last 96 years. A pervious event in the historical periodical series was a pneumonic plague outbreak in Manchuria in 1920–1921 when several tens of thousands people got infected with the disease and died [16].

Today we can consider an epidemic situation that existed in Madagascar prior to the outbreak in 2017 quite "normal". According to the WHO data, from 2000 to 2016 there were 9,869 registered disease cases, their annual average quantity being equal to 580. There were two peaks in morbidity during that period, the 1st one occurred in 2000 when 1,333 people got infected with plague and 63 out of them died; the 2nd one was in 2004, 1,214 disease cases and 98 deaths accordingly. Starting from 2010 Madagascar has been occupying the 1st place in the world as per morbidity with plague [17]. A share of people who have pneumonic plague varies from 2 to 25 % [18]. All the plague cases in Madagascar are caused by *Y.pestis* strains that belong to the eastern type of biological plague agents. Overall, a rather unfavorable epidemic situation in terms of plague that exists in Madagascar is due to poorly organized and inefficient epidemiologic surveillance. However, the pneumonic plague outbreak that occurred in 2017 can't be explained only by epidemiologic surveillance being insufficient. Obviously, unusual functional peculiarities of epidemiologic risk categories had their own specific role in the epidemic process development.

According to data provided by the WHO Regional Office for Africa and results obtained via operative epidemiologic research, the pneumonic plague outbreak started on August 23, 2017, when a 31-year old man from Tamatave visited Ankazobe area on the Central plateau (a territory which was enzootic as per plague) and then had symptoms of a disease that was similar to malaria. When he went to Tamatave through Antananarivo using public transport (a fixed-run taxi), he suddenly felt himself seriously sick and was taken to Monmoranga district hospital where he died and was buried without any safety precautions taking during the burial. Overall, 31 people contacted him and got infected with the disease; later 4 out of them died. The Madagascar Public Healthcare Ministry officially informed the WHO about the pneumonic plague outbreak on September 13, 2017 after a 47-year old woman had died from a respiratory disease in Soavinandriana Hospital in Antananarivo on September 11, 2017. This secondary case was the reason to start epidemiologic research that allowed detecting the above-mentioned traveler who had been the first to get infected with the disease.

According to the above data, formal step-by-step defects in accomplishing epidemiologic surveillance oriented at timely detection of people who got infected with plague (and not epizooty) were as follows: experts missed the first person who got infected and fell sick with bubonic plague on a territory which was enzootic as per this infection; they neglected bubonic plague complications with the secondary pneumonic type and a death caused by the secondary pneumonic plague in hospital; the first person who died from the disease was buried without any safety precautions; a secondary pneumonic plague case and lethal outcome in hospital in Antananarivo was also missed.

So, defects in epidemiologic surveillance in 2017 resulted in two interrelated pneumonic plague cases and the disease being transferred from an enzootic territory into Antananarivo.

Those events could be interpreted as being quite usual for Madagascar, especially bearing in mind that the epidemiologic situation as per plague had already been rather unfavorable in 2000–2016.

But still, there were several signs that the pneumonic plague epidemic was large-scale and developed too rapidly; they were a large cluster of registered pneumonic plague cases among people who had contacted the first infected person (secondary cases); occurrence of tertiary cases and avalanche-like growth in pneumonic plague cases without any clear epidemiologic links registered in different parts of the country including areas which were not endemic as per plague and large cities (Antananarivo and Tuamasino).

As for epidemiologic risk categories, "risk groups" category should be the first focus of attention, in particular, the first infected person being unusually active; he was already sick with pneumonic plague and still moved around Antananarivo on his own for a long period of time taking public transport (a fixed-run taxi). Pneumonic plague in people usually tends to have grave clinical course and rapidly occurring lethal outcome. A closed space inside a vehicle and too many passengers getting on and off it created critically significant conditions for absolutely efficient transfer of the plague microbe from the first infected person to all people around him. The situation can be compared to an aerosol chamber where contagion of all live objects placed into it amounts to 100 %. It was the "aerosol chamber effect" that allowed creating a critical "pool" of people who fell sick with pneumonic plague; later on it developed into a full-scale epidemic and ES.

The first infected person, being a source of the infection, was simultaneously a risk factor in urban environment that became an anthropogenic risk territory, and all urban population who contacted the infected person became a risk group. When we speak about risk factors in this case, we, above all, should bear in mind the meaning of the word "factor" that is Latin in its origin and means "doing, making", a driving force of any process, in this case, an epidemic one[4].

Unusual functional properties of "risk territory" category were determined with the epidemic process spreading into large cities with high population density, developed transport infrastructure, and high migration activity of the population. Usually functionality of a risk territory in Madagascar is restricted to the Central plateau region that is endemic as per plague [19, 20] and where multiple cases of predominantly bubonic plague occur among population living in rural areas.

Epidemiologic significance of 'risk group" category is due to risk territories extending into urban areas, and as a result urban population being involved into the epidemic process and most pneumonic plague cases being registered exactly among people living in cities. 68 % people who got infected and fell sick with pneumonic plague were detected in Antananarivo. Usual functionality of "risk groups" category in Madagascar is related to rural population that account for more than 80 % diseases cases and predominantly suffer from bubonic plague that is annually aggravated with the secondary pneumonic plague in limited number of cases [21].

Unusual functionality of "risk period" category is due to an earlier start of an epidemic season, on August 23, 2017 (the date when the first person with pneumonic plague was registered) and the plague outbreak occurring in cities already in September; it was probably the reason why the first person with plague wasn't noticed and plague spread into Antananarivo earlier than it was expected. It then led to a rapid spread of the diseases, a lot of people falling sick with pneumonic plague, relatively rapid development of the process into a large-scale epidemic and ES. Usual functionality of "risk period" category in Madagascar is related to a seasonal growth in morbidity with plague that normally occurs in October and lasts up to late April.

When speaking about the pneumonic plague epidemic in Madagascar in 2017, we

[4] The foreign words dictionary. The 18th edition. Moscow, Russkiy yazyk Publ., 1989, 624 p.

should also remember that there could have been a potential predictor of its extremely unfavorable development. This predictor was plague microbes being resistant to antibiotics that were recommended to be used within the National Anti-plague Program. It had already happened in Madagascar in 1995–1998 [22]. In 2017 all plague microbe strains were sensitive to antibiotics and it was extremely important for establishing epidemic control over the disease.

Conclusion. We took two epidemics caused by infectious agents belonging to I pathogenicity group as our examples; both epidemics had specific features at the moment they started that were fixed in the IHR (2005) and considered to be signs of a future ES; to be exact, the epidemics were unusual, unexpected, and grave. We detected specific functionalities of epidemiologic risk categories and their combinations with predictors that were obvious ES precursors. Obtained data give grounds for more profound research on examples of a wider range of epidemic events in order to reveal the whole variational series of critical combinations of epidemiological risk categories and predictors; to create a relevant database; to monitor necessary data and work out targeted activities that would allow enhancing preventive potential of the IHR (2005) as regards emergency situations.

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RISK-ORIENTED APPROACH IN ANALYZING EPIDEMIOLOGIC SITUATION WITH INCIDENCE WITH TICK-BORNE ENCEPHALITIS ON ENDEMIC TERRITORIES

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Sverdlovsk region is a zone with a strenuous natural-anthropogenic focus of virus tick-borne encephalitis (TBE). Incidence with the disease has decreased by 5 times over the last 20 years due to mass vaccination among population. Since 2015 incidence with TBE has remained steady at fewer than 3 cases per 100,000 people. However, over the last 10 years incidence with TBE has been decreasing at a significantly slower rate due to a considerable growth in number of immune people (from 68 % in 2007 to 84.99 % in 2018). Analysis revealed that probability of the disease after a person had been bitten by a tick was quite different on different territories in the region.

Our research goal was to develop a procedure for ranking administrative territories as per risks of clinical TBE occurrence among people bitten by ticks.

We took a number of people bitten by ticks per one TBE case as our risk parameter. Our analysis revealed that average regional risk reached its maximum values (1:40–1:50) in years prior to implementation of mass vaccination against TBE. As a number of immune people grew, risk fell by 6 times (just 1 TBE case per 319 bitten people in 2018). Average regional risk was taken as to be equal to 1. We ranked administrative territories as per their risk index values (a ratio of a territorial risk to average regional one).

We showed that ranking of TBE-endemic territories as per their risk index allowed implementing a differentiated approach to planning and organizing efficient prevention.

Key words: *virus tick-borne encephalitis, epidemic process, incidence, mass vaccination, people bitten by ticks, disease risk index, risk index, ranking of endemic territories.*

Over the last 50 years there have been considerable changes in epidemiology of virus tick-borne encephalitis (TBE) due to occurrence of anthropogenic foci around cities located on endemic territories and urban population becoming involved into the epidemic process as a major risk group [1–4]. It resulted in changes in vaccination strategies involving implementation of extended vaccinal prevention up to mass immunization among popula-

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tion taking into account risks of contagion with TBE virus on this or that territory; those risks manifested themselves via levels of morbidity [5–8]. Impressing results of mass preventive vaccination against TBE among population were first achieved in Austria; when more than 80 % population were vaccinated against TBE, it allowed reducing morbidity by 15 times [9–11].

In the Russian Federation risks of TBE contagion among population differ significantly both on TBE-endemic regions and on specific territories within those regions [12]. The Urals Federal District is a region where morbidity with TBE is rather high; Sverdlovsk region is included into this Federal District. The whole territory of the region is a zone with intense natural-anthropogenic focus of tick-borne infections, virus tick-borne encephalitis (TBE) being one of the most significant [13]. Over more than 70 years of observation (1944–2018) there have been significant cyclic fluctuations in morbidity with TBE detected in Sverdlovsk region [14]. These cycles occur due to biological factors (periodical changes in numbers of natural tick carriers); weather and climatic factors (air temperature and humidity during a period when ticks are most active); and socioeconomic factors as well [15–17].

From 1956 to 1999 rises and falls in morbidity took their turns with 3–4 year intervals and their turns coincided with periods during which ticks were most active. After 1999 mass immunization among population was implemented, and dynamics of morbidity changed. Thus, from 1999 to 2011, duration of cycles involving rises and falls in morbidity with TBE taking their turns grew to 6 years; from 2012 to 2018 there was a clear trend in morbidity stabilizing at a low level with insignificant annual fluctuations (2.4–3.6 per 100 thousand people) [18].

A considerable amount of people who get infected and fall sick with TBE during annual rises in morbidity with TBE in spring and summer are those who haven't been vaccinated or their vaccination hasn't been completed. Over the last 25 years picks in morbidity with TBE were detected in 1993, 1996, 1999, 2005, and 2011 (24.0, 42.9, 19.3, 10.1, and 6.1 per 100 thousand people accordingly).

Morbidity with TBE among population living on endemic territories is well-known to directly depend on ticks being most active in spring and summer. There is a regular dependence between a number of people applying for medical aid due to tick bites and morbidity [12].

Over the last 20 years the epidemiologic service of the Regional Rospotrebnadzor Office in Sverdlovsk region has been actively performing targeted prevention activities and it has allowed achieving substantial results as regard reducing morbidity with TBE [19].

Since 1996 mass vaccination against TBE has been implemented within the regional prevention program; due to it starting from 2000 there has been a persistent descending trend in morbidity [20]. Since 2015 morbidity with TBE has remained insignificant, namely lower than 3 cases per 100 thousand people. In 2017 a share of regional population vaccinated against TBE reached 87.4 %. However, even now TBE cases are registered on 80 % of all administrative territories in the region. Average long-term morbidity (ALTM) with TBE amounts to 2.85 per 100 thousand people and it is by 1.65 times higher than on average in the country (1.72 per 100 thousand people). Annually up to 5 % of all people sick with TBE are those who have completed their anti-TBE vaccination. Having analyzed the structure of TBE clinical forms among vaccinated and non-vaccinated people, experts revealed that a specific weight of focal forms was by several times lower among vaccinated people than among non-vaccinated ones, and in some years (2011–2013) 100 % of vaccinated people had only fever TBE. Nevertheless, lethality among people suffering from TBE has been varying from 0.6 to 2.7 % over a long period of time.

We analyzed dynamics of the TBE epidemic process in Sverdlovsk region over the last 20 years; the analysis revealed that mass vaccination among population that started in 1998 allowed reducing morbidity with TBE in

Sverdlovsk region by more than 8 times by 2018 (down to 2.35 per 100 thousand people). At the same time, morbidity on 11 administrative territories was still 1.5–2 times higher than on average in the region; it was more than 2 times higher on 6 territories.

In 2018 84.99 % people living in Sverdlovsk region were vaccinated against TBE. On half of administrative territories the figure was even higher as more than 90 % people living there were vaccinated. However, over the last 10 years, in spite of a considerable growth in a share of vaccinated population (from 68 % in 2007 to 84.99 % in 2017), reduction in morbidity with TBE has slowed down considerably.

Except from 1996 and 2011, when there was a drastic rise in activity of ticks (2,027.8 and 1,325.7 bitten people per 100 thousand accordingly), over 1995–2018 a number of people bitten by ticks varied from 489.7 (the minimum in 2002) to 983 (the maximum in 2015) per 100 thousand people. A square where annual acaricide treatments took place increased by 10 times from 2000 to 2018 (from 969 hectares in 2000 to 9,498 hectares in 2018); however, it didn't result in any decrease in number of people bitten by ticks (565 and 749 per 100 thousand people in 2000 and 2018 accordingly). It indicates that a system of planning and organizing acaricide treatments on endemic territories needs substantial revising and adjusting.

A share of vaccinated people in Yekaterinburg (88.88 %) is higher than on average in the region (84.99 %). At the same time morbidity with TBE among population living in Yekaterinburg is annually higher than on average in the region (average long-term morbidity is 3.94 and 2.85 per 100 thousand people accordingly). About 30 % of the overall region population live in Yekaterinburg. The Yekaterinburg city square amounts to 0.6 % of the overall region territory, but people living in Yekaterinburg account for about 50 % of all patients with TBE (average long-term morbidity is equal to 55 out of 118 TBE cases in the region). The above data indicate it is necessary to analyze why people living in Yekaterinburg are not efficiently protected from TBE even though there is annual mass vaccination.

Comparative analysis performed with maps that showed how territories in the region were ranked as per such parameters as morbidity with TBE and average numbers of ticks revealed that morbidity with TBE among population living on this or that territory didn't correlate with high, average, or low numbers of ticks on it in most cases. Thus, zones with high quantities of ticks were located on the south and west-south of the region whereas terrorizes where morbidity with TBE among population was high were located along the meridian from north to south in sub-zones of mid-latitude and southern taiga. The analysis also revealed that a number of people bitten by ticks didn't correspond to morbidity with TBE on most territories, that is, a probability (risk) that a manifested disease could occur after a tick bite was different for population living on different territories.

Our research goal was to analyze long-term dynamics of the TBE epidemic process in Sverdlovsk region and, basing on the analysis results, to work out a procedure for ranking administrative territories as per a risk of a manifest disease among people bitten by ticks.

Data and methods. We took necessary data from the State Statistical Report Form No. 2 "Data on infectious and parasitic diseases" filled in by the Rospotrebnadzor's Federal center for Hygiene and Epidemiology, Sverdlovsk regional office. Data on a number of people bitten by ticks and a share of population who got vaccinated against TBE were obtained from annual reports on sanitary-epidemiologic welfare of the population issued by the Rospotrebnadzor Sverdlovsk Regional Office.

We took probability of a manifest TBE case after a tick bite as our risk parameter. We calculated average regional risks of manifest TBE among bitten people and those for specific administrative territories via finding a ratio between a number of bitten people and morbidity with TBE per 100 thousand people on a given territory.

We calculated risk index via determining a ratio between a risk calculated for an admin-

istrative territory and the average regional risk which was taken as being equal to 1.

Results and discussion. Collective immunity among population living on a given territory, share of infected ticks in a local tick population, and virulence of circulating TBE strains are basic factors that determine how high a risk of TBE occurrence is after exposure. This parameter is integral as it allows objectively assessing whether population living on a given territory is well-protected. Therefore, determining a risk of TBE occurrence in people who were bitten by ticks in different years and on different territories provides additional data for epidemiologic analysis and efficiency assessment for specific and non-specific prevention of the disease.

We can examine dynamics in such risks taking into account mass vaccination against TBE on the example of Sverdlovsk region. Figure 1 shows long-term dynamics of morbidity with TBE and risks of the diseases occurrence in people bitten by ticks.

As we can see from the data given above, risk was on average equal to 1 TBE case per 50 bitten people prior to mass vaccination and during first years after it has been started (1995–2002). This value is well in line with results obtained via monitoring over shares of infected ticks in populations in Sverdlovsk region which were on average equal to 2 %.

In 2018 risk decreased by 7.6 times in comparison with the period prior to vaccination (from 1:42 to 1:319). Over the same period of time morbidity went down by 5 times (average long-term morbidity being equal to 14.6 and 2.85 per 100 thousand people accordingly).

Figure 2 shows long-term dynamics of risks taking into account growing shares of people who got vaccinated against TBE in the region.

As we can see from the data given above, after 60 % of population got vaccinated, risk of the diseases started to fall dramatically. However, as more than 70 % of population got vaccinated in 2008, it leveled off at 1:200–1:250 (there were no statistically significant discrepancies, $p > 0.05$). Therefore, an increase in a share of population who got vaccinated over the last 10 years from 73 % to 85 % didn't have any significant influence on risk of TBE occurrence after a tick bite. But still, there was a persistent descending trend in morbidity. From 2008 to 2014 morbidity with TBE varied from 6.1 to 3.4 per 100 thousand people, and starting from 2015 it stabilized at fewer than 3 cases per 100 thousand people (Figure 1).

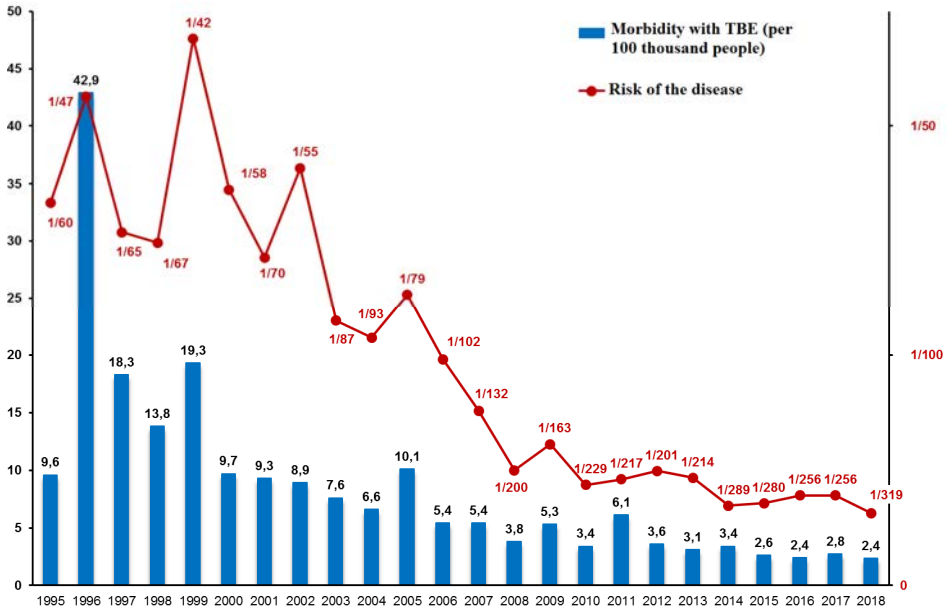

Figure 1. Long-term dynamics of morbidity with virus tick-borne encephalitis in Sverdlovsk region (per 100 thousand people) and risk of the disease (number of bitten people per 1 disease case)

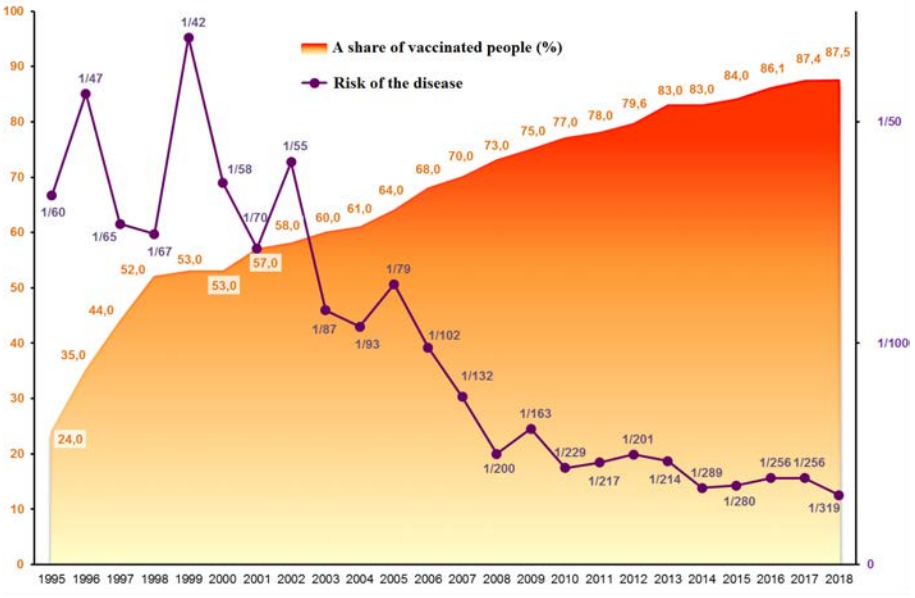

Figure 2. A share of people living in Sverdlovsk region who got vaccinated against virus tick-borne encephalitis and risk of the disease (number of bitten people per 1 disease case)

Figure 3 shows long-term dynamics of a number of bitten people and the risk of TBE occurrence among them.

As we can see from the given data, risk didn't change significantly from 2008 to 2018 even though there were considerable fluctuations in numbers of people bitten by ticks. It indicates there is no correlation between a number of people bitten by ticks and risks of TBE among those who were bitten.

Risk-oriented approach to analyzing epidemiologic situation with TBE allows spotting out administrative territories where there is certain divergence in parameters related to a number of bitten people and morbidity with TBE, in particular:

– territories where a number of bitten people is high but there are no TBE cases;

– territories where a number of bitten people is high but morbidity with TBE is close to average regional values;

– territories where morbidity is high but a number of bitten people is close to average regional values.

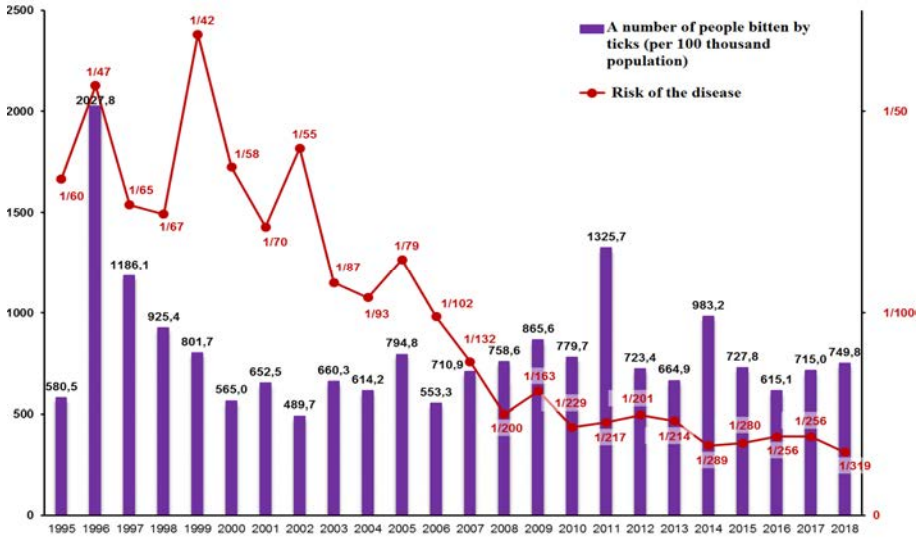

Figure 3. Long-term dynamics of a number of bitten people per 100 thousand people living in Sverdlovsk and risk of virus tick-borne encephalitis among them

Table 1

Parameters showing numbers of people bitten by ticks and morbidity with virus tick-borne encephalitis in specific municipal districts in Sverdlovsk region in 2017

Territory	A number of people bitten by ticks ($^0/_{0000}$)	A ratio of a number of bitten on a specific territory to average regional one	Morbidity with TBE ($^0/_{0000}$)	A ratio to average regional morbidity with TBE
Sverdlovsk region	715.00	1.00	2.75	1.00
Asbest district	1,528.50	2.10	1.50	0.50
Revda district	740.40	1.04	4.60	1.67
Polevsk district	855.60	1.20	7.10	2.60

Table 2

Risk of TBE occurrence among people bitten by ticks and a share of vaccinated people in specific municipal districts in Sverdlovsk region in 2017

Territory	Risk of manifest TBE among bitten people	Risk index	A share of people vaccinated against TBE (%)
Sverdlovsk region	1:260	1.0	87.4
Asbest district	1:1,019	0.3	96.3
Revda district	1:160.9	1.6	86.5
Polevsk district	1:120.5	2.2	73.1

The procedure includes:

1. Calculating average regional risk of TBE occurrence and risks for specific administrative territories via finding a quotient from dividing morbidity with TBE per 100 thousand people by a number of people bitten by ticks on a given territory.

2. Calculating risk index via calculating a ratio between risk for a given administrative territory and average regional risk that is taken as being equal to 1.

3. Ranking administrative territories as per risk index:

– high risk (index is ≥ 1.3);

– moderate risk (index is $0.70 < 1.0 < 1.3$);

– low risk (index is ≤ 0.70).

Should a territory be ranked as having high risk index, it is advisable to pay special attention to analyzing volumes and quality of vaccinal prevention, urgent immune TBE prevention, and examining collective immunity among population on such a territory.

In case risk is moderate (morbidity with TBE is equal to average regional one or higher and a number of bitten people is high), it is necessary to analyze volumes and efficiency of acaricide treatments as well as efficiency of sanitary-educational activities among population aimed at commutating how to keep safe in the forest and prevent tick bites.

Tables 1 and 2 contain an example on how to present and analyze data on risk-oriented approach to ranking territories that are endemic as per TBE.

As we can see from data given in the tables, a probability of catching TBE among people bitten by ticks can be different on different territories where a share of people who got vaccinated against TBE is also different. Thus, people living in Asbest district run the lowest risks of TBE even though a number of people bitten by ticks on that territory was 2.1 times higher than on average in the region, but still morbidity with TBE amounted to 1.5 per 100 thousand people and it was 1.8 times lower than on average in the region (2.75 per 100 thousand people). Therefore, a risk of manifest TBE occurrence was 3.3 times lower in Asbest district than in the region as a whole and it indicates that a high share of vaccinated

people (96.3 %) exerts significant influence on morbidity with TBE; on the contrary, there is no correlation between a number of people bitten by ticks and a number of TBE cases.

It means that it is advisable to implement specific and non-specific prevention activities on the territory; they should be scheduled and focus on selective immunization of risk groups with the highest risks of manifest TBE occurrence, namely, those age cohorts where a share of vaccinated people hasn't reached its target level (95 %). It is also necessary to pay special attention to assessing quality of performed acaricide treatments and sanitary-educational activities aimed at informing people about the necessity of timely immunization against TBE and rules of safe behavior in the forest that will protect them from tick bites.

In Revda district in 2017 morbidity with TBE amounted to 4.6 per 100 thousand people and it was 2 times higher than on average in the region although numbers of people bitten by ticks were relatively similar, 740.4 on the territory against 715.0 per 100 thousand people on average in the region. At the same time risk of manifest TBE occurrence among people bitten by ticks was 1.6 times high in Revda district than on average in the region. A share of people who got vaccinated against TBE was quite similar in Revda district to that in the region, 86.5 % against 87.4 %. In this case, it is clear that not enough people have been vaccinated in Revda district and it is advisable to take additional efforts aimed at scheduled immunization among children combined with immunization among adults who haven't been vaccinated in due time; it is also necessary to perform stricter control over timely revaccination among vaccinated people and to examine intensity of collective immunity against TBE.

In Polevsk district in 2017 there was a high risk of manifest TBE, 2.2 times higher than on average in the region; also a number of people bitten by ticks was by 20 % higher, and morbidity with TBE was 2.6 times higher against on average in the region. Only 73.1 % people living on the territory were vaccinated against TBE. All the above indicates that a low share of vaccinated people can't have any significant influence on a further decrease in morbidity with TBE in Polevsk district as the latter directly depends on a number of people bitten by ticks.

Therefore, a leading prevention activity in Polevsk district should be scheduled immunization among children accomplished within the Regional prevention vaccination schedule and mass immunization among those adults who haven't been vaccinated in due time. Not less than 95 % people should be vaccinated against TBE in all age cohorts.

To sum up all the above stated, we can conclude that a risk parameter gives an objective assessment of new TBE cases probability among population living on a specific territory and allows choosing priority prevention activities (specific or non-specific prevention) aimed at decreasing morbidity with TBE.

Conclusions:

1. Determining post-exposure risk of TBE occurrence on different territories (a number of people bitten by ticks per 1 TBE case) allows obtaining additional significant data for epidemiologic analysis and planning efficient prevention activities.

2. Ranking territories that are endemic as per TBE as per risk parameter allows objectively assessing whether population living there is well protected and implementing differentiated approach to planning and organizing efficient prevention activities.

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ISOLATION AND IDENTIFICATION OF β-LACTAMASE PRODUCING PSEUDOMONAS SPP. IN READY-TO-EAT RAW VEGETABLES

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Contamination of antibiotic-resistant bacterial pathogens in raw food is becoming an increased health risk in numerous countries, including Vietnam where raw herbs and vegetables are used daily in many dishes as a flavor enhancer and a source of vitamin and nutrients. However, raw vegetables can also be a reservoir of various foodborne pathogens such as Pseudomonas spp. and Enterobacteriaceae. In this study, we evaluated the extent of Pseudomonas spp. contamination in 180 ready-to-eat (RTE) vegetables samples from restaurants in Hanoi, examined their antibiotic susceptibility profiles and determined the ability to produce β-lactamase enzymes of Pseudomonas spp. strains. Our results showed that 21.67 % (n = 39) of ready-to-eat vegetables samples in Hanoi were contaminated with Pseudomonas spp.. Of those, sixteen samples were determined to be β-lactamase producing strains including Pseudomonas putida, P. mendocina and P. aeruginosa. Further analysis revealed six strains (37.50 %) producing extended spectrum β-lactamase (ESBL) enzyme, five strains (31.25 %) producing ampC β-lactamase enzyme and five strain (31.25 %) producing both ESBL and ampC β-lactamases. It can be concluded that ready-to-eat vegetables in Hanoi would be a source of contamination of β-lactamase producing Pseudomonas spp. that could pose a threat to public health in the community.

Keywords: *food, ready-to-eat vegetables, contamination of antibiotic-resistant bacterial pathogens, Pseudomonas spp., health risks.*

Ready-to-eat raw vegetables have become a favorable product among many consumers thanks to their high vitamin contents and low energy. In Vietnam, vegetables are common food in the daily life. According to the World Bank's report (2017), the consumption rate of vegetables in Vietnam is 0.4 kg/day/person, therefore, it can be estimated that 2,800 tons of vegetables are consumed everyday in Hanoi [1]. With a consumption of about 1 million tons per year, Hanoi must use supplies from vicinity provinces in the Red River Delta. However, vegetables can become a source of bacterial contamination to animals and humans due to polluted irrigation water and improper washing before they are sold at a marketplace. Thus, it is difficult to control hygiene and monitor safety for food product.

Pseudomonas spp. are versatile gram-negative bacteria that occur in various habitats such as soil, water and living organisms including animals, insects and human. In the genus, P. aeruginosa are a risk to human health as they cause serious diseases such as septicemia, liver damage or necrosis at in-

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jured sections. Other *Pseudomonas* species including *P. fluorescens, P. luteola, P. putida*, and *P. stutzeri* possess lower toxicity, but they can cause many infectious diseases in immune-compromised patients [2, 3]. Pseudomonas spp. often invade host tissue and cause infection and septicemia in immune-compromised hosts such as patients suffering from HIV/AIDS, cystic fibrosis, bronchiectasis and severe chronic obstructive pulmonary, burn, or patients after malignant or urinary removal [4]. Recently, pathogenic *Pseudomonas spp.* have become one of the prominent public health concerns due to their fast-evolving multi-drug resistance. Studies on disease treatment in patients show difficulties in combatting high multi-drug resistant *Pseudomonas spp*. [5].

Studies on bacteria extracted from raw vegetables sold at different markets and restaurants that have antibiotic resistant agents have been previously reported [5]. The prominent group of antibiotic resistant bacteria contaminants from raw vegetables is Pseudomonas spp. They are well known to have multiple antibiotic resistances due to gene scarring genetic factors that originated from other Gram-negative bacilli families such as *Enterobacteriaceae* through gene exchange [6]. Among these, the β-lactamase producing strains especially extended-spectrum β-lactamase (ESBL) and plasmid-encoded β-lactamase (AmpC) producers are of greatest interest. The emergence and spread of β-lactamase have become a significant global health problem, as they inactivate a wide group of antibiotics used in various hospital treatments [3]. However, until now, there have been no data on Pseudomonas spp. and β-lactamase producing Pseudomonas spp. in ready-to-eat vegetables in Vietnam. In this study, we aim to evaluate the presence of β-lactamase producing *Pseudomonas spp*. in ready-to-eat raw vegetables from restaurants in Hanoi.

Materials and methods. ***Vegetables sampling.*** A total of 180 ready-to-eat raw vegetables samples were collected at restaurants in six urban districts of Hanoi from July to October, 2018. Six districts including CauGiay, Hoang Mai, Ha Dong, Dong Da, Hai Ba Trung, Long Bien were selected based on the high rate of population. After being acquired from restaurants, vegetables samples were finely-sliced without any further washing but brown-leaf removing. For every sample, the amount of 25 grams of sample was added into 225 mL of buffered peptone water (BD, America). The mixture was then homogenized for 30 seconds using a homogenizer. Weight 25 grams of each finely-sliced leaf-sample and homogenizing for 30 seconds using homogenizer. The mixtures were then diluted to desired concentration by using peptone (0.1 %) saline diluent.

Bacterial isolation and identification. *Pseudomonas spp*. are extracted with a conventional method according to ISO 13720: 2010 [7]. Briefly, 0.1 mL of homogenized sample was spread on *Pseudomonas* CFC/CN (Merck, Germany) agar supplemented with CFC Selective supplement (Merck, Germany) and the plates were incubated under aerobic conditions at 25 $^{\circ}$C for 44 ± 4 hours. After incubation, colonies were confirmed using Oxidase Strips (Merck, Germany). Oxidase-positive colonies identified as *Pseudomonas spp*. *E. coli* ATCC25922 (ATCC, America) were used as negative control while *P. aeruginosa* ATCC 27853 (ATCC, America) strain was used as the positive control. Confirmed *Pseudomonas spp*. strains were further analyzed using Vitek®MS system (BioMerieux, France) to identify their species. Vitek®MS procedure was performed according to manufacturer's recommendations.

Identification of β-lactamase producing Pseudomonas spp. To determine *Pseudomonas spp*. producing β-lactamase, susceptibility testing with disc diffusion method was performed and interpreted following the technique recommended by the World Health Organization and the guidelines by

the American Institute for Testing and Clinical Standards (CLSI) [8, 9]. The antibiotics being examined included cefotaxime (CTX, Liofilchem, Italy), cefotaxime combined with clavulanic acid (CTL, Liofilchem, Italy), ceftazidime (CAZ, Liofilchem, Italy) and ceftazidime combined with clavulanic acid (CAL, Liofilchem, Italy). The strains are confirmed to be extended-spectrum β-lactamase (ESBL) producing ones when the observed CTL or/and CAL resistance zones are at least 5 mm greater than the observed CTX and CAZ resistance zones.

The presence of AmpC β-lactamase enzyme was determined by testing strains against cefotaxime (CTX, Liofilchem, Italy), cefotaxime combined with cloxacillin (CTC, Liofilchem, Italy), ceftazidime (CAZ, Liofilchem, Italy) and ceftazidime combined with cloxacillin (CAC, Liofilchem, Italy) following CLSI guidelines. When the observed CTC or/and CAC resistance zones are at least 5 mm greater than the observed CTX and CAZ resistance zones, the tested bacteria are confirmed to be AmpC β-lactamase producing strains.

Results and discussions. *Pseudomonas spp. in raw vegetables.* The obtained results revealed that 39 over 180 samples of ready-to-eat vegetables collected at restaurants (Grilled duck, bun cha, bread, spring rolls, pho, vermicelli, and noodle) were found to be positive with *Pseudomonas spp*. assuming oxidase-positive colonies. The finding of this study revealed that *Pseudomonas spp*. were prevalent (Figure 1).The difference in *Pseudomonas spp*. prevalence among sampling locations (districts) was not significant ($P > 0.05$).

Similar to our results, the prevalence of *Pseudomonas spp*. on ready-to-eat vegetable was also reported in former studies in several countries such as Italy ($n = 24$), India ($n = 12$ isolates were collected from tomato, cucumber and potato), and Nigeria ($n = 82$) [5, 10, 11]. *Pseudomonas spp.* are known to be the most common bacteria involved in spoilage of many kinds of foods, due to their very simple nutritional requirements and metabolic versatility that allows them to live in variable environments. The soil and irrigation water contamination may cause the prevalence of *Pseudomonas spp*. in ready-to-eat vegetable. Numerous studies have demonstrated that water plays important role in directly introducing bacteria into food during pre-harvest and postharvest stages [12–14]. It is during the pre-harvest phase that vegetable might be contaminated with *Pseudomonas spp*. due to infected irrigation water. Furthermore, for postharvest processes, ranging from storage and washing before selling, might lead to contamination.

Identified Pseudomonas spp. by using Vitek®MS. The obtained colonies were identified on the Vitek®MS. We detected 3 strains of *Pseudomonas spp*. including opportunistic

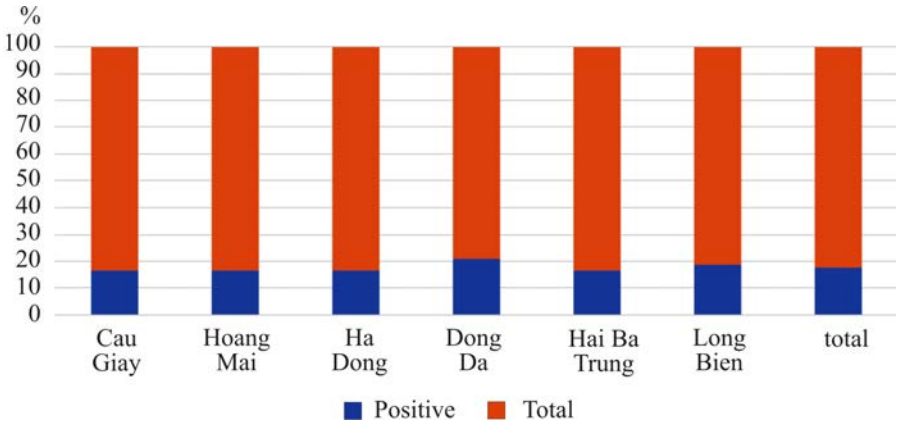

Figure 1. Percentage bar chart illustrating the prevalence of *Pseudomonas* spp. on ready-to-eat vegetable in restaurant in Hanoi

pathogens (*Pseudomonas aeruginosa, Pseudonasmendocina and Pseudomonas putida*). Among them, *P. putida*are accounted for the majority with 36 out of 39 strains (92 %). There were also 2 strains of *P. aeruginosa* (5 %) and 1 strain of *P. mendocina* (3 %) (Figure 2).

The identification results show a similarity of more than 90 % compared to the source library of the VITEK MS identifier system, from 99.4 % to 99.9 % for *P. putida* and 99.9 % for *P. aeruginosa* and *P. mendocina* (Figure 3).

The results in this study are similar to those obtained in the previous research on *Pseudomonas* spp. presence in raw vegetables by Caldera et. al. (2016), and the study by Franzetti et. al. performed on vegetable samples collected in Milan in 2007 as well [10, 15]. Another study conducted by Devarajan et. al. (2017) in Congo, India and Switzerland revealed occurrence of 141 strains of *Pseudomonas* spp. in untreated hospital wastewater samples and domestic wastewater sources; *P. putida* (42 %) and *P. aeruginosa* (39 %) were the majority in detected *Pseudomonas spp*. Another study by Kittinger et. al. conducted in 2016 on water of the Danube river (Austria) showed that 66.0 % (n = 520) isolates were identified as *Pseudomonas putida* and 27.1 % were *Pseudomonas fluorescens*, 2 *Pseudomonas aeruginosa*s strains, and less than five other *Pseudomonas* strains. A large number of *Pseudomonas spp*. was also found in raw and fermented vegetables in previous studies [16, 17]. The presence of animal dung containing *Pseudomonas spp*. used as a fertilizer or accidentally in the soil can be a source of contamination of Pseudomonas spp. on vegetables. Moreover, in most developing countries as well as Vietnam, wastewater is discharged from sewage directly into rivers and lakes that are used as irrigation water sources for cultivation, leading to *Pseudomonas spp*. occurrence in raw vegetable products. Meanwhile, should improper cleaning steps be applied at restaurants they are not effective enough and don't allow removing bacteria from food products making ready-to-eat vegetables infected.

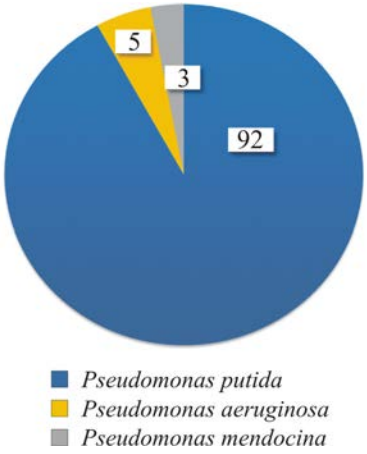

Figure 2. Percentage of *Pseudomonas* spp. isolates in this study

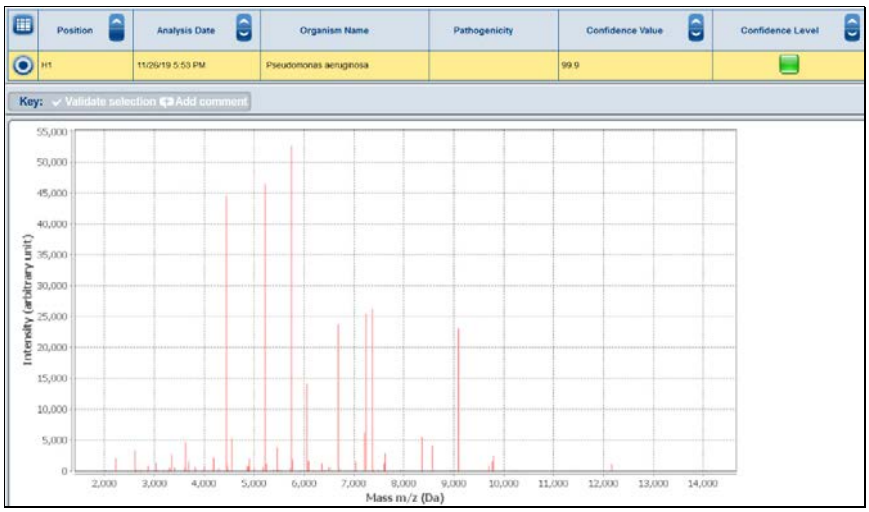

Figure 3. *Pseudomonas aeruginosa* spectrum of isolate from ready-to-eat vegetable

Table 1

Pseudomonas spp. producing β-lactamase enzyme

Pseudomonas spp.	District	Vegetables	ESBL	AmpC	ESBL &AmpC
P. aeruginosa	CauGiay	Saw-leaf, Perilla leaf, Vietnamese basil	+	+	+
P. putida		Thai basil	+	-	-
P. putida		Buttercrunch lettuce, Vietnamese basil, Mint leaf	+	+	+
P. putida	Hoang Mai	Perilla leaf, mint leaf	-	+	-
P. putida		Vietnamese Balm, Perilla leaf, mint leaf, cucumber	+	-	-
P. putida		Cilantro, Vietnamese Balm, Buttercrunch lettuce	-	+	-
P. putida	Ha Dong	Mint leaf, Buttercrunch lettuce	+	-	-
P. putida		Vietnamese Balm, Buttercrunch lettuce	-	+	-
P. putida	Dong Da	Mint leaf, Lettuce, Vietnamese Balm	+	-	-
P. putida		Buttercrunch lettuce, Perilla leaf, Cilantro	-	+	-
P. putida		Green Onion, Mint leaf	+	+	+
P. putida	Hai Ba Trung	Thai basil, Saw-leaf	+	+	+
P. aeruginosa		Thai basil, Saw-leaf	+	+	+
P. putida	Long Bien	Cilantro, Green onion	+	-	-
P. putida		Buttercrunch lettuce	-	+	-
P. putida		Buttercrunch lettuce, Perilla leaf	+	-	-

Note: + Positive
- Negative

3.3 Pseudomonas spp.producing β-lactamase enzyme. 39 identified strains of *Pseudomonas spp.* were used for antimicrobial testing corresponding to each β-lactamase enzyme. The results showed that 16 strains of *Pseudomonas spp*., which were detected in vegetables from restaurants in 6 districts, produced β-lactamase enzyme, of which 6 strains produced an expanded spectrum of β-lactamase enzyme (ESBL), 5 *Pseudomonas* strains produced enzyme β-lactamase AmpC, and 5 strains produced enzyme ESBL and AmpC as well. Whole 16 strains, were *P. putida and P. aeruginosa*, in which 2 *P. aeruginosa* and 3 *Pseudomonas putida* produced both ESBL and β-lactamase enzyme (Table 1).

In the study conducted in Nigeria in 2016 by Odumosu et. al. *P. aeruginosa* were detected in 54 out of 82 vegetable samples; 10 out of these 54 strains were identified with ESBL enzymes [5]. The result of Odumosu's study is higher than ours, due to the difference in geography, sample size, conditions of cultivation and sanitation as well. Despite of the fact that ready-to-eat vegetables are a good source of vitamins and nutrients, they can also be a source of pathogenic contamination hazardous for humans and transfer antibiotic-resistant bacteria to them.

Conclusion. The study showed prevalence of 16 *Pseudomonas spp.* strains producing β-lactamase enzyme in 180 samples of ready-to-eat vegetables collected in Hanoi. The number of *Pseudomonas* member found in ready-to-eat vegetable in our study was lower than in former studies from some countries worldwide. The difference in geography, sample size and sample collection location might lead to different results among studies.

It is necessary to conduct further studies on genetic characteristics and ability to transfer antibiotic resistant genes to other gram-negative bacteria in same ecological conditions.

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PROCEDURE FOR ASSESSING RISKS OF AN INFECTIOUS DISEASE BEING IMPORTED AND SPREAD IN THE RF REGIONS EXEMPLIFIED WITH MEASLES IN 2018

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The authors describe a procedure for assessing risks of the measles virus being imported and spread in certain RF regions; they also give results of test risk assessment in 2018 in relation to the FIFA World Cup 2018.

Risks of the virus being imported and spread in the country were assessed in accordance with a score estimation procedure aimed at assessing both internal and external risks. The procedure was developed basing on the document issued by the WHO "Rapid risk assessment for acute public health events. WHO-2012".

We detected risk factors that could make for the measles virus being imported and factors that made for secondary spread of the virus once it was imported. All the RF regions were assessed as per each factor and assigned into three categories, namely regions with high, average, and low risks of the measles virus being imported and spread. We visualized the results and presented them on maps.

As per our research results, "risk territories" are RF regions located at the state borders as well as regions where epidemiologic surveillance over measles is inefficient and there hasn't been sufficient immunization against the disease. These regions are Moscow city, Saint Petersburg, Voronezh region, Moscow region, Kaliningrad region, Samara region, Amur region, Rostov region, Sverdlovsk region, Krasnodar region, the Chechen Republic, Dagestan, Primorye, and Khabarovsk region.

Applying risk assessment in epidemiology involves developing such a technique for examining and forecasting an epidemiologic situation which will allow determining influence exerted on them by risk factors. This procedure enables ranking problems as per their significance and minimizing or eliminating possible risks.

Key words: *risk assessment, epidemiology, measles, risk-oriented surveillance, infectious diseases being imported into a country, aerogenic contagion, FIFA World Cup, tourism.*

Social and economic structure of the contemporary world society involves growing inter-continental and inter-state migration as well as intensifying international relations; it exerts significant influence on spread of communicable diseases from country to country [1].

Mass international events play a substantial role in tourist flows becoming more and more intense. Such events are trade fairs, congresses, festivals, exhibitions, and sport events and they usually involve short-term but mass inflow of participants or sport fans from a great number of countries including those that are endemic as per extremely dangerous quarantine infections. Therefore, it is truly vital to promptly assess epidemiologic risks in the contemporary world with life in it becoming faster and faster.

Respiratory viruses occupy the most significant place in human infectious pathology as they tend to cause mass communicable diseases. Wide spread of acute viral respiratory infections makes it necessary to develop rapid and efficient procedures for predicting new mass contagions in order to improve epidemiologic surveillance and accelerate elimination of viral infections.

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Measles is one of the most contagious diseases among known communicable ones. Almost 100 % people are susceptible to this infection and vaccination remains the most efficient way to fight against measles. People who were not vaccinated remain susceptible to measles during their lifetime and can catch it at any age. As per data provided by the WHO, the situation with measles is unfavorable in many countries all over the world [2].

The WHO European Office felt inspired with successes achieved in eliminating poliomyelitis; its experts developed and implemented a strategic program aimed at preventing measles and congenital rubella in the Europe in 2002.

The basic goal of the program was to eliminate measles and to prevent congenital rubella by 2010[1] [3–4]. Prior to these initiatives aimed at eliminating measles and rubella, 562,000 children died annually all over the world due to complications caused by measles.

As per data provided by the WHO, as more and more people got vaccinated, an apparent descending trend appeared in morbidity with measles in different regions in the world. Predicted long-term morbidity with measles was so low that it allowed believing measles would be eliminated completely in 2015. Despite significant successes achieved due to initiatives aimed at eliminating measles and rubella, 115,000 children, mostly those younger than 5, annually die from these diseases all over the world. It is approximately 314 people per day or 13 deaths per hour.

Initially, experts planned to eliminate measles in 2010–2015; but now elimination seems to move into distant future. Morbidity with measles registered in Europe in 2016–2018 was considered by the WHO as epidemic. Thus, in 2018 approximately 60 thousand measles cases were registered in European countries. In 2018 high morbidity was registered in Ukraine (1,209.25 per 1 million people), Serbia (579.3 per 1 million people), Albania (499.6 per 1 million people), Georgia (563.8 per 1 million people), Monte Negro (322.6 per 1 million people), and Greece (196.8 per 1 million people) [5–8]. 43 % patients were adults older than 20. Measles epidemic process in Russia also became more active. Morbidity with measles grew by 3.5 times in 2018 against 2017 and amounted to 17.3 per 1 million people (4.9 in 2017). 2,538 measles cases were registered. Low protection against the infection among adult population is the basic reason for this growth. Over recent years European countries have faced certain difficulties including a reduction in scales of scheduled immunization, stably low immunization among marginal population groups, troubles with vaccine supplies, and inefficient performance by epidemiologic surveillance authorities.

Countries take complex measures aimed at eliminating existing outbreaks and preventing new ones. Such measures include more intense communication about the problem with the society; immunization among medical personnel and other adult population groups who run high risks of the disease; elimination of any obstacles preventing a person form being vaccinated; better planning and material support for vaccine supplies [9].

In the present work the authors first selected and then analyzed criteria for assessing external and internal risks of measles viruses being imported onto the RF territory.

Our research goal was to work out a procedure for a score estimate of risks that a communicable disease would be imported and then spread in the country; the next goal was to assess risks of measles virus being imported and spread in certain RF regions.

The following tasks were to be solved:

– to work out criteria for assessing external and internal risks that an epidemiologic situation as per measles would deteriorate;

– to assess external risks that measles virus would be imported onto the RF territory, in particular, migration flows and communications with countries where the situation with measles is adverse;

[1] "The Program "Elimination of measles and rubella in the Russian Federation" (2016–2020)" (approved by Rospotrebnadzor on December 28, 2015, The RF Public Healthcare Ministry on December 31, 2015). Laws, codes and legal acts of the Russian Federation. Available at: https://legalacts.ru/doc/programma-eliminatsija-kori-i-krasnukhi-v-rossiiskoi-federatsii-2016/ (10.06.2019).

– to assess internal epidemiologic risks that measles virus would spread, in particular, quality of epidemiologic surveillance over measles and number of people vaccinated against the disease; to reveal "risk territories" (RF regions that were the most susceptible to risks of measles virus being imported and spread across them);

– to develop a draft procedure for score estimate of risks that measles virus would be imported and spread.

Data and methods. We took data from the Federal Statistic Service on population migration; data from the Statistic Form No. 2 "Data on infectious and parasitic diseases" over 2015–2018; data from the Statistic Form No. 6 "Data on children and adult groups vaccinated against communicable diseases" over 2015–2018; data on a number of border checkpoints in RF regions where people can cross the RF State Border; data of "The national vaccination schedule" on mass events with international participation.

We applied a procedure for score estimate of external and internal risks. When selecting criteria and working out our risk assessment procedure, we followed the Guide entitled "Rapid risk assessment of acute public health events" developed by the World Health Organization (WHO) in 2012 [9]. A direct choice on these or those assessment criteria depended on properties of data that characterize an epidemic process and risk factors in their quantitative equivalent; such data were to be used in operative risk assessment and therefore they should be available, reliable and easy to obtain in due time. Digital values that characterized factors of a communicable diseases import and spread were taken from state observations and other open data sources. Each risk factor was given a score estimate, from 0 (minimum) to 2 (maximum) depending on how active it was. We chose this exact factor gradation based on epidemically significant values in order to make the model simpler and calculations more rapid. A similar procedure for score estimate of parameters was used by several researchers to quantitatively assess potential epidemiologic threats; in particular, when mass sport events took place [11–13]. Each RF region was assessed as per all the criteria for assessing external and internal risks. Then those scores were summed up and ranked as per their value; after that we calculated sigma (standard deviation) and a rank was assigned to each region depending on its value. We created a "calculator" based on Microsoft Excel for scores being automatically distributed as per input data and preset criteria. Having performed all the necessary calculations, we divided all the RF regions into three categories: territories with high, average, and low risks of measles virus being imported and spread; we also visualized our results on maps with specific GIS software (Map Info 16 Pro).

Results and discussion. Risk assessment is a systematic process of collecting, analyzing, and documenting data in order to determine levels of examined risks. It gives grounds for efficient measures aimed at reducing and eliminating consequences of events that imposed direct threats for population health.

In order to assess risks related to measles virus being imported and spread across the RF territory according to the procedures developed by the WHO, we determined internal and external risk factors that could make for deterioration of the epidemiologic situation in the country (Tables 1 and 2). Depending on values of these factors, all the RF regions were to be assigned into three categories:

– low risk territories (the lowest probability that the epidemiologic situation would deteriorate in comparison with other regions in the country);

– average risk territories (a risk that the epidemiologic situation might deteriorate is equal to average country level or differs from it insignificantly);

– high risk territories or "risk territories"; they are territorial units (geographic or administrative ones) where morbidity and/or its consequences are higher or a certain epidemiologic phenomenon is more prevailing than on other comparable territories [14]).

International migration is a primary criterion of external risks. According to official Rosstat reports, annually people from countries where the situation with measles is adverse come to the Russian Federation (Table 3). These data don't include illegal migration and

transit passengers from these countries. At present world airlines annually transport more than 2 billion passengers and it creates greater opportunities for rapid spread of communicable diseases and their carriers. Contemporary tourism is one of the largest and most rapidly developing spheres in the world economy due to intensifying globalization and development of international transportations [15].

As per data taken from Rosturism report entitled "Selected statistic data calculated according to the official statistic methodology for assessing a number of in and out tourist trips", annually more than 24 million foreign citizens come to the Russian Federation as tourists. In 2017 more than 39 million Russian citizens made tourist trips abroad and it was by 25 % higher than in 2016.

Table 1

Criteria for external risk assessment

An international air border checkpoint to cross the RF State Border	0 scores, absent; 1 score, cargos and goods movement; 2 scores, people movement.
An international motorway border checkpoint to cross the RF State Border	0 scores, absent; 1 score, cargos and goods movement; 2 scores, people movement.
An international railway border checkpoint to cross the RF State Border	0 scores, absent; 1 score, cargos and goods movement; 2 scores, people movement.
An international marine border checkpoint to cross the RF State Border	0 scores, absent; 1 score, cargos and goods movement; 2 scores, people movement.
An international river border checkpoint to cross the RF State Border	0 scores, absent; 1 score, cargos and goods movement; 2 scores, people movement.
Long-distance railways	0 scores, absent; 1 score, passenger transportation on the RF territory; 2 scores, international passenger transportations.
Migration	0 scores, migration is lower than on average in the country; 1 score, migration is equal to that on average in the country; 2 scores, migration is higher than on average in the country.
Mass events with international participation to take place in a region	0 scores, not scheduled; 1 score, one or more such events are scheduled

Table 2

Criteria for internal risk assessment

Average morbidity with measles over 2009–2017	0 scores, morbidity is lower than 1 per 1 million people; 1 score, morbidity is higher than 1 but lower than 5 per 1 million people; 2 scores, morbidity is higher than 5 per 1 million people
Measles outbreaks in a region over the last year	0 scores, no measles outbreaks; 1 score, one or more measles outbreaks
Number of people vaccinated against measles in a region	0 scores, 97 % or more people vaccinated; 1score, 95–97 % people vaccinated; 2 scores, less than 95 % people vaccinated
Territories (districts) in a region where less than 95 % population are vaccinated against measles	0 scores, no such districts; 1 score, one such district in a region; 2 scores, more than 1 such district in a region
Number of infants younger than 1 year per 100 thousand people over the last year	0 scores, fewer than 10,000 infants in a region; 1 score, more than 10,000, but fewer than on average in the country (20,714); 2 scores, more infants than on average in the country (20,714)
Deaths from measles over the last five years	0 scores, no deaths; 1 scores, there are deaths from measles

Table 3

Number of people who came to the Russian Federation from countries that are endemic and adverse as per measles virus over 2010–2017

	2010	2011	2012	2013	2014	2015	2016	2017
Ukraine	27,508	43,586	49,411	55,037	126,819	194,180	178,274	150,182
France	150	322	326	352	351	360	303	346
Greece	298	614	835	995	694	557	450	419
Germany	2,621	4,520	4,239	4,166	3,743	3,976	4,153	3,704
Bulgaria	214	371	353	419	346	392	293	238
Czechia	112	157	193	192	160	180	148	151
Spain	140	201	253	364	303	279	218	227
Serbia	159	600	576	943	860	682	589	769
India	110	1,390	1,068	1,451	1,850	2,894	4,768	5,622
Tajikistan	18,188	35,087	41,674	51,011	54,658	47,638	52,676	63,467
Kirgizia	20,901	41,562	34,597	30,388	28,543	26,045	28,202	41,165
Turkmenistan	2,283	4,524	5,442	5,986	6,038	6,539	7,242	8,734
The United Kingdom	125	166	182	221	185	273	226	375

Also, we took another external risk criterion and it was activity of international migration in a region and reasons for its occurrence. First of all, we considered its possible pathways or international border checkpoints where the RF State Border could be crossed. Then, we considered mass events with international participation that took place in a region.

As per results obtained via score estimate, all the regions were ranked and it allowed revealing regions with the highest risks that measles virus could be imported there in 2018. They were Kaliningrad region (6 scores); Krasnodar region (6 scores); Moscow city (5 scores); Saint Petersburg (5 scores); Rostov region (5 scores); Dagestan (5 scores); Primorye (5 scores); Moscow region (4 scores); Khabarovsk region (4 scores); Crimea (4 scores). 4 scores were a threshold for assigning a region into high external risk category.

For better visualization, analysis results are given on a map in Figure 1.

Figure 1. RF regions distributed as per external risks of measles virus import in 2018

GIS technologies are now a significant element in epidemiologic surveillance. Medical-geographic maps are a relatively new type of subject maps. Drawing up such a map requires specific knowledge and approaches [16]. Bearing in mind, that a map is always a better visual than a table, we applied a GIS software program Map Info Pro 16 to create a specific cartographic model as per results of our calculations; the model gave a tentative picture of an actual situation.

When analyzing possible outcomes resulting from import of communicable diseases, it is necessary to differentially assess probable secondary spread of a disease and potential threat that it could persist in new conditions as probability of such outcomes is determined by different sets of factors [17].

We selected several internal risk factors that could make for a measles outbreak; they were criteria describing quality of epidemiologic surveillance in each specific region:

– average morbidity with measles per 1 million people over 2009–2018; measles outbreaks in the last year;

– a share of people vaccinated against measles in a region;

– districts within a region where less than 95 % people were vaccinated against measles;

– number of infants younger than 1 year per 100 thousand people.

Sanitary-epidemiologic authorities can influence external risks only indirectly; but as for internals ones, obviously quality of sanitary-epidemiologic surveillance is a direct responsibility of sanitary-epidemiologic authorities and public healthcare organizations in a RF region.

Certain RF regions ran the highest risks of measles outbreaks in case the virus was imported into them in 2018. They were Chechen Republic (6 scores); Moscow city (5 scores); Dagestan (5 scores); Ingushetia (5 scores); Kabardino-Balkaria (5 scores); Karachai-Cherkess (5 scores); Yakutia (5 scores); Voronezh region (4 scores); Kaluga region (4 scores); Moscow region (4 scores); Krasnodar region (4 scores); Astrakhan region (4 scores); Rostov region (4 scores); Samara region (4 scores); Sverdlovsk region (4 scores); Tomsk region (4 scores); Amur region (4 scores) (Figure 2). 4 scores were a threshold for assigning a region into high internal risk category.

Then we combined both groups of criteria, determined combined risks, and ranked territories as per them (Figure 3).

“Risk territories” in terms of measles virus import and transfer in 2018 were Moscow city (10 scores); Krasnodar region (10 scores); Dagestan region (10 scores); Rostov region (9 scores); Moscow region (8 scores); Kaliningrad region (8 scores); Voronezh region (7 scores); Saint Petersburg (7 scores); Chechen Republic (7 scores); Samara region

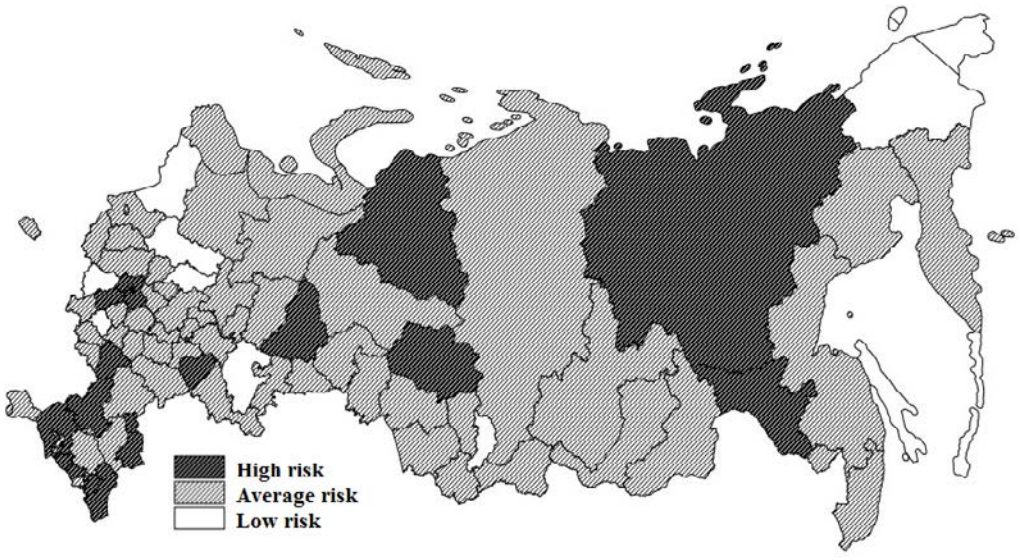

Figure 2. RF regions distributed as per internal risks of measles virus spread in 2018

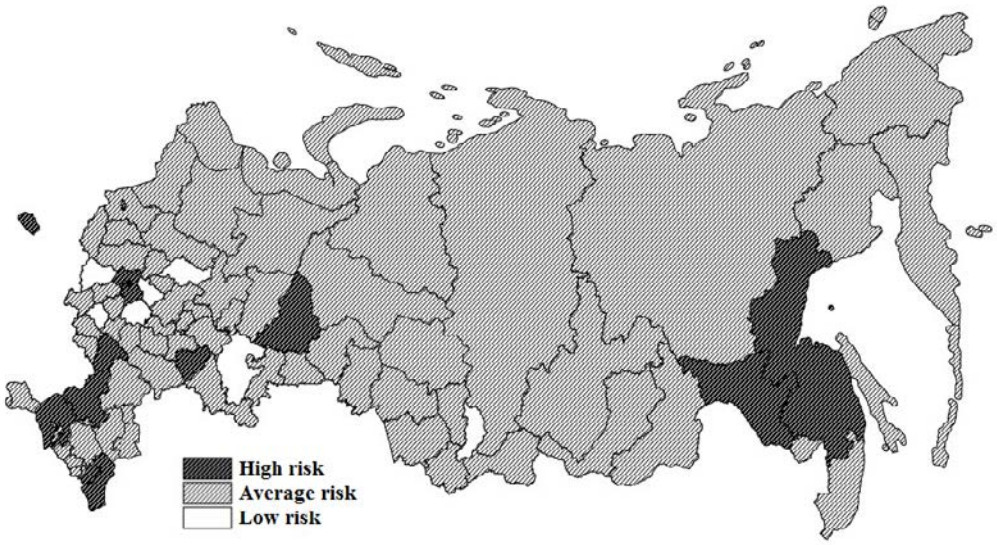

Figure 3. RF regions distributed as per external and internal risks of measles virus import and spread in 2018

(7 scores); Sverdlovsk region (7 scores); Khabarovsk region (7 scores); Amur region (7 scores). 7 scores were a threshold value to assign a region into "risk territory" category.

We should note that Moscow city is the only RF region where both external and internal risks are high. Obviously it is due to most international communications being concentrated in the city.

Therefore, our research allowed us to reveal regions with high, average, and low risks basing on score estimates given for external and internal risk parameters. As expected, border regions in the country turned out to be risk territories with both external and internal risks being high; the category also included regions where epidemiologic surveillance over measles was inefficient and not enough people were vaccinated against the disease.

Having analyzed morbidity with measles in RF regions in 2018 (data taken from the Statistic Form No. 2 "Data on infectious and parasitic diseases"), we revealed regions where morbidity was higher than 5 cases per 1 million people (Table 4).

Then, we assessed whether the model was relevant. To do that, we compared predictions and actual morbidity on the assumption that if actual figures differ from risk levels (being lower), it can't be interpreted as errors in results obtained with the prediction model. Coincidence amounted to 68 %.

Also, when comparing predictions with actual figures, we revealed three RF regions where morbidity differed drastically from their risk group. They were Kaluga region, Kostroma region, and Bashkortostan. Morbidity was unexplainably high in these three regions bearing in mind criteria obtained for them via assessing risks of the disease spread in case measles virus was imported into them and data obtained via statistic observations. It can be probably due to some unaccounted factors or a dramatic deterioration of epidemiologic surveillance over measles virus in 2018 in these regions. It can also indirectly imply that statistic data are collected and present rather improperly in these RF regions.

It was also important to analyze morbidity in 11 regions (data taken from the Statistic Form No. 2 "Data on infectious and parasitic diseases") where events of FIFA World Cup 2018 took place, namely:

- Moscow, 926 registered measles cases;
- Krasnodar region, 66 registered measles cases;
- Saint Petersburg, 54 registered measles cases;
- Rostov region, 49 registered measles cases;
- Samara region, 22 registered measles cases;
- Tatarstan region, 12 registered measles cases;
- Sverdlovsk region, 8 registered measles cases;

Table 4

Regions where morbidity with measles was higher than 5 cases per 1 million people in 2018

RF region	Morbidity per 1 million people	RF region	Morbidity per 1 million people
Dagestan	89.42	Leningrad region	11.65
Kaluga region	85.85	Rostov region	11.60
Moscow city	74.42	Kalmykia	10.85
Moscow region	53.59	Kostroma region	10.84
Karelia	46.42	Astrakhan region	10.80
Adygei	39.70	Tula region	10.70
North Ossetia	34.16	Saint Petersburg	10.16
Ingushetia	33.04	Yamal-Nenets Autonomous Area	9.31
Chechen Republic	28.75	Bashkortostan	8.61
Stavropol region	26.76	Karachai-Cherkess	8.58
Transbaikalia	25.10	Penza region	8.23
Vladimir region	24.57	Primorye	7.82
Tambov region	24.11	Sevastopol	6.93
Novosibirsk region	19.40	Magadan region	6.90
Crimea	19.34	Samara region	6.88
Khanty-Mansi Autonomous Area	15.15	Kamchatka	6.35
Krasnodar regiom	11.81	Khabarovsk	5.26

- Volgograd region, 6 registered measles cases;
- Nizhniy Novgorod region, 4 registered measles cases;
- Kaliningrad region, 2 registered measles cases;
- Mordovia, 1 registered measles case.

Seven out of these regions were “risk territories” as per measles virus according to the prediction; four were assigned into “average risk” category in terms of measles virus being imported and spread across them. Undoubtedly, the FIFA World Cup made a significant contribution into an increase in morbidity with measles in the regions but it was higher than 5 cases per 1 million people in only five of them (Moscow city, Krasnodar region, Saint Petersburg, Rostov region, and Samara region). Still we should point out that, apart from the World Cup, other risk factors were rather high in those regions. Therefore, it can indirectly imply that epidemiologic surveillance over measles virus was quite satisfactory in those regions during a great mass event with international participation.

Authenticity of the risk assessment was determined according to the document developed by the WHO and entitled “Rapid risk assessment of acute public health events. WHO 2012”. It is very important to document level of confidence in an assessment performed by an assessment group as well as reasons for not making an assessment more precise.

Precision of any assessment depends on applied data being authentic, complete and qualitative as well as on correctness of basic assumptions regarding hazards, exposure and context of an event.

Level of confidence in results obtained by an assessment group is directly proportionate to quantity of actual data on hazards, exposure, and context of an event.

We can state that the level of confidence in our assessment and its results is high due to use of official statistic data, documents, and statistic reports that were issued basing on data obtained by authorities responsible for epidemiologic surveillance over measles virus in the Russian Federation.

Our procedure was developed on the basis of the WHO document entitled “Rapid risk assessment of acute public health events”; the document was originally developed to assess

risks related to spread of poliomyelitis across the European countries. The procedure is aimed at rapid operating risk assessment and prediction how a situation will develop in a year following the examined one. This procedure for calculating risks that a communicable disease will be imported and spread in RF regions has some similarities with analogue procedures developed by Russian authors but it also has some differences [18–20]. A difference is a complex approach to analyzing social, economic, demographic, and epidemiologic risk factors. Another difference is that our examination involved assessing risks related to import and spread of an infection that could be managed via immune prophylaxis. Most previous research accomplished by Russian scientists focused on mapping and revealing "risk territories" regarding natural foci infections and parasitic diseases that are endemic in their essence [16]. The procedure has certain drawbacks as it assigns RF regions only into three categories, with high, average and low risks; it also has a rather generalized nature as it is designed for an operating assessment and developing preventive activities as the federal level.

The results which we obtained can be applied as independent data in activities performed by Rospotrebnadzor authorities and aimed at developing targeted plans of preventive measures in administrative districts assigned into "risky territories" category. Such activities can be related to creating stocks of anti-measles vaccine and medications for treating the infection; making laboratory support ready for clinical examinations; organizing workshops on epidemiology and clinical issues regarding measles in order to increase epidemiologic suspicion among primary medical personnel.

It is also advisable and highly desirable to add various criteria to the procedure and apply it to perform rapid assessment of other epidemic (biological) risks as it was shown on the example of wild poliomyelitis virus.

The next stage in developing this prediction model will be an attempt to assess risks of measles virus as well as other communicable diseases being imported and spread in 2019 and to create an online service for rapid calculation and public use of this analytical model.

Conclusion. Significant migration flows and insufficient vaccination against measles both in Russia and abroad create a threat that measles virus can be imported, spread across the RF territory and then persist in the country.

When a risk assessment is applied in epidemiology, it involves developing a procedure for examining and predicting an epidemiologic situation that will allow determining influence exerted on it by risk factors. Such a procedure provides an opportunity to rank problems as per their significance and minimize or even eliminate risks completely. In other words, to manage a risk means to monitor and assess it and implement systems designed for managing risks.

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PECULIARITIES OF TOXIC EFFECTS PRODUCED BY DIISONONYL PHTHALATE AND REGULATION OVER IT IN POLYMER MATERIALS AND MEDICAL PRODUCTS

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We chose diisononyl phthalate as our experimental research object; this compound is an isononyl ester of phthalic acid based on n-butene (CAS No. 28553-12-0).

Our research goal was to establish peculiarities and regularities related to toxic effects produced by diisononyl phthalate on experimental animals' bodies and give scientific substantiation for fixing its permissible quantities that could migrate from polymer materials and medical products.

We accomplished our research applying a set of toxicological, physiological, hematologic, biochemical, immunologic, cytogenetic, morphological, organoleptic, sanitary-chemical, and statistical techniques. We were the first to establish peculiarities of toxic impacts exerted by diisononyl phthalate after intragastric introduction into white rates. Those impacts were dose-dependent toxic effects produced both on a whole body and specific organs and systems with typical disorders in functional and morphological parameters, cell differentiation, and an increase in number of cells with changes in their genetic apparatus.

We revealed that overall toxic effects as well as specific damaging ones produced by diisononyl phthalate on the endocrine and reproductive systems in experimental animals' bodies became apparent via functional disorders in the thyroid gland and gonads, embryogenesis, and offspring post-natal development. A chronic experiment allowed us to determine quantitative regularities and how toxic effects produced by diisononyl phthalate manifested themselves; so we were able to determine limiting parameters and a biological marker as well as fix a threshold of its hazardous impacts on a body, and substantiate fixing permissible quantities that could migrate from polymer materials and medical products. Our experiment results gave grounds for creating a hygienic standard for diisononyl phthalate that provided hygienic assessment and safe distribution of materials and medical products made of polymers that contained the compound.

Key words: *diisononyl phthalate, plasticizer, toxicity, hazard, biological effects, laboratory animals, hygienic standard, medical products.*

Modern policies in the sphere of chemical safety are aimed at reducing health risks caused by exposure to hazardous chemical factors; given that, it becomes vital to accomplish relevant primary prevention and to work out hygienic standards for such substances [1, 2]. A special attention should be given to issues related to new polymer-based materials and products made of them being safely applied in medical practices [3]. Polyvinylchloride (PVC) is a widely used material; to produce it, phthalate plasticizers (PPs), or phthalic acid ethers, are applied as they make products made of PVC more elastic and flexible. When products made of PVC are used, PPs migrate into media that contact them hence there are no covalent bonds between a plasticizer and polymer molecules. Phthalates migration from medical products (blood storage containers, transfusion systems, tubes, catheters, etc.) is especially hazardous due to their direct impacts on internal media in a patient's body[1] [4–7].

Over many years low-molecular PPs have been used in polymer products manufacturing;

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[1] Guide on designing polyvinylchloride-based compositions. R.F. Grossman ed. The 2nd edition, with amendments and corrections. Saint Petersburg, Nauchnye osnovy i tekhnologii Publ., 2009, 608 p.

the most widely spread ones were dioctyl phthalate (DOP) and dibutyl phthalate (DBP). Both these substances had high migration capacities [8, 9]. Multiple research results indicate that the said phthalates produce apparent toxic effects under long-term exposure and they primarily damage the reproductive and endocrine systems [10–14]. There are differences in state regulations over phthalates application in different countries including use of health risk assessment and scopes of permissible migration from products; DOP and DBP are completely forbidden in certain countries or their use is significantly limited.

Search for alternative PPs with improved technological properties and low migration capacities resulted in wide use of new high-molecular plasticizers; diisonyl phthalate (DIIP) is one of them. It has two isomers (CAS No. 68515-48-0 and No. 28553-12-0). Data taken from literature indicate that DIIP isomer CAS No. 28553-12-0 has a more branching carbon chain and is more widely used nowadays in manufacturing PVC and products made of it. The substance doesn't have a complete toxicological profile; regularities related to its dose-dependent toxic and specific effects produced on a body, specifically on the reproductive and endocrine systems, under long-term exposure haven't been studied; safe scopes of its migration from medical products haven't been substantiated.

Our research goal was to establish peculiarities and regularities of toxic effects produced by diisonyl phthalate on experimental animals and scientifically substantiate a standard for permissible scopes of its migration from polymer materials and medical products.

Data and methods. All the experiments were performed according to methodical guides[2,3] on 3 types of experimental animals: 106 white mice weighing from 18 to 22 grams; 352 random-bred white rats weighing from 180 to 210 grams; and 5 white rabbits weighing from 4.2 to 4.5 kilos.

We determined DIIP toxicometric parameters in acute experiments under intragastric, intraperitoneal, epicutaneous, and inhalation introduction into experimental animals. Capabilities to cumulate were examined in a sub-chronic experiment on white rats when DIIP was introduced into the stomach in doses varying from 100.0 to 10,000.0 mg/kg for 60 days. A chronic experiment that lasted 6 months involved intragastric DIIP introduction into white rats in doses varying from 0.01 to 1,000.00 mg/kg.

All the experiments were accomplished in full conformity with the European Convention for the Protection of Vertebrae Animals used for Experimental and other Scientific Purposes (ETS No. 123) and Good Laboratory Practice (GLP) rules. Laboratory animals were taken out of experiments according to requirements fixed in the Use of Research Animals (1997).

Impacts exerted by DIIP on the reproductive function were examined as per a procedure developed by A.A. Dinerman [15]. We applied sagittal sections technique developed by W. Wilson and modified by A.P. Dyban [16] to determine and examine anomalies in internal organs of white rats embryos. Any possible irritating effects produced on undamaged skin and eye mucous membranes as well as sensitizing effects were examined according to the Guide No. 1.1.11–12–35–2004. We applied Ames test to estimate mutagenic activity [17]. Meta-phase analysis of chromosome aberrations in bone marrow cells and spleen cells was performed on mice[4]. We examined ge-

[2] Guide 2.1.5.11-10-199-2003. Substantiation of hygienic standards for chemicals contents in water taken from water objects used for communal and drinking water supply. Approved by the Order of the Belarus Chief Sanitary Inspector on December 12, 2003 No. 160. The collection of sanitary rules and standards on drinking water supply. Minsk, 2005, Part 2, pp. 120–167.

[3] Guide 1.1.11-12-35-2004. Requirements to accomplishing experimental research for primary toxicological assessment and hygienic standardization of substances. Approved by the Belarus Public Healthcare Ministry on December 14, 2004. Minsk, 2004, 43 p.

[4] Guide 055-1215. How to determine mutagenic effects produced by chemical products (chemical substances and their mixtures). Approved by the Order of the Belarus Chief Sanitary Inspector on August 30, 2016. E.S. Yurkevich eds. Minsk, Theoretical and practical center for hygiene Publ., 2015, pp. 10–15.

netic structures of white rats' leucocytes in sub-chronic and chronic experiments via making smears as per a procedure developed by J.N. Mills [18]. Organoleptic and sanitary-chemical parameters applied for determining limiting hazard properties were examined according to the Guide[5].

All the obtained data were statistically processed with conventional analysis techniques applying MS Excel and STATISTICA 10 software. Discrepancies between experiment and control groups were taken as statistically significant at $p<0.05$.

Experimental data on DIIP toxic properties can be applied when establishing safety parameters as per acceptable health risk.

Results and discussion. We didn't register any intoxication and deaths among experimental animals in acute experiments involving intragastric DIIP introduction into animals of both sexes in maximum possible doses. The same results were obtained when DIIP was either applied on skin or introduced intraperitoneally. We didn't establish any potential hazards caused by inhalation exposure and subsequent acute intoxication in experiments on mice due to low volatility of the substance. We couldn't calculate average lethal doses and concentrations due to absence of any deaths in the above mentioned experiments (Table).

We didn't reveal any irritating effects produced on undamaged skin and eye mucous membranes of laboratory animals after a single epicutaneous exposure to DIIP. We also didn't detect any sensitizing effects produced by DIIP in experiments that modeled delayed hypersensitivity in white mice.

In another experiment DIIP was repeatedly introduced intragastrically into white rats in doses equal to 10,000.00, 1,000.00, and 100.0 mg/kg; the substance didn't have any apparent cumulative properties that could be equal to lethal effects but exerted general toxic polytropic impacts. Disorders in the nervous system became apparent via changes in motor activity of laboratory animals: there was 2.9 times decrease in a parameter called "passing through sectors" after exposure to 10,000.00 mg/kg of DIIP, but the parameter grew by 1.8 times under exposure to 100.00 mg/kg ($p<0.05$). We analyzed parameters of experimental rats' peripheral blood under exposure to 10,000.0 mg/kg and revealed signs of iron-deficiency anemia, namely, erythropenia, decreased haematocrit contents, lower erythrocytes volumes, lower hemoglobin concentration and contents in an erythrocyte. There was also a statistically significant 3.0 times decrease in iron contents in blood serum under exposure to the maximum dose and 2.4 times decrease after the dose was 10 times lower against the control group.

We revealed some disorders in the mineral metabolism in a sub-chronic experiment when DIIP was introduced into rats in doses equal to 10,000.0 and 1,000.0 mg/kg; there was 1.2–1.4 times decrease in calcium contents in blood serum ($p<0.05$) due to its 5.2–8.6 times more intense excretion with urine ($p<0.05$) against the control group. Phosphor contents, on the contrary, grew by 30.6–33.3 % ($p<0.05$) as its excretion with urine went down by 2.3–9.1 times

Table

Acute DIIP toxicity

Parameters	Animal type	Dose, mg/kg	Classification
Average lethal dose under intragastric introduction	rats	>5,000.0	IV hazard category, low hazard[6]
	mice		
Average lethal dose under epicutaneous introduction	rats	>2,500.0	
Average lethal dose under intraperitoneal introduction	rats	>3,000.0	VI toxicity category, relatively harmless [19]
	mice		

[5] Guide 1.1.10-12-41-2006. Hygienic assessment of medical products, medical appliances and materials used in their manufacturing / Approved by the Order of the Belarus Chief Sanitary Inspector on November 22, 2006 No. 155. The collection of regulatory documents on toxicology. Minsk, 2007, part. 3, pp. 27–93.

[6] GOST 12.1.007-76. Hazardous substances. Classification and overall safety requirement (Introduced on January 01, 1977). Minsk, BelGISS Publ., 2008, 8 p.

against the control group ($p<0.05$) after exposure to all experimental doses.

There were also disorders in kidney functions after repeated exposure to DIIP in the above mentioned doses; it became obvious due to a statistically significant 1.9–3.9 times growth in diuresis in the test groups together with a decrease in uric acid contents (by 2.5–4.2 times), urea (by 1.5–2.1 times), and creatinine (by 2.0–3.1 times) against the control group. After exposure to DIIP in a dose equal to 10,000.00 mg/kg we revealed a 1.2 times decrease in kidneys volume in animals from the test group ($p<0.05$) and a 1.7 times increase in excretion of magnesium with urine ($p<0.05$) against the control group. All the above mentioned changes were accompanies with structural disorders in the kidneys, in particular, dystrophy and inflammation.

We analyzed enzyme activity of white rats' blood serum after DIIP doses were introduced into them and statistically processed the results; it allowed us to reveal that gamma-glutamyl transpeptidase contents grew by 12.5–17.6 % ($p<0.05$), asparagine aminotransferase contents went down by 1.5 times, and α-amylase contents grew by 1.4 times against the control group at $p<0.05$.

We performed morphologic examinations of experimental rats' livers and revealed dystrophic changes in hepatocytes, interstitial hepatitis signs, and a 1.2–1.6 times increase in the organ volume ($p<0.05$) under exposure to DIIP in doses equal to 10,000.0 and 1,000.0 mg/kg. Functional disorders in the spleen became obvious via hyperplasia of lymphoid follicles in white pulp, hyperemia, and reactive proliferation of red pulp together wuth 1.4 times growth in the organ volume ($p<0.05$) against the control group. Signs of gastritis and interstitial pancreatitis were also morphologically revealed in animals from the test group.

We analyzed hormonal state of experimental animals under sub-chronic oral exposure to DIIP in doses ranging from 10,000.00 to 100.0 ms/kg and didn't reveal any statistically significant changes. However, morphologic and morphometric examinations revealed a dose-dependent decrease in the diameter of the thyroid gland follicles that was 1.3–2.3 times smaller ($p<0.05$) and there were signs of diffuse toxic goiter and hyperplasia of the adrenal cortex as it became 1.3–1.8 times thicker ($p<0.05$).

We performed cytogenetic analysis of experimental rats' peripheral blood after a sub-chronic experiment was over; the analysis revealed there was an increase in number of leucocytes with morphological signs of necrotic death; this number was 5.1–6.1 times higher than in animals from the control group ($p<0.05$) after exposure to DIIP in doses equal to 10,000.0 and 1,000.0 mg/kg.

We performed a 180-day experiment on white rats (6 test groups and 1 control group) that involved intragastric DIIP introduction in doses ranging from 0.01 to 1,000.0 mg/kg; there were no external evidence of intoxication or any behavioral disorders during the experiment. As the experiment was over, we revealed toxic effects produced by DIIP that had poly-systemic nature; their evidence depended on introduced doses. Thus, after exposure to doses varying from 1,000.0 to 1.0 mg/kg, experimental rats had statistically significant changes in their peripheral blood: leucopenia; lymphocytes contents decreased by 1.5–2.0 times; monocytes, 1.3–1.4 times; granulocytes, 1.5–1.6 times; average hemoglobin concentration in erythrocytes went down by 5.0–5.8 %; average thrombocytes volume, by 7.9–13.3 %; thrombocytosis occurred after exposure to a dose equal to 1,000.0 mg/kg.

Exposure to DIIP in doses ranging from 1,000.0 to 0.1 mg/kg resulted in renal toxic effects that became obvious via lower iron excretion with urine, glycosuria occurrence, and morphological changes in the kidneys such as dystrophy of proximal tubules epithelium with interstitial nephritis signs. Special attention should be paid to more intense excretion of magnesium from a body as its contents was 2.0–3.3 times higher in urine of animals from all the test groups ($p<0.05$) against the control group after exposure to all the experimental doses varying from 1,000.0 to 0.01 mg/kg.

We analyzed biochemical parameters of experimental animals' blood serum under exposure to DIIP in doses varying from 1,000.0 to 10.0 mg/kg and revealed a statistically significant 2.1–5.4 times decrease in iron contents

and 1.2–1.4 times decrease in phosphor contents together with 2.0 times increase in calcium contents against the control group at $p<0.05$.

DIIP produced obvious toxic effects on the liver in doses equal to 1,000.0 and 100.0 mg/kg as the organ became smaller in its size by 19.1 and 15.8 % ($p<0.05$), contents of high density lipoproteins went down by 7.1 and 8.3 % accordingly ($p<0.05$), and urea contents grew by 1.2 times ($p<0.05$); there were also signs of interstitial hepatitis and dystrophic changes in hepatocytes.

As for hormonal state of white rats after exposure to DIIP in doses varying from 1,000.0 to 1.0 mg/kg, the most apparent changes were revealed in contents of thyroxin and thyrotrophic hormone in blood serum as they increased by 2.1–3.3 times and 1.8–2.5 times accordingly against the control group ($p<0.05$). An increase in free thyroxin contents was statistically significant only after exposure to doses equal to 10.0 mg/kg and higher; free triiodothyronine, after exposure to a dose equal to 1,000.0 mg/kg. Pathomorphological disorders were revealed in the thyroid gland of experimental animals after exposure to DIIP in doses varying from 1,000.0 to 1.0 mg/kg; they were an increase in follicles size and changes in their epithelium. There were no such changes after exposure to smaller doses.

Anti-androgenic effects produced by DIIP in doses varying from 1,000.0 to 1.0 mg/kg became apparent via an authentic 2.6–3.9 time decrease in testosterone contents in blood serum of experimental animals and an ascending trend in contents of progesterone, estradiol, lutropin, and collicle-stimulating hormones ($p\geq0.05$) against the control group.

We didn't detect any changes in contents of hydrocortisone and dehydroepiandrosterone (adrenal cortex hormones) in blood serum of experimental animals. Morphometric examinations revealed that adrenal cortex increased by 3.0–3.8 times in experimental animals ($p<0.05$) and the organ volume grew by 1.6 times under exposure to DIIP in a dose equal to 1,000.0 mg/kg at $p<0.05$. We didn't reveal any changes in hormonal state of white rats under multiple exposure to DIIP in a dose equal to 0.1 mg/kg and it allows us to consider this dose as a non-effective one as per a specific sign.

We didn't reveal any disorders in non-specific humoral immunity as per contents of complement components (C3, C4) and immunoglobulins A, G, and M in blood serum of white rats after DIIP introduction in all series of experiment.

We performed morphological examinations of the gastric mucosa and pancreas mucosa of rats under exposure to DIIP in doses varying from 1,000.0 to 1.0 mg/kg and revealed reactive hyperplasia in the gastric epithelium and chronic gastritis occurrence with progressive 1.2–1.4 times growth in thickness of multi-layer pavement epithelium of the gastric mucosa ($p<0.05$); we also detected signs of interstitial pancreatitis with Langerhans cells hyperplasia.

We performed cytogenetic analysis of leukocytes taken from blood of experimental animals after a chronic experiment was over and established that DIIP in doses varying from 1,000.0 to 1.0 mg/kg was able to produce damaging effects on genetic structures such as enhanced cellular proliferation with simultaneous growth in numbers of micronucleuses and cells with necrosis signs and a decrease in contents of young forms.

Consequently, the results obtained via complex clinical-biological, morphological, morphometric and cytogenetic research indicate that DIIP doses which vary from 1,000.0 to 0.1 mg/kg and are intragastrically introduced in chronic experiments produce general toxic effects on a body. A dose equal to 0.01 mg/kg can be taken as chronic exposure threshold and its limiting parameter is a nephrotoxic effect related to excretion of magnesium with urine (a biological marker). Toxic effects produced on the endocrine system were revealed under exposure to DIIP when it was introduced into experimental animals in a dose which was 100 times higher than the chronic exposure threshold.

Experimental study of remote effects produced by exposure to DIIP on a body involved examining reproductive toxicity (embryotropic, teratogenic, and gonadotropic effects) as well as occurrence of mutagenic and cytotoxic properties.

Exposure to DIIP after intragastric introduction in a dose equal to 10,000.0 mg/kg during pregnancy resulted in statistically signifi-

cant 3.5 times growth in overall embryonic and post-implantation mortality (up to 29 %) and occurrence of multiple (combined) malformations.

When introduced DIIP doses were reduced to 100.0 mg/kg, there was also a decrease in quantity of embryonic malformations (microphthalmia in 4.8 %); there were no such malformations when doses went down to 10.0 mg/kg.

We observed post-natal development of offspring born by female white rats that had been exposed to DIIP in a dose equal to 10,000.0 mg/kg during pregnancy; mortality among infants rats grew by 22.7 % ($p<0.05$). We also examined hormonal state of male offspring on the 60^{th} day of the experiment after exposure to DIIP in doses equal to 1,000.0 and 100.0 mg/kg and revealed functional disorders in the thyroid gland and gonads. Those disorders became apparent via a growth in crude thyroxin contents (by 2.0 and 2.3 times, $p<0.05$) and thyrotrophic hormone (by 2.1 and 2.3 times, $p<0.05$) in blood serum as well as a decrease in testosterone concentration (by 2.3 and 2.9 times, $p<0.05$) accordingly against the control. But still, there were no significant changes in morphofucntional parameters of male rats' gonads (volumes of testicles and appendages, overall sperm concentration, motile and immotile sperm concentration, average velocity of motile sperm) [20, 21].

We didn't detect any functional disorders in pubescent male white rats in experiments involving research on effects produced on gonads after 2-month intragastric DIIP introduction in doses varying from 10,000.0 to 100.0 mg/kg. However, morphological research on testicles performed after 60-daty exposure revealed a decrease in spermatogenesis of experimental rats and reduced sizes of testicular tubules together with reduced number of layers and cellularity. At the same time spermatogenesis ended with spermatocides and spermatids. The same changes were detected when exposure to DIIP in doses varying from 1,000.0 to 1.0 mg/kg was extended to 6 months. Under exposure to the maximum experimental dose a number of mature sperm in testicular tubules of experimental animals went down by 5.4 times ($p<0.05$) against the control group.

Consequently, experiments allowed establishing that there was a cause-and-effect relation between reproductive function disorders in white rats under exposure to DIIP and introduced doses, 0.1 mg/kg being the non-effective one.

We estimated mutagenic effects produced by DIIP with Ames test on *Salmonella typhimurium* strains *TA 98, TA 100, TA 1535, TA 97,* and *TA 102* in concentrations equal to 0.3, 0.6, 13., 2.5, and 5.0 mg/ml with metabolic activation and without it; the results indicated there were no such effects. We analyzed cytogenetic specimen of the bone marrow and spleen after 24 hours had passed from a single intraperitoneal introduction of DIIP into white mice in a dose equal to 2,000.0 mg/kg; we didn't reveal any increase in chromosome aberrations. We also didn't establish any discrepancies between the test and control groups as per quantity of cells with apoptosis signs (interphase death) and polyploids in the bone marrow. However, spleens of white mice from the test groups contained a statistically 5.0 times lower number of cells with apoptosis signs and 4.0 times higher number of polyploids against the control group ($p<0.05$).

Therefore, DIIP didn't produce any mutagenic effects as per number of chromosome aberrations after a single intraperitoneal introduction into white mice; still, the substance caused changes in division, differentiation, and death of cells in the spleen. It can possibly occur due to active proliferating cells in a body of a warm-blooded animal, including embryonic ones, being most susceptible to cytotoxic effects produced by DIIP. It can be a possible mechanism of embryotoxic (teratogenic) effects produced by DIIP. Impacts on the reproductive and endocrine systems revealed in our experiments are secondary in their nature and occur due to damaging effects produced by DIIP on a body as a result of structural and functional changes in cells.

Permissible DIIP migration into model media (distilled water) is a parameter that determines safety of polymer-based materials and medical products. A safe standard was calculated according to methodical approaches on substantiating hygienic standards for chemicals contents in water with determining a limiting hazard criterion as per organoleptic, overall sanitary, and toxicological parameters.

We examined impacts exerted by DIIP on standardized organoleptic and overall sanitary water parameters under its maximum attainable concentration in water that was equal to 0.6 mg/dm^3. We didn't reveal any impacts exerted by DIIP in this concentration on such water parameters as smell, taste, turbidity, color, foaming, permanganate oxidability, susceptibility to being bromated, and some others. Therefore, the tested concentration was non-effective.

At the same time, a threshold dose equal to 0.01 mg/kg was established in a chronic experiment on white rats that involved examining toxic effects produced by DIIP under intragastric introduction as per a limiting parameter related to nephrotoxic impacts.

Maximum non-effective concentration calculated as per toxic hazard criterion amounted to 0.02 mg/dm^3 taking into account safety coefficient equal to 10, average human body weight, and a volume of daily water consumption. However, it seems impossible to determine this DIIP concentration in model media as contemporary techniques for control over its contents have sensitivity equal to 0.05 mg/dm^3 [7]. Bearing that in mind, a standard for permissible DIIP migration was substantiated and fixed; it contained a "non-permissible" value (<0.05 mg/dm^3) and it ensures sanitary-hygienic control and safe use of polymer-based materials and medical products that contain DIIP.

Conclusions. Complex toxicological and hygienic research allowed obtaining complete toxicological assessment of DIIP in acute, subchronic, and chronic experiments under intragastric introduction into experimental animals. We determined polytropic nature of overall toxic dose-dependent effects produced by DIIP on organs and tissues and specific damaging effects produced on the endocrine and reproductive systems of experimental animals. Chronic experiments allowed revealing quantitative regularities and specific features of toxic effects produced by DIIP; they also allowed establishing a nephrotoxic limiting parameter and biological markers of leading adverse impacts exerted by DIIP on a body. We took a toxicity criterion for substantiating and fixing a standard for permissible DIIP migration from polymer materials that could ensure relevant hygienic assessment and safe use of polymer-based materials containing DIIP and medical products made of such materials.

Revealed peculiarities and regularities of toxic effects produced by DIIP can be applied to determine reference safe contents and concentrations as per acceptable health risk criteria.

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TNF GENE POLYMORPHISM AS A RISK FACTOR THAT CAN CAUSE ARTERIAL HYPERTENSION IN PATIENTS SUFFERING FROM GASTROESOPHAGEAL REFLUX DISEASE

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Comorbidity of arterial hypertension (AH) and gastroesophageal reflux disease (GERD) is widely spread (from 20.6 % to 29 %); despite that fact, risk factors that can cause AH in patients suffering from GERD have still not been examined completely. Experts are discussing a role played by anti-inflammation cytokine of tumor necrosis factor alpha (TNFα) both in AH occurrence and GERD pathogenesis as it is its activity that is to a great extent determined by a patient having certain alleles of tumor necrosis factor (TNF) gene. Therefore, it seems vital to study TNF gene G308A polymorphism in patients with combined AH and GERD.

Our research goal was to study frequency and variants of TNF gene G308A polymorphism relations with AH risk and AH phenotypic peculiarities in patients suffering from GERD.

We examined 58 people who had AH (29 patients with isolated AH, average age being 53 [46; 62], and 29 patients with combined AH and GERD, average age being 56 [51; 59]). Patients from both groups were comparable in terms of sex, age, and examined factors of cardiovascular diseases risks. We applied allele-specific polymerase reaction with test systems produced by "Sintol" LLC (Moscow) to determine G308A (rs1800629) polymorphism of TNF gene. To assess relations between alleles and genotypes and disease risks, we calculated odds ratio (OR) with 95 % confidence interval (CI).

We revealed a relation between G308A polymorph marker of TNF gene and systolic blood pressure and disorders in tolerance to dextrose among patients with comorbid AH and GERD. Patients with combined AH and GERD had unfavorable allele A and G/A genotype authentically more frequently (OR 5.14; 95 % CI – 1.06–24.95; p = 0.03, OR 6.08; 95 % CI – 1.18–31.25; p = 0.02 accordingly).

We showed that G308A polymorphism was related to AH occurrence in patients suffering from GERD. These data confirm that endothelial dysfunction plays a significant role in AH pathogenesis among such patients.

***Key words**: arterial hypertension, gastroesophageal reflux disease, comorbidity, endothelial dysfunction, risk factor, genetic polymorphism, TNF gene, arterial hypertension phenotype.*

Over the last decades arterial hypertension (AH) has been a most widely spread and socially significant cardiovascular disease [1]. As per data obtained via epidemiologic research high blood pressure (BP) is registered in 39–40 % population of the Russian Federation [2]. Today more and more patients with AH have one or more associated diseases and comorbidity and poly-morbidity tend to grow among older patients. Concomitant pathology

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determines variability of clinical symptoms that a basic pathology might have, influences prediction and life quality of a patient and in some cases makes it necessary to change therapeutic tactics in treating a patient [3]. Cases in which AH combines with pancreatic diabetes, ischemic heart disease, or chronic kidney failure have been studied in detail and given pathogenetic substantiation. But still, an issue related to comorbid pathology regarding AH and other socially significant diseases, gastric ones in particular, is becoming truly vital at the moment.

Gastroesophageal reflux disease (GERD) is among the most frequent pathologies occurring in the gastroduodenal area; it is widely spread (up to 60 %) among adult population in the RF. Results of a multicenter trial «Epidemiology of Gastroesophageal Reflux Disease in Russia» [4] indicate that 61.7 % men and 63.6 % women in Russian have heartburn that is the primary GERD symptom. According to foreign researchers, AH prevalence among patients suffering from GERD varies from 20.6 % to 29 % [5].

The above data allow considering AH and GERD comorbidity being quite possible; frequent combination of the said nosologies allows assuming there is a specific pathogenetic regularity in their associated clinical course.

Apart from traditional risk factors that cause AH and GERD occurrence and development, a lot of attention is given to a pathogenetic role played by inflammatory mediators; another issue under discussion is a relation between anti-inflammatory cytokines and endothelial dysfunction occurrence together with vascular walls becoming more rigid [6, 7].

Tumor necrosis factor alpha (TNFα) is a multifunctional anti-inflammatory cytokine that participates in regulating a wide range of biological processes; its activity to a great extent depends on carrying specific alleles of tumor necrosis factor (*TNF*) gene.

Therefore, *TNF* gene can be examined among candidate genes that can take part in AH occurrence among patients with GERD; it can be interesting from a practical point of view to examine polymorphism of the said gene in patients who suffer from combined AH and GERD.

All the above mentioned determined **the research goal** that was to examine prevalence and variants of a relation between G308A polymorphism in *TNF* gene and a risk of AH occurrence among patients suffering from GERD as well as phenotypic peculiarities of the disease.

Data and methods. Overall, 58 patients with AH took part in the research; 29 patients (the test group) also suffered from GERD. The reference group was made up of 29 patients who had only AH. All the patients were comparable in terms of their sex, age, and duration of the main disease. The examination was performed in the Cardiology and Prevention Department of the Regional Cardiologic Clinic (Perm). All the patients gave their written informed consent to take part in the examination. The research was also granted permission by the local ethical committee of the E.A. Vagner's Perm State Medical University (Protocol no. 8, October 02, 2018).

There were certain criteria for a patient to be excluded from the research; they were AH being only a secondary disease; cardiac infarction in a case history or exertion angina higher than II category; pancreatic diabetes; chronic kidney failure higher than C3a stage; hepatic failure; chronic heart failure higher than IIA category as per New York Cardiology Associates classification; malignant neoplasms of any localization; absence of a written informed consent to take part in the research.

AH was diagnosed according to recommendations on AH given by the Russian Medical Society for Arterial Hypertension (the 4th edition) [8]. To initially screen GERD, we applied GerdQ questionnaire (method sensitivity is 91.7 %; method specificity, 65.4 %) which allowed diagnosing GERD during an initial contact with a patient [9]. Later on GERD was confirmed according to the up-to-date clinical guidelines given by the Russian gastroenterological association [10].

Laboratory and instrumental examination of patients was performed in conformity with the standard for providing medical and sani-

tary aid to patients with primary AH (supplement to the RF Public Healthcare Ministry Order No. 708n issued on November 09, 2012). Additionally all the patients had their blood pressure monitored for 24 hours uninterruptedly with devices produced by BPLab (Russia) prior to any anti-hypertension therapy was prescribed.

A smear from a cheek mucosa was taken from each participant in the examination; it was done with a dry sterile probe with a cotton pellet. Then, allele-specific polymerase chain reaction with the use of test systems produced by "Syntol" LLC (Moscow) was applied to determine G 308A (rs1800629) polymorphism in tumor necrosis factor (*TNF)* gene.

The results were statistically processed with certified computer software. All the obtained data were given as Me (25; 75) where Me was a median, 25 and 75 were 25^{th} and 75^{th} percentiles. Non-parametric values were compared with Mann-Whitney test. To statistically analyze how frequencies of alleles and genotypes were distributed, we applied contingency tables with calculating χ^2. Discrepancies were considered authentic at $p<0.05$. To assess a relation between alleles and genotypes and a risk of the diseases occurrence, we calculated odds ratio (OR) with 95 % confidence interval (CI). Conformity of genotypes frequencies distribution with Hardy-Weinberg principle was determined with a specific software package called "Calculator for calculating statistics in case-control studies".

Results and discussion. It was a simple open comparative study. Median age of patients from the test group was 56 (51; 59); in the reference group, 53 (46; 62) ($p = 0.392$). Men prevailed in both groups (82 % and 79 % accordingly). Patients were comparable as per their body mass index (BMI), smoking status, and AH duration ($p = 0.733$). Patients with the II stage increase in blood pressure prevailed in both groups (51.21 % among patients with AH only and 55.55 % among patients with AH and GERD combined), II stage AH (56.09 % and 59.25 %), the 3^{rd} risk degree (48.14 % and 43.9 %) accordingly. Having examined G308A polymorphism, we determined that G/G and G/A genotypes prevalence amounted to 77.94 % and 22.05 % accordingly among patients with AH, frequencies distribution didn't deviate from Hardy-Weinberg principle ($\chi^2 = 0.79$, $p = 0.37$). These results are well in line with data obtained by other researchers and don't have any significant discrepancies from distribution of genotypes in most European populations [11, 12].

Table contains data on frequency of G308A polymorph marker genotypes in *TNF* gene among patients suffering from AH only and AH combined with GERD.

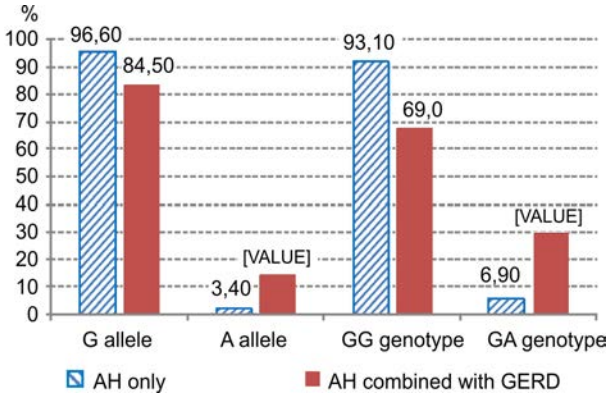
Table

Distribution of -308GA (rs1800629) polymorph marker frequent alleles and genotypes in *TNF* gene among patients from the test and reference group (multiplicative and additive inheritance model) chi-square test, df = 1

Tumor necrosis factor alpha gene (*TNF*) Alleles and genotypes	Distribution of alleles and genotypes frequencies		χ^2	p	OR	
	The test group (AH and GERD) n = 29	The reference group (AH) n = 29			value	CI
G	49 (84.5 %)	56 (96.6 %)	4.92	0.03*	0.19	0.04–0.94
A	9 (15.5 %)	2 (3.4 %)			5.14	1.06–24.95
G/G	20 (69 %)	27 (93.1 %)			0.16	0.03–0.85
G/A	9 (31 %)	2 (6.9 %)	5.5	0.02*	6.08	1.18–31.25
A/A	0	0			1.0	0.02–52.10

Note: *TNF* is tumor necrosis factor alpha gene;
AH is arterial hypertension;
GERD is gastroesophageal reflux disease;
OR is odds ratio;
* means significance of discrepancies.

Mutant allele A in TNF gene was determined in 15.5 % cases among patients who suffered form both AH and GERD; in 3.4 % cases, among patients who had AH only. Therefore, adverse allele A prevailed among patients with AH and GERD combined (OR 5.14; 95 % CI 1.06–24.95; p = 0.03). Prevalence of G/A heterozygotes among allele A carriers was also authentically higher among patients with comorbid AH and GERD (OR 6.08; 95 % CI 1.18–31.25; p = 0.02) (Figure). We didn't reveal patients with A/A genotype in either group.



Note: *TNF* is tumor necrosis factor alpha gene;
OR is odds ratio;
* means significance of discrepancies

Figure. Frequency of G308A polymorph marker alleles and genotypes in *TNF* gene among patients with AH and AH and GERD combined

The next stage in our research was a search for possible relations between G308A genotype in *TNF* gene and clinical peculiarities and risk factors that could cause AH in the examined groups.

We didn't establish any relations between *TNF* gene G308A polymorphism and BMI, lipid metabolism, TNF and C-reactive protein contents in plasma, or echocardiographic parameters. At the same time, we revealed a relation between minor allele A and possible disorders in carbohydrate metabolism. Heterozygote genotype G/A in TNF gene was higher (27.6 %) among patients with combined AH and GERD and abnormal glycemia on an empty stomach (dextrose contents in blood plasma is 6.1–6.9 mmol/l) than among people with normal glycemia (6.7 %, p = 0.03). G/A genotype carriers among patients with combined pathologies ran 5 times higher risk of increased dextrose contents than their counterparts with G/G genotype (OR 5.33; 95 % CI 1.02–27.76; p = 0.03). We didn't reveal any similar discrepancies among patients who suffered only from AH. Despite the fact that patients from both groups were comparable as per an increase in their blood pressure and prescribed medications, we revealed a relation between minor allele A in *TNF* gene and a possibility of AH developing into severe one (as per systolic AH). A risk of systolic blood pressure rising higher than 150 mmHg was almost 4 times higher among G/A genotype carriers than those with G/G genotype (OR 3.92, CI 0.97-15.90; p = 0.04) among patients with combined AH and GERD. We didn't reveal any similar discrepancies among patients who suffered from AH only.

TNF gene is mapped on a shirt arm of the 6th chromosome (6r21.33) and belongs to a locus of genes in the basic histocompatibility complex. There are established relations between TNF gene polymorphism and pancreatic diabetes occurrence, insulin resistance [13, 14], respiratory organs diseases, atherosclerosis [15, 16], and oncologic diseases. There is also an established relation between a polymorph variant of G308A in *TNF* gene and an elevated risk of acute coronary syndrome and high risks of adverse coronary outcomes after a cardiac infarction [17]. Several research works dwelled on determining precisely what relation existed between G308A polymorphism and AH development. A meta-analysis that included 2,224 works [12] revealed a relation between carrying allele A of polymorph G308A variant and an elevated risk of AH occurrence (OR 1.45). The same research indicated that a relation with AH was 2 times stronger for AA genotype in *TNF* gene (OR 3.454).

A role played by TNF gene polymorphism in diseases occurring in the gastroduodenal area has also been proven. Foreign researchers are now discussing a role played by G308A polymorphism in occurrence of erosive reflux-esophagitis and esophagus cancer;

however, results obtained by them are still contradictory and require further investigation in the area [18, 19].

Allele variants of *TNF* gene are known to regulate TNF expression. A role played by TNF-α in cardiovascular pathology occurrence is undisputable. There is evidence that TNF-α contents in blood plasma go up proportionate to blood pressure. There are also data in literature on a predictor role played by TNF-α in GERD and Barrett's esophagus development as per long-term research data [20]. It was established that when metaplasia progresses, TNF-α expression grows in epithelial cells in the esophagus [21].

An increase in TNFα contents produces an instant cytotoxic effect easing off degranulation of neutrophilic granulocytes and inducing lipid peroxidation. It results in structural integrity of endothelial cells being violated and their functions being disrupted; a decrease in vasodilators synthesis and release; an increase in endothelin-1 and angiotensinogen production. All this makes for endothelial dysfunction occurrence [22].

We didn't reveal any relations between G308A polymorphism in TNF gene and TNF-α activity in blood plasma in our research; it can be due to its low concentration in the examined patients (0.085 (0; 0.525) pg/ml). But we were the first to attempt to reveal a relation between G308A polymorphism and a risk of AH occurrence in patients suffering from GERD. The obtained data indicate that AH occurrence in patients with GERD is to a certain extent determined by a patient carrying G/A genotype in *TNF* gene. It is quite possible to assume that endothelial dysfunction in patients with GERD occurring due to impacts exerted by G308A polymorphism can be a pathogenetic mechanism that induces AH in such patients. Though, we established relations between polymorphism and systolic blood pressure and carbohydrate metabolism disorders in patients with combined AH and GERD; it allows us to assume that G308A polymorphism in *TNF* gene influences clinical and metabolic peculiarities of AH in such patients.

Conclusion. Given high prevalence of comorbid AH and GERD, it is rather necessary to further search for any relations between molecular-genetic mechanisms of AH occurrence in patients with both pathologies combined. The results of the present work supplement already existing data on AH and GERD comorbidity and highlight a significant role played by endothelial dysfunction in pathogenesis of the said nosologies. Allele A in *TNF* gene (G308A) in a form of G/A heterozygote allows considering this genotype an additional marker of specific AH phenotype occurring in patients with GERD. Genetic profile determination can be additionally applied when patients with GERD are screened in order to reveal those running elevated AH risks among them. The results presented in this work not only allow enhancing an insight into a genetic component in AH occurrence among patients with GERD but also give grounds for working out prevention programs aimed at reducing cardiovascular risks among patients suffering from GERD.

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ECONOMIC LOSSES DUE TO ONCOLOGIC DISEASES RELATED TO MODIFIABLE RISK FACTORS

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As per data provided by the WHO, about one third of death cases due to cancer are caused by risk factors that can be modified. Also, according to data provided by several authors, 4 modifiable risk factors cause 70% of malignant neoplasm cases that could have been prevented. Malignant neoplasms occupy the 2nd rank place in mortality structure in the Russian Federation. A significant share of analyzed malignant neoplasms can potentially be prevented provided that risk factors are corrected; therefore, investments into prevention should be a core in any activity aimed at public health improvement.

Our goal was to evaluate economic losses that occurred due to most common oncologic diseases related to behavioral risk factors among population in Russia in 2016 (including costs borne by the public healthcare and losses in the economy).

Our analysis comprised direct expenses borne by the public healthcare or, in other words, money spent on oncologic diseases treatment, direct funds allocated to pay disability allowances and temporary disability payments as well as indirect losses in the economy associated with untimely deaths and disability among employable population.

Aggregated economic losses that occurred due to 10 most common malignant neoplasms included into our analysis amounted to 241.3 billion rubles or 0.3% of the country GDP in 2016. Direct expenses accounted for 71.7% of the total losses while GDP losses caused by untimely deaths and disability amounted to 28.3%. The highest direct expenses borne by the public healthcare were due to colorectal cancer (52 billion rubles) and cancer in the trachea, bronchial tubes, and lungs (50 billion rubles).

Our data indicate it is necessary to invest into oncologic diseases prevention and treatment. These investments can produce a significant economic effect in long-term period thus making for economic growth.

***Key words**: economic losses, risk factors, oncologic diseases, public healthcare, expenses, economic losses, direct costs, indirect costs.*

Malignant neoplasms (MNs) take the 2nd rank place in the structure of mortality in the RF [1]. In 2017 in the RF a number of MNs cases exceeded 600 thousand for the first time [2] and the overall number of diagnosed MNs is growing each year. But at the same time as per data provided by the World Health Organization (WHO) [3], approximately one third of all death cases due to cancer are caused by 5 primary risk factors. These factors are high body mass index, fruit and vegetables consumed in insufficient quantities, low physical

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activity, and alcohol intake; but the most significant factor is tobacco smoking since approximately 22% death cases due to cancer are related to it. As per data obtained by British researchers, four modifiable risk factors (obesity, smoking, excessive alcohol intake, and low physical activity) are reasons for 70% of preventable MNs such as lung cancer, rectal cancer, breast cancer, etc. [4]. At the same time it is these MNs localizations that cause high morbidity and mortality in the RF [1]. A significant share of MNs in these localizations is potentially preventable should risk factors be corrected; therefore, investments into prevention should be given specific attention when activities aimed at public health improvement are planned and implemented. Determination of economic losses caused by risk factors and related diseases is a convincing argument for authorities responsible for making decisions on investments into prevention activities [5].

Economic losses caused by MNs are being examined in the EU, both overall and in separate countries, the USA [6–8], Canada, and some other states. Estimation of economic damage caused by MNs is a bit complicated due to difficulties related to taking into account all the components in such damage and estimating costs on treatment, both in our country and abroad. Methodical issues related to estimating economic burdens caused by MNs, first of all, estimating direct costs, are described in the work by V.I. Ignatyeva [9]. There have been several research works performed in the RF that focused on estimating economic losses caused by certain MNs [10–18], but they were accomplished in different time periods and are based on different methodological approaches; though some of them were accomplished as per similar procedures by the same authors [9, 14, 16, 18].

Our research goal was to estimate economic losses that occurred due to main oncologic diseases related to behavioral risk factors among population in Russia in 2016 (including costs borne by the public healthcare system and indirect economic losses).

Data and methods. Our analysis included several oncologic diseases that were authentically related to behavioral risk factors and caused high morbidity and mortality among population in Russia (Table 1). They are lung cancer; breast cancer; stomach cancer; pancreas cancer; kidney cancer; cervical cancer; ovarian cancer; prostate cancer; rectal cancer [19–24].

Table 1

Oncologic diseases included into the analysis

Oncologic disease	ICD-10 code	Number of cases in 2016 [1]	
		New	Overall
Stomach cancer	C16	37,135	139,800
Rectal cancer	C18–C21	69,500	357,050
Liver cancer and intrahepatic biliary ductules cancer	C22	8,320	7,829
Pancreas cancer	C25	18,517	18,511
Trachea, bronchial tubes, or lung cancer	C33, C34	60,467	137,381
Breast cancer	C50	69,095	642,720
Cervical cancer	C53	17,212	177,876
Ovarian cancer	C56	14,017	108,188
Prostate cancer	C61	38,371	202,604
Kidney cancer	C64	23,908	159,427

Our analysis included direct costs borne by the public healthcare on treating oncologic diseases; direct costs on permanent disability and temporary disability payments; indirect costs occurring in the economy and related to untimely deaths and permanent disability among employable population.

Data on direct costs borne by the public healthcare in 2016 were determined on the basis of previous research on a cost of each disease in the analyzed group (Table 2). Basing on literature data on expenses spent on treating patients suffering from oncologic pathologies, we recalculated direct costs borne in 2016. Costs borne by the public healthcare and related to pancreas cancer are not included into the calculations due to absence of relevant data in the domestic research works.

To reduce data obtained for different years to a level relevant for 2016, we adjusted direct medical costs according to actual inflation.

Table 2

Sources of relevant data for analyzing direct medical costs on MNs borne in 2016 and related to modifiable risk factors

Oncologic disease	ICD-10 code	Year of the analysis	Direct medical costs per 1 patient a year (rubles)	Source
Stomach cancer	C16	2013	262,216	[10]
Rectal cancer	C18–C21	2010	395,175	[11]
Liver cancer and intrahepatic biliary ductules cancer	C22	2008	446,252	[12]
Pancreas cancer	C25	No data in domestic research works		
Trachea, bronchial tubes, or lung cancer	C33, C34	2009	1,202,675	[13]
Breast cancer	C50	2014	40,275	[14]
Cervical cancer	C53	2008	122,500	[15]
Ovarian cancer	C56	2009	15,460	[16]
Prostate cancer	C61	2009	10,758	[17]
Kidney cancer	C64	2009	34,455	[18]

Calculations were performed as per the following formula:

$$COST_{dmc16} = COST_{dmc0} * In_0 * In_1 * \ldots * In_{16}, \quad (1)$$

where

$COST_{dmc16}$ are reduced direct medical costs in 2016;

$COST_{dmc0}$ are direct medical costs at the moment research was performed;

In_0 is growth rate for consumer prices indexes (CPI) in the RF in the year in which direct medical costs were analyzed;

In_1 and In_{16} are CPI growth rates over years up to 2016.

Costs borne by the public healthcare per 1 patient in 2016 calculated according to the above procedure were multiplied by a number of people who suffered from an analyzed oncologic disease in 2016 (Table 1).

To calculate number of people with disability as per a specific nosology, we multiplied an overall number of people who were recognized as being disabled for the first time as per data provided by the Rosstat in 2016 [25] by a share of this specific MN in the overall number of the first diagnosed MNs with the given localization (Report Form No. 7 "Data on malignant neoplasms in 2016). When determining a disability group, we took into account stages of MN: Stage IV meant a person had the 1st disability group (the highest payments); Stage III, the 2nd disability group; Stages I and II, the 3rd disability group accordingly. When calculating annual economic losses related to disability payments, we took 6 months as it was an average period during which patients received such payments and average disability payment granted for disabled people by the RF State Pension Fund in 2016 [26].

To calculate a number of days of temporary disability (TD) and a number of people with it as per a specific nosology, we took data from the Statistic Report Form No. 16-td issued in 2016.

Indirect costs (economic losses) included costs of products that were not manufactured as a lost contribution into the country GDP due to untimely deaths and disability among employable population.

Mortality was analyzed basing on data provided by the WHO and data on MNs-related mortality provided by the Herzen's National Medical Research Center for Radiology" of the RF Public Healthcare Ministry [1]. To calculate potential years of life that were lost, we multiplied an absolute number in a specific age group by a number of years that a person failed to live to reach 72 years of age in each specific age group. Losses related to untimely deaths among employable population included non-manufactured GDP due to years of life in a relevant age group that were lost due to deaths caused by MNs taking into account employment rates. Future losses were calculated with the use of 'net present value" with 3% discounting.

GDP losses due to disability were determined according to the following procedure: first, we calculated a number of people with persistent disability in each disability group taking into account employment rates; then a calculated number of disabled people among employable population was multiplied by GDP net present value per capita.

Data were statistically analyzed with Microsoft Excel 10.0.

Results and discussion. To determine economic losses related to MNs, we calculated the following parameters: number of deaths among people younger than 72; potential years of life lost before a person reached 72 years of age; number of days during which a person was temporarily disabled; number of temporary disability cases (Table 3).

Table 3

Number of deaths, lost years of life, duration and number of temporary disability cases related to MNs in 2016

MN	Number of deaths	Years lost before age of 72	Temporary disability duration (in days)	Number of temporary disability cases
Stomach cancer	16,341	106,058	607,616	17,134
Rectal cancer	18,690	112,533	1,137,183	32,067
Liver cancer and intrahepatic biliary ductules cancer	5,514	35,928	136,135	3,839
Pancreas cancer	10,470	66,338	302,982	8,544
Trachea, bronchial tubes, or lung cancer	34,619	205,530	989,382	27,899
Breast cancer	14,204	104,565	1,130,556	31,880
Cervical cancer	5,174	53,600	281,629	7,941
Ovarian cancer	5,150	37,925	229,351	6,467
Prostate cancer	4,831	22,233	627,840	17,704
Kidney cancer	5,394	32,888	391,191	11,031

Trachea, bronchial tubes and lung cancer caused the greatest number of overall deaths (34 thousand); a number of lost years of life was also the greatest for this nosology (205 thousand). Rectal cancer, stomach cancer, and breast cancer also caused a significant number of deaths and lost years. Cervical cancer caused a relatively low number of deaths (5 thousand) but a related number of lost years of life was rather great (53 thousand) due to people dying from this cancer at a relatively young age. Temporary disability was the longest in case of breast cancer, rectal cancer, and cancer in the trachea, bronchial tubes and lungs (approximately 1 million days for each nosology).

Economic losses caused by MNs included into the analysis are given in Table 4. The greatest direct costs borne by the public healthcare were caused by rectal cancer (52 billion rubles) and trachea, bronchial tubes and lung cancer (50 billion rubles). Breast cancer caused substantially lower costs (20 billion rubles) as direct costs on treating 1 breast cancer case taken from initial data were substantially lower than costs on treating rectal cancer or lung cancer (Table 2). Overall direct costs also turned out to be the greatest for rectal cancer and trachea, bronchial tubes and lung cancer (56 and 54 billion rubles accordingly).

Lung cancer caused the highest GDP losses due to untimely deaths (18.5 billion rubles), and it was practically two times higher than losses caused by rectal cancer (10 billion rubles). Untimely deaths caused by stomach cancer and breast cancer also resulted in significant GDP losses (9 billion rubles and 8 billion rubles accordingly).

Overall costs borne by the public healthcare in 2016 on treating 10 analyzed MNs related to modifiable risk factors amounted to 152 billion rubles; overall direct costs taking into account permanent and temporary disability payments were equal to 173 billion rubles.

Overall GDP losses related to 10 MNs amounted to 65.7 billion rubles; GDP losses caused by disability amounted to 2.6 billion rubles.

Total economic losses caused by 10 MNs included into our analysis amounted to 241.3 billion rubles or 0.3% RF GDP in 2016.

In 2016 in the RF the greatest economic losses were caused by malignant neoplasms in

Table 4

Economic losses due to oncologic diseases caused by modifiable risk factors in the Russian Federation in 2016 (million rubles)

Disease	ICD-10 code	Direct medical costs	Temporary disability payments	Permanent disability payments	Total direct costs	GDP losses due to disability	GDP losses due to untimely deaths	Total GDP losses	Total costs and losses
Stomach cancer	C16	11,140.0	733. 3	1,264. 1	13,137. 5	282. 9	8,926. 4	9,209. 4	22,346. 9
Rectal cancer	C18–C21	52,173.7	1,372. 4	2,488.4	56,034. 5	454. 9	10,064. 3	10,519. 2	66,553. 7
Liver cancer and intrahepatic biliary ductules cancer	C22	5,656.5	164.3	366.2	6,186. 9	106. 7	3,016. 6	3,123. 3	9,310. 3
Pancreas cancer	C25	–	365.7	881.7	1,247. 3	230. 8	5,684. 9	5,915. 8	7,163. 1
Trachea, bronchial tubes and lung cancer	C33, C34	50,626.6	1,194.1	2,487.2	54,307. 8	628. 7	18,564. 6	19,193. 3	73,501. 2
Breast cancer	C50	20,903.9	1,364.4	2,541.0	24,809. 3	314. 5	7,950. 7	8,265. 3	33,074. 6
Cervical cancer	C53	4,362.8	339. 9	618.2	5,320. 9	85. 8	3,160. 6	3,246. 3	8,567. 2
Ovarian cancer	C56	1,049.2	276.8	493.3	1,819. 2	126. 8	2,886. 5	3,013. 2	4,832. 3
Prostate cancer	C61	5,431.4	757. 7	1,394.9	7,584. 2	233. 2	2,491. 2	2,724. 4	10,308. 5
Kidney cancer	C64	1,146.9	472.1	985.9	2,604. 8	126. 7	2,908. 1	3,034. 7	5,639. 6
Total		152,491.0	7,040.7	13,520.9	173,052.5	2,591.0	65,653.9	68,244.9	241,297.4

the trachea, bronchial tubes and lungs (73.5 billion rubles); rectal cancer took the 2nd rank place (66.5 billion rubles); breast cancer took the 3rd rank place (33.1 billion rubles) (Figure 1).

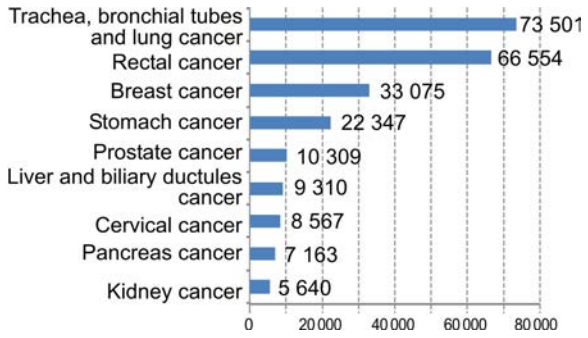

Figure 1. Economic losses due to MNs caused by modifiable risk factors (million rubles)

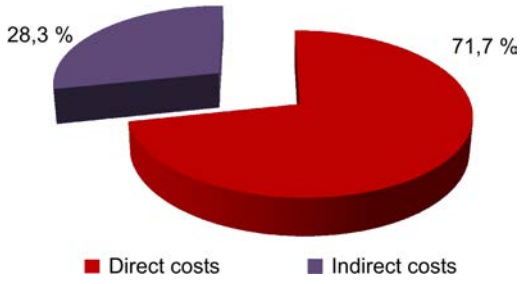

Figure 2. Structure of economic losses due to oncologic diseases caused by modifiable risk factors in 2016

Figure 2 shows the structure of economic losses due to 10 MNs caused by modifiable risk factors; thus, direct costs accounted for 71.7% of the total losses whereas GDP losses due to untimely deaths and disability accounted for 28.3%.

Oncologic diseases cause substantial losses borne by public healthcare systems in different countries all over the world. Our analysis revealed that total economic losses caused by 10 MNs included into the research amounted to 241.3 billion rubles or 0.3% RF GDP in 2016. Expenses borne by the public healthcare accounted for the biggest part of these losses (more than 70%). It differs from the results obtained via analyzing economic losses caused by cardiovascular diseases in 2016: the analysis was performed as per a similar procedure and it revealed that indirect economic losses prevailed in that case, primarily due to untimely deaths [27]. When compared, economic losses in the RF caused by oncologic diseases turned out to be smaller than those caused by cardiovascular diseases. However, one should take into account that our analysis didn't include all oncologic diseases; it focused only on those caused by modifiable risk factors of chronic non-communicable diseases. In its turn, mortal-

ity due to oncologic diseases is lower and high morbidity occurs among older people and it determines not so significant losses due to employable population being disabled permanently or temporarily.

In the EU overall expenses on MNs amounted to 126 billion euro in 2009; 51 billion euro or 40% were direct expenses borne by public healthcare systems; losses due to untimely deaths amounted to 42.6 billion euro; losses due to temporary disability, 9.43 billion euro; expenses on so called "informal relatives' help", 32.2 billion euro [6]. Our research examined costs as per other components, besides, a significant share of indirect costs in the EU could be due to high GDP in European countries.

In 2012 in Canada 7.5 billion dollars were spent by public healthcare solely on treating oncologic diseases [28].

In Russia in 2016 malignant neoplasms in the trachea, bronchial tubes and lungs accounted for the highest economic losses among all the examined MNs; the 2nd place belonged to rectal cancer; the 3rd place, breast cancer. Lung cancer also caused the greatest economic losses in the EU (15% out of expenses on all MNs), breast cancer (12%) and rectal cancer (10%) followed. Therefore, relative economic burdens related to MNs with various localizations turned out to be quite similar.

There was a research work performed in Canada that focused on estimating economic losses due to MNs caused by such risk factors as smoking, obesity, low physical activity, and excessive alcohol intake [29], and it is similar to approaches adopted in the present work as we assessed losses due to not all MNs but only the most significant ones and caused by modifiable risk factors. In Canada economic losses due to MNs caused by such risk factors amounted to 9.6 billion dollars, including 1.7 billion dollars direct costs and 8.0 indirect ones; that is, structure of economic losses turned out to be different from that described in the present work. It can also be due to high GDP in Canada. It was also stated in the same research that a decrease in risk factors prevalence would allow preventing 13.2% MNs and reduce economic losses by 1.3 billion dollars a year.

When economic losses due to MNs are calculated, actual damage, as a rule, tends to be underestimated thanks to several factors. Thus, for example, not only a patient loses his or her productivity but the same goes for his or her family members as they have to quit job or take some time off to take care of a sick relative. Our research didn't cover such issues due to absence of any data. Immaterial losses related to pain and anxiety, social limitations and poorer life quality are also very difficult for estimating; therefore they are hardly ever included into any calculations [7]. So, the present work also has certain limitations as economic losses due to MNs caused by modifiable risk factors are underestimated thanks to absence of data on certain components and impossibility to include them into any analysis. Besides, though most components in losses were calculated as per a unified procedure (indirect GDP losses, permanent and temporary disability payments), direct expenses borne by the public healthcare were calculated basing on results obtained via previous research works that could differ both methodologically and as per time periods and it could exert significant influence on their results.

Nevertheless, it is the first research work accomplished in the RF that focused on estimating economic losses due to 10 MNs with different localizations caused by modifiable risk factors over a calendar year. These data can give grounds for planning investments into preventing and treating the analyzed MNs in order to reduce burdens occurring due to oncologic diseases. It is economic arguments that are the most significant for authorities responsible for decision-making and selecting activities aimed at improving population health and determining investment volumes. Besides, calculation results can be applied for determining economic losses caused by risk factors related to the given MNs.

Conclusion. Total economic losses caused by the analyzed MNs amounted to 241.3 billion

rubles and it was equal to 0.3% RF GDP in 2016. Direct expenses borne by the public healthcare prevailed in the structure of losses (more than 70%) and they were primarily caused by rectal cancer (52 billion rubles) and trachea, bronchial tubes and lung cancer (50 billion rubles). The obtained data determine the necessity to invest into preventing and treating oncologic diseases. Such investments can result in significant economic effects in a long-term period thus making for the economic growth.

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NANOCLAYS IN FOOD PRODUCTS: BENEFITS AND POSSIBLE RISKS (LITERATURE REVIEW)

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Nanoclays (NC) are aluminosilicates that consist of layers (nano-plates) being 1–2 nanometers thick and having a diameter over 1 μm, nanotubes, and nano-disks. Due to such structure and their ion-exchange and sorption properties as well as gas permeability NC are widely used in industries, agriculture, and medicine. Gas-barrier composite packages are made from hydrophobic NC modified with cation-active surface-active substances. A person can be orally exposed to NC due to their migration from packages into food products and drinks, when NC are applied in medicine as enteric sorbents and antibacterial preparations, they can be introduced with food additives and residual quantities of technological auxiliaries as well as in case when food products and agricultural raw materials are accidentally contaminated with clays. Multiple research works dwell on experiments with NC performed with model systems in vitro when NC turned out to be cytotoxic for various cell types, and it was more apparent for hydrophobic NC than for their non-modified analogues. Minimum effective NC dose varied from 0.001 to 1 mg/ml in various in vitro tests. In vitro research on NC toxicity yielded somewhat contradictory results. Though NC didn't seem to have apparent acute toxicity (IV hazard category, LD_{50} > 5,000 mg/kg), results obtained via sub-acute and chronic experiments with their duration being up to 196 days and single clinical observations revealed a number of both toxic and non-toxic effects. Organic NC modifiers were highly toxic in vitro. Besides, NC produce anti-microbe effects and it may result in dysbiotic disorders when they are introduced orally. Model experiments revealed that NC and their organic modifiers could possibly migrate from packages into food products. NC are able to free silicon and aluminum that are partly biologically available. A contribution made by NC that are contained in packages into overall exposure to toxic aluminum should be examined profoundly given an adverse situation caused by clay minerals being introduced into a human body as components contained in food additives. Assessment of aluminum consumption with food rations in Russia and several foreign countries revealed it was necessary to exclude potassium and calcium aluminosilicates, bentonite, and kaolin (E555, E556, E558, and E559) from the list of additives that are permitted for use in food industry.

Key words: *nanoclays, aluminum, food additive, exposure, biological availability, toxicity, intestinal microbiocenose, risks.*

Introduction. At present food products are manufactured with wide use of food additives and technological auxiliaries; they are usually packed into innovative packaging. It creates certain functional and economic advantages that justify using these food additives and technological auxiliaries provided that food products still remain safe for health of this and future generations. In this relation technologists and hygienists are paying greater attention to clay minerals applied in food manufacturing including various non-modified and chemically modified **nanoclays** (NCs). Aluminum occurs in most clay minerals; this

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metal is known to produce overall toxic and neurotoxic effects. So it is vital to have a closer look at risks caused by this element migrating into edible components of food products [1]. Besides, there are certain issues related to hypothetic toxic effects produced by clays as nanomaterials and occurring due to small sizes and peculiar physical and chemical properties of particles they are made of (so called "nano-toxicity") [2, 3]. And finally, a specific issue is hygienic assessment of synthetic organic modifiers that are applied in producing certain NCs.

In the present work we have analyzed and generalized data on NCs application, effects they produce on biological systems including a human body and microorganisms, as well as data on potential risks caused by NCs application in food manufacturing. Our research technique was searching, selecting, and analyzing literature sources (articles published in revised scientific journals, theses, and monographs, as well as reports issued by international organizations) using open databases including PubMed, Scopus, Google Scholar and RSCI; a period selected for the research was 1993–2019.

Clays classification and structure. Clay minerals are widely spread in the upper lithosphere, soils, and bottom sediments; they occurred due to long-term physical-chemical and biotic transformation of igneous volcanic rocks [4]. There are such varieties of clays as sedimentary rock as bentonite, kaolin, etc.; apart from clay minerals, they contain significant admixtures of quartz, cristobalite, calcite (chalk and marble), rutile, and other minerals. Clay minerals are divided into aluminosilicates and silicates (complex silic acid salts) as per their structure. The former are the most widely spread among clay minerals; their classification is given as a diagram in Figure 1 [5].

Bearing technological properties in mind, phyllosilicates made from layered aluminosilicates structures are the most interesting, especially smectites in which these layers are comparatively weakly bonded to each other and quite mobile. This peculiarity determines physical and chemical properties of smectites, namely their strong hydrophilic nature, ability to swell in water, non-linear rheological characteristics (thixotropy) and great adsorption capability to various molecules and ions. Montmorrilonite (MMT) is the most widely spread smectite which can be found in natural bentonite clays. Its chemical structure is shown in Figure 2a. MMT is made of layers (plates) with their diameter, as a rule, being equal to 1–10 μm and their thickness varying

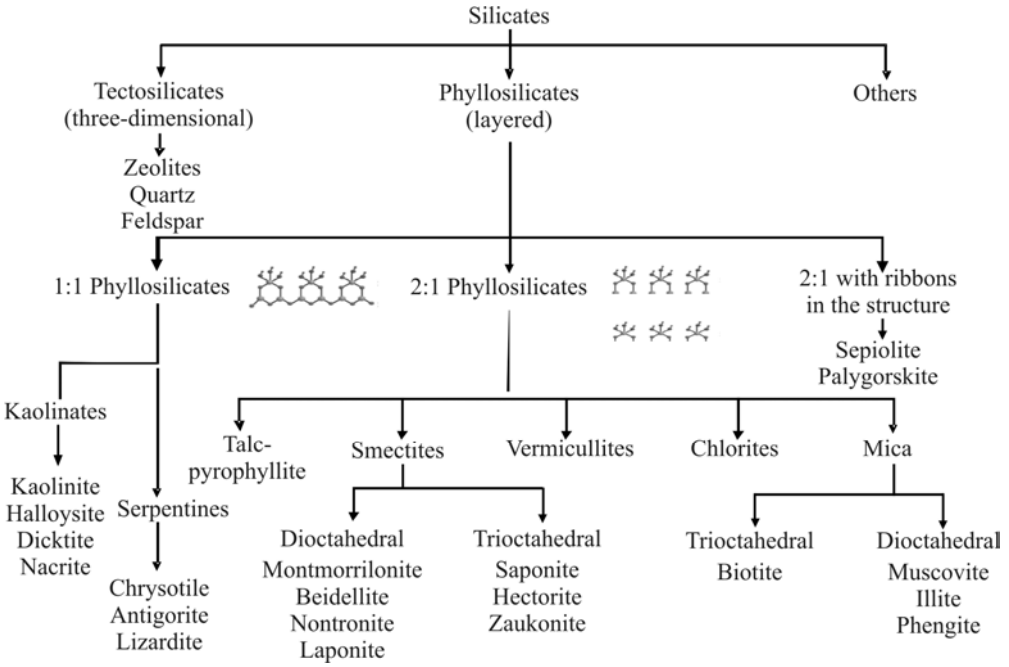

Figure 1. Silicates classification (as per data taken from [5])

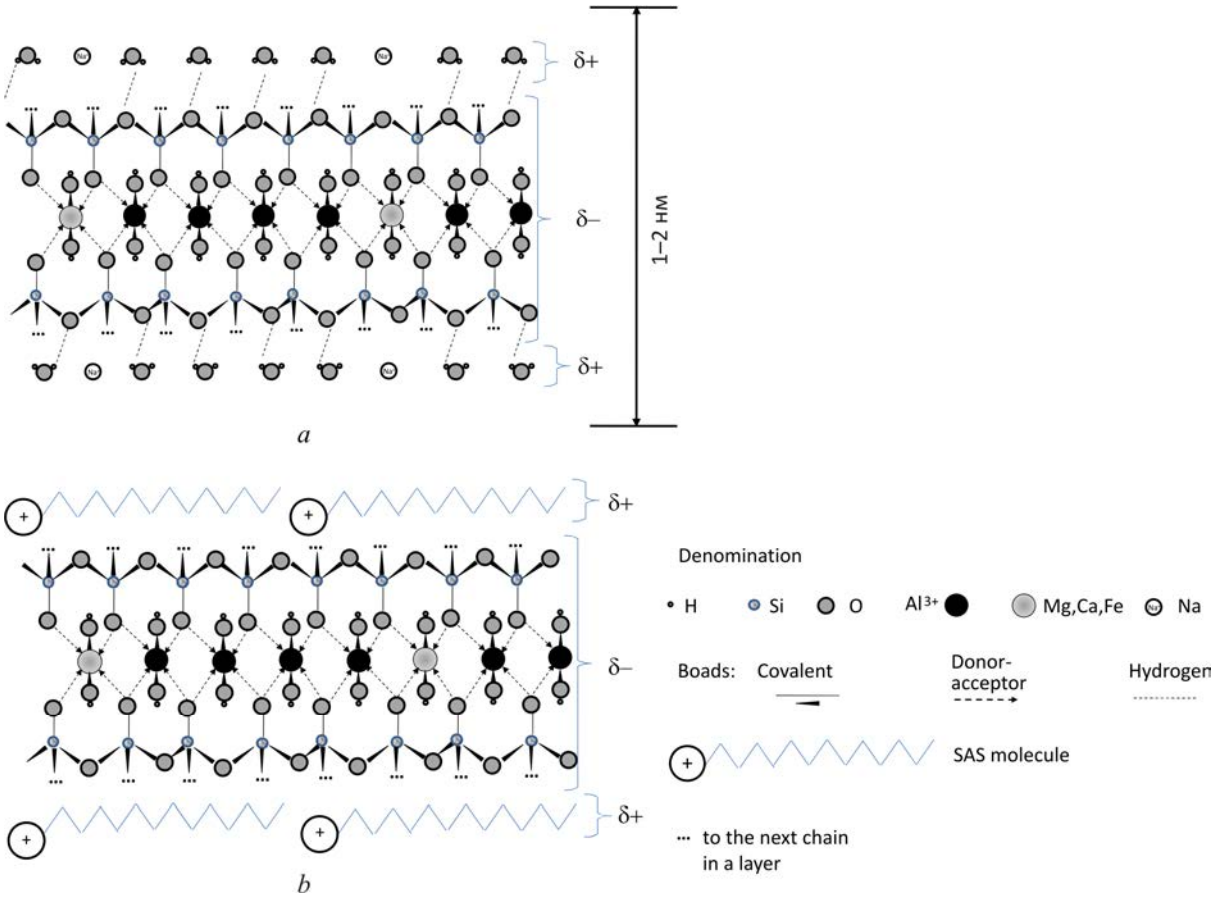

Figure 2. Chemical structure of montmorrilonite nanoclay (*a*) and its organically modified form (*b*)

within 1–2 nm depending on medium properties and as per data obtained with different measuring techniques. A MMT plate is a three-layer structure consisting of two sheets made of conjugated silica-oxygen tetrahedrons and a sheet made of aluminum ions between them; all the sheets are octahedral-coordinated with oxygen atoms in silicate tetrahedrons and hydroxyl ions. An outer layer of each plate consists of water molecules held by hydrogen bonds. Interplanar spacing between MMT layers is significantly greater than a layer thickness and is equal to approximately 7 nm in dry MMT [6, 7].

Some Al^{3+}ions in MMT are able to undergo isomorphous substitution and be replaced with an uncertain number of bivalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}); due to the process, a three-layer structure overall becomes negatively charged. This charge is compensated for with electrostatic binding within solvate shell of a plate that contains one-valent cations (usually Na^{+}). These cations can be comparatively easily exchanged for other positively charged particles and it determines MMT having cation-exchanging properties. Its empiric gross-formula can be given as $(M^{+}_{x}\cdot nH_2O)\cdot[Al_{2-x}Z_x)\ Si_4O_{10}(OH)_2]$, where M is a one-valent cation (sodium), Z is bivalent cation (magnesium, calcium, or iron), $x < 0.5$. MMT extracted from natural bentonite, as a rule, contains insignificant admixtures of quartz sand and mica and can also contain certain toxic elements in trace quantities, for example, Pb, Cd, Be, Ba, Sr, Ni and others.

Technological processing (making the material hydrophobic) involves sodium in MMT being replaced with ions of cation-active surface-active substances (SAS) which are aliphatic amines or quaternary ammonium bases; that is, substances with such composition as $R_1R_2R_3CH_3N^{+}Hal^{-}$, where R_i are aliphatic (from C1 to C16) or aromatic residues or hydrogen, and Hal^{-} is chlorine or bromine anion (Figure 2b) [7–9]. SAS molecules create new bonds in interplanar space in MMT and

displace water from it thereby replacing weak hydrogen bonds between layers with even weaker hydrophobic interactions; it results in spontaneous MMT splitting into separate plates and an organically modified NC is created [7, 10]. Similar splitting is possible also with native watered MMT; however, it involves intensive outer mechanic exposure (ultrasound).

Hydrophobic organically modified NCs are basic raw materials applied in manufacturing nanocomposites with organic polymers which are used in packing and packages for food products [5].

There are some other clay minerals that are applied in food manufacturing and therefore should be given attention. One of them is **kaolinite**, a phyllosilicate with its structure close to MMT but the difference is that its structure has only two layers instead of three, one layer is silicon oxide and the other is aluminum oxide. Potassium prevails as an outer cation in kaolinite as opposed to MMT where this role belongs to sodium. Steric strains in the structure of kaolinite plates make them bend cylinder-like and the extreme case here is a nanotube formation. Another mineral, so called **halloysite**, is made of such aluminosilicate nanotubes. As opposed to carbon nanotubes, halloysite nanotubes are not closed; they are composed of kaolinite layers folded into rolls (Figure 3). A typical diameter of such nanotubes amounts to 20–50 nm and their length exceeds 1 μm. Another phyllosilicate is **laponite** that is close to MMT in its chemical structure but is made of nanoparticles (nanodisks) with a rather small diameter (it is usually 25–30 nm) and 1 nm thick; it is prone to swelling and thixotropic gels formation.

Other clay minerals that are given in Figure 1 are applied in food manufacturing in rather insignificant quantities and have low practical importance.

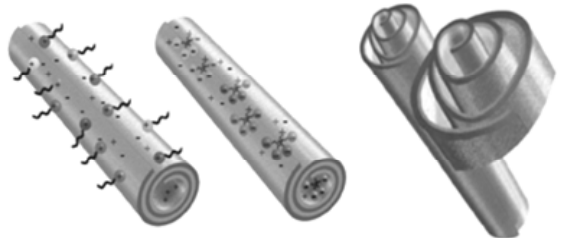

Figure 3. Structure of halloysite nanotubes

There are data in literature on probable practical application of so called “anionic” clays that are artificial layered mineral materials; they are usually created with other non-metal element participating in their structure instead of silicon (usually carbon) or with chloride anion [11]. Any issues related to biological properties of those artificial materials might have not discussed in the present work.

How clays are applied in food manufacturing. MMT and some other phyllosilicates are widely used in various industries. There are data on more than 100 different areas where clays can be applied [5, 12].

NCs have high specific surface area and ion-exchange properties; due to that their adsorption capacity is also great and it provides an opportunity to intercalate organic molecules between their layers. Organically modified NCs are easily built into volumes of hydrophobic organic polymers thus creating planar structures (nanocomposites) [13]. A most significant property of clay nanocomposites is that NC plates are impermeable for gas molecules (oxygen or carbon dioxide) as well as for water; due to it gas or water molecules have to overcome a much longer path in their diffusion through a polymer [14] (Figure 4). Apart from this barrier function, nanocomposites are usually more solid and rigid, have greater thermal stability and resistance to swelling [15–18]. On the other hand, there are developments that allow obtaining clay nanocomposites with natural biopolymers (proteins or polysaccharides) that are able to degrade biologically and therefore are environmentally “friendly” [19, 20]. Biologically degradable nanocomposites with antimicrobial properties were obtained via combining vegetative polysaccharides, NCs and natural essential oils [21, 22]. There are data on creating a biological nanocomposites based on montmorrilonite clay that had antioxidant properties and contained silibin, an extract of holy thistle [23]. Barrier properties typical for nanoclays are widely used in producing film packages and packing that are able to prevent packed products from drying up and oxidation-induced spoilage, as well as prevent carbonated drinks from degassing [16, 24, 25]. Nano-

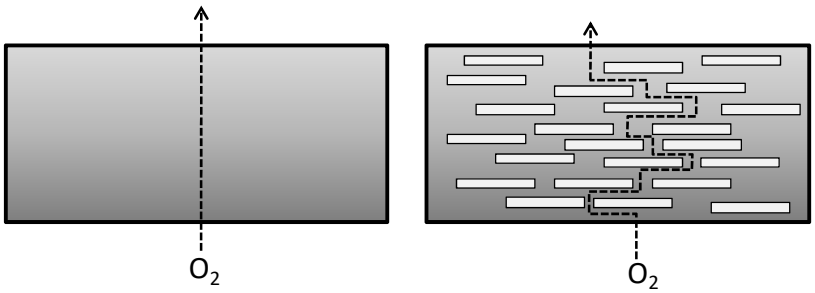

Figure 4. A scheme that explains gas-barrier properties of nanoclays composites

composites based on clay with nisin are considered to be a useful compromise when this preservative is applied to prevent food products from spoilage taking into account insignificant migration of nisin from the nanomaterial into a food product [26, 27].

Overall, an effect produced by NCs and their modified forms applied in packing materials is that food products become safer and their quality improves [28]. NCs are becoming more and more widely used in food products packages and packing and this promising trend is developing rapidly. As per data given in [29] a market of nanocomposites applied in food products packages and packing in the USA was equal to 4.13 billion $ in 2008 and it grew to more than 7 billion $ in 2014. Clay-based composites accounted for the biggest share in this market [30].

NCs can be also used as medication carriers as it was discussed in a review [31]. Akbari A.M. et al. revealed an opportunity to use montmorrilonite clay as vitamin B_{12} carrier [32].

Some other ways to apply clays in food manufacturing are, for example, their use as technological auxiliaries (filter media, adsorbents, or flocculants) in manufacturing vegetable oils, beer, or non-alcoholic drinks. Clays, especially in their nanoform, are efficiently applied in this sphere due to their great adsorption capability regarding various ions and polar organic molecules [33, 34]. We should note that technological auxiliaries should be completely removed from ready food products during a production process. As opposed to that, food additives based on aluminosilicate clays that act as carriers and anti-caking agents can persist in certain food products. The legislation existing in the RF and EAEU countries in 2019 allows using some aluminosilicates as additives to food; they are sodium, potassium, and calcium aluminosilicates (E554–E556), bentonite (E558), and kaolin (E559)[1]. Standardized contents of the said food additives (separately or in a combination) in spices and products tightly wrapped in foil should not exceed 30 g/kg; sugar powder, 15 g/kg; cheese and its substitutes, 10 g/kg; salt, 10 g/kg. Use of aluminosilicate food additives in producing pelleted food products and biologically active additives (BAA), as well as sugary confectionary excluding chocolates (for surface treatment) is regulated in accordance with technological standards accepted by a manufacturer (according to technological chats in quantities necessary to produced desirable technological effects).

Use of clay minerals (MMT or kaolinite) as additives to forage implies that these substances have certain enterosorbent properties; it allows efficient preventing fusariose-induced toxicosis and acidosis in animals and makes for less active hepatic transaminases and lower lactate and biogenic amines (histamine and spermine) contents in blood serum [35, 36].

We should also note that so called “edible” clays are actually used as nutrition components in certain regions. Some African and Asian tribes with their socioeconomic and cultural development being rather low tend to eat mineral substances occurring in soils (so called

[1] CU TR 029/2012. The Customs Union Technical Regulations "Requirements to safety of additives to food, flavoring agents, and technological auxiliaries" (last edited on 18.09.2014). Appendix 2. *KODEKS: the electronic fund for legal and reference documentation*. Available at: http://docs.cntd.ru/document/902359401 (14.02.2020).

"pica" phenomenon) [37]. There is an opinion that it helps them obtain certain mineral substances in additional quantities as these substances (potassium, magnesium, and iron) are found in clays in a form that is partially biologically available [38]. Besides, clays have apparent enterosorbent properties and it can make for prevention of enteric infections and aflatoxicosis [39]. When bentonite was consumed with food, it helped people living in Ghana excrete aflatoxins and T2 toxin out of their bodies [40]. It is interesting to note that there are specially designed (and certified as being safe) traditional food products based on "edible" African clay that are sold in some African countries (Nigeria, Cameroon, Ghana, Togo, the SAR, and others) as well as in the USA and Western Europe [37]. As we can see from available literature sources, this phenomenon is almost absent in the RF and EAEU countries.

NCs toxicity for live organisms. Though it is apparent that all the above mentioned ways to apply NCs can produce truly favorable technological effects, probable toxic impacts exerted by both modified and non-modified NCs on people and environmental objects can cause potential risks [41].

Data obtained via experiments in vitro. Experiments *in vitro* that were usually performed on human and animal cells cultures applied as test objects (both primary cultures of standard cells and transformed ones) allow obtaining data on cytotoxic properties of nanomaterials such as NCs; screening of presumably the most toxic nanomaterials out of the examined group; and getting an insight into probable nanotoxicity mechanisms [5, 42].

The WHO document [4] contains a review of early research works (published in 1969–1996) in which their authors described various effects produced by particles of MMT, kaolinite, and other clays in *in vitro* systems.

Some research works revealed that native MMT turned out to be cytotoxic. Four types of aluminosilicates were toxic for the human umbilical vein endothelium (HUVE) under exposure in concentrations varying from 0.01 to 0.1 mg/ml that lasted for 6–24 hours; toxicity given in descending order: MMT > native bentonite > kaolinite > zeolite [43]. Kaolinite and zeolite induced a dose-dependent increase in fatty acids production and cell lysis. Two other cell lines, neuroblastoma N1E-115 and oligodendrocytes ROC, were resistant to effects produced by clays.

Non-modified MMT in a concentration equal to 1 ml/mg in a 24-hour exposure resulted in weaker survivability of ovary cells taken from a CHO hamster [44]. The same results were obtained for intestinal cells of INT-407 line [45]. Non-modified MMT, Cloisite Na trademark, didn't have any cytotoxic effects on transformed intestinal epithelium cells Caco-2 after 24-hour exposure in a concentration equal to 0.17 mg/ml, didn't cause any DNA breaks in them according to comet assay and was not mutagenic for *Salmonella spp*. in Ames test [46]. As per data obtained by Gao et al. [47] kaolin turned out to be cytotoxic for a primary culture of rat's lung macrophages and its cytotoxicity was similar to that of quarts; however, it caused less significant damage to DNA. Along with nano-plates, some data were obtained for nanotubes of native clays. Verma et al. [48] applied transformed lung epithelium cells A549 in their experiment and revealed that tubular clay structures were less toxic than nano-plates. An issue related to interpreting these data is different chemistry of these nanomaterials; to be exact, nano-plates were MMT-based, and nanotubes, halloysite. It should be noted that, according to Vergaro et al [49], native halloysite nanotubes were cytotoxic for HeLa and MCF-7 cells in a concentration higher than 0.075 mg/ml. On the other hand, Lai et al. [50] didn't reveal any toxic effects produced by halloysite nanotubes on intestinal epithelium cells in concentrations varying within 0–0.1 mg/ml range.

Rawat et al. [51] compared cytotoxic effects produced by the natural MMT and synthetic laponite on HEK (human embryo kidneys) cells and SiHa (uterine neck carcinoma) cells as well as on *E.coli* culture. They also showed that clay nano-disks (laponite) had more apparent cytotoxic and antimicrobial effects than long nano-plates (MMT).

When an organic modifier consisting of cation-active SAS is introduced into nanoclay

in order to make it hydrophobic, it can lead to considerable changes in cytotoxicity of the nanomaterial. It is related to both changes in physical and chemical properties of NC itself (its hydrophobic properties, zeta-potential, and aggregative stability) and, presumably, effects produced by a modifier that migrates from NC into biological media. Thus, organically modified MMT Cloisite 30B, as opposed to its native precursor, was genotoxic for Caco2 cells and mutagenic for *Salmonella sp.* [46]. The same effects were produced by Cloisite 30B that was filtered through a 2-μm thick membrane and didn't contain any clay; consequently, occurring effects were most likely due to a migrating modifier. Organically modified Cloisite 93A nanoclay was more cytotoxic than native MMT in a culture that contained transformed liver cells HepG2 after a 24-hour exposure [52].

An increase in cytotoxicity of organically modified NCs depended on a modifier and its essence. Thus, when a modifier was diethylbenzyldialkyl-ammonium based on fatty acids obtained from tallow, it resulted in greater toxicity of modified MMT for certain cell lines (Ramos, Burkitt lymphoma; A-549, lung adenocarcinoma; HCT116, colorectal carcinoma; SK-MEL 28, melanoma; HepG2, hepatocellular carcinoma; and HUVEC human umbilical veil endothelium) than when a modifier was dimethylalkyl-ammonium base [53]. Those data were well in line with cytotoxic effects produced by those modifiers themselves on the said cell lines. Concentration of 50 % – inhibiting (LC_{50}) didecyldimethyl-ammonium saccharinate in a test with tetrazolium salts (MMT-test) was within 1.44–5.47 mM range for certain lines of human cells [54]. Native MMT turned out to be less toxic than organically modified one (Cloisite 30B) in experiments performed on HepG-2 and Caco-2 cells [55, 56]. Threshold toxic concentrations of two organically modified NCs varying in a modifier structure changed from 8 to 30 μg/l. Modified NCs in concentrations higher than 30 μkg/l caused DNA fragmentation in cells of both lines and depletion of reduced glutathione resources in HepG-2 cells. Effective concentrations of various hydrophobic NC modifiers differed also according to data obtained from [7, 55–58].

When bentonite was "activated" by the treatment with sulfuric acid, it resulted in its higher cytotoxicity for human B-lymphoblasts [59, 60]. The process, together with reduced ability of the cells to survive, also involved greater oxidants production and damage to DNA. Water extracts from clay didn't apparently have any genotoxic effects as it was revealed in those experiments.

On the other hand, there are data that certain modifications of nanoclays don't result in their increasing cytotoxicity. Thus, Han et al. [61] stated there was no damage done to membranes and no reduction in survivability of cells from 4 lines when they contacted organically modified calcium and magnesium phyllosilicates. Native and functionalized halloysite nanotubes had the same cytotoxicity for HeLa and MCF-7 cells in concentrations higher than 0.075 mg/ml [49].

There are relatively few assessments of cytotoxicity that NC composites might have in available literature. When MMT was built into oligo co-polymer of styrene with acrylonitrile, it resulted in its lower cytotoxicity [62]. The same authors revealed [63] that a composite that contained halloysite nanotubes with chitosan was not cytotoxic for fibroblasts from NIH3T3 line and was quite biologically compatible such as films made from pure chitosan. L-929 fibroblasts were more adhesive and grew better on chitosan without clay in comparison with chitosan that contained MMT in quantity from 1 to 8 % [64].

Kevadiya et al. [65] stated that clay composites were able to act as reservoirs for cytotoxic medications, and their side toxic effects produced on normal cells could decrease whereas medications could be delivered to their destination more efficiently.

The fact that nanocomposites have different toxicity may result from quantities of NCs and organic modifiers that migrate from them. In particular, Cloisite 30B nanoclay that contains quaternary ammonium base can be extracted from a biopolymer nanocomposite based on

soya polysaccharide [66]. Cloisite 30 B NC was cytotoxic for Hep 2, C26 and HTC cells in concentrations varying from 39.1 to 90.17 μkg/l. Cloisite 30B particles migrated from packing film into the liquid media in significant quantities. It was concluded that a nanocomposite with such structure could be applied for packing dry products only and it could protect them to a certain extent from bacterial contamination but not from mold fungi.

It is rather alarming that people may be exposed to aerosols occurring when packing wastes with NCs in their structure are burnt at combustion plants. Wagner A. et al. [67] performed their experiments on BEAS-2B cells of human lung epithelium and revealed that burning under 900 ^{o}C didn't exert any significant impacts on cytotoxicity of native MMT and drastically reduced cytotoxicity of organically modified Cloisite 30B nanoclay; it corresponded to relevant burning out of its organic component. The same authors performed similar experiments [68] to examine cytotoxicity of solid products occurring after burning of a polymer nanocomposite that contained three different organically modified nanoclays. They showed that combustion products that had powder-like or spongy structures with their particles size significantly exceeding 100 nm didn't produce any apparent cytotoxic effects in a concentration up to 0.3 mg/ml. The authors made a conclusion that although any organically modified NC was more cytotoxic than native one its cytotoxicity went down considerably during packing materials combustion due to modifiers in its structure having been burnt out.

Data obtained via *in vitro* tests allow making assumptions as regards mechanisms of cytotoxic effects occurring in direct contacts between NC and cells. Thus, lysis occurred in HUVE cells after 24-hour exposure to MMT, bentonite, and kaolinite [43]. Short-term exposure (60 minutes) to MMT and bentonite resulted in complete lysis of neuronal cells [69]. Morphological changes appeared in HepG-2 and Caco-2 cells exposed for 24–48 hours to organically modified Cloisite 93A NC in a concentration varying from 0.05 to 1 mg/ml; among those changes were damages to mitochondria, nucleus, and endoplasmic reticulum [55]. Degenerated mitochondria in HepG2 and Caco-2 cells exposed to organically modified NCs are consistent with data obtained via MTT-test performed on many cell types exposed to both modified and native halloysite nanotubes [44, 45, 49, 50, 52, 55–58, 63]. Fatty impurities in HepG-2 and Caco-2 cells cultivated with C30B present during cultivation indicate that lipid metabolism is violated [43]. All the above stated facts allow one to assume that a direct contact between non-modified MMT and different cell types results in damage to them with prevailing antioxidant stress caused by catalytic synthesis of free radicals at interphase borders whereas the basic factor that makes organically modified clays toxic is their organic component (a modifier) that produced membranetropic effects [5].

Therefore, as per data obtained from numerous research works, negative effects produced by NCs on different cells are undoubted. But it is impossible to determine whether significant these effects are in terms of toxic impacts produced by NCs on a human body without exact data on exposure scenarios, quantities in which nanomaterials migrate from package and packing into products and NCs ability to penetrate through biological barriers.

Data obtained via experiments in vivo. Research works on clay minerals toxicity performed *in vivo* are not so numerous as compared to data obtained via *in vitro* systems; available results are hardly comparable due to experimental models and tested objects being different form each other. A review on early data on clay mineral toxicity is given in works [4, 70].

Overall, exposure to clay materials can stimulate both toxic and non-toxic response. The latter can occur due to favorable effects produced by clays as enterosorbents as they are able to reduce burdens related to penetration of aflatoxins, heavy metals and microbe metabolites into a body; they produce antacidic effects; and serve as a source of essential microelements that can be consumed with them in certain additional quantities [5, 71, 72]. Most early

works revealed that clays didn't induce overall toxic and histopatologic changes, both under single exposure in an acute experiment [45, 73, 74] and in a sub-acute or chronic one lasting up to 196 days [75–78] performed on rodents. For example, Baek et al. [45] estimated LD_{50} of non-modified MMT for mice and it amounted to 41,000 mg/kg of body weight. Wiles et al. [79] stated that MMT was not toxic or produced negligible toxic effects on pregnant Sprague–Dawley female rats and they didn't reveal any embryotoxicity either. However, previously Patterson and Staszak (1977), cited as per [5] revealed certain reproductive toxicity as there was anemia in female rats and lower body weight of newborn offspring after exposure to kaolin in quantity amounting to 20 % in the overall forage mass.

There have been few *in vivo* research works on clay minerals toxicity in the last 10 years. There was a suspicious case when cats got poisoned by bentonite after swallowing bentonite-containing cat litter; they suffered from hypokaliemia and hypochromic anemia, lethargy and muscle weakness. Orally introduced bentonite resulted in lower calcium contents in bone tissue of goats. Bentonite didn't compensate for a decrease in calcium contents in shinbones of broilers with alimentary deficiency. A 3-year old girl who had been exposed to bentonite orally and rectally as a home-made medication suffered from severe hypokaliemia [40].

On the other hand, Maisanaba et al. [78] didn't reveal any increase in lipoperoxides, or changes in activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in the liver or kidneys of rats after 40-day exposure to organically modified MMT; although, catalase became more active, and there was an increase in its protein contents and gene expression in the kidneys. EFSA [76] gives some data on chromosome aberrations revealed in rats that were exposed to bentonite for 15 days. However, Sharma et al. [80] didn't detect any breaks in DNA chains in cells or any inflammatory response in the large intestine, liver or kidneys of Wistar rats that had been twice exposed to organically modified Cloisite 30B via a stomach tube in a dose 250–1,000 mg/kg of body weight. This result is quite consistent with data obtained by Hsu et al. [81] in their experiments on Sprague–Dawley rats that were subcutaneously exposed to a suspension containing MMT nano-plates.

V.V. Smirnova et al. [82] performed their experiments on male rats that were exposed to non-modified hydrophilic NC "Nanoclay Nanomer PGV" via a stomach tube for 28 days in a dose equal to 1 or 100 mg/kg of body weight. A lot of biochemical and hematologic parameters were then analyzed and the authors didn't reveal any changes in animals from the test group that could be considered unfavorable. Moreover, exposure to NC had certain effects indicating that antioxidant protection system became more active (diene conjugates contents in plasma went down and glutathione peroxidase became more active). Clay consumption didn't stimulate liver cells apoptosis or make intestinal walls more penetrable for macromolecules.

There was an experimental model that involved inducing hypercreatinemia in mice; montmorrilonite applied within it reduced creatinine contents in blood serum and accelerated its excretion from the intestines. Bentonite consumed by animals with renal failure made for urea diffusion out of blood vessels into the intestines and inhibited its absorption there [40]. Mice suffering from hyperthyrosis were given montmorrilonite and it reduced thyroxine and triiodothyronine contents, made for longer sleep, increased tolerance to hypoxia and reduced spontaneous motor activity [40].

Some researchers argue that absence of toxic effects produced by NCs is probably due to them being practically not biologically available under enteral introduction [80]. However, Baek et al. [45], although they didn't observe any toxic effects produced by MMT in a dose up to 1,000 mg/kg of body weight, still reported that clay minerals could be absorbed in a body during 2 hours with certain marker elements (silicon and aluminum) accumulating in specific organs. Mascolo et al. [74] detected an increasing accumulation of NCs marker elements in rats' urine and tissues, and the organs where

they accumulated were distributed as per their quantities given in the descending order in the following way: kidneys > liver > heart > brain. Reichardt et al. [83] showed that kaolinite could dissociate into the intestine lumen and escaping aluminum ions could be absorbed. Particles of organically modified Cloisite 30B nanoclay can be absorbed by epithelium cells in rats' esophagus [66]. However, Sharma et al. [80] didn't reveal any aluminum accumulation in the liver and kidneys of rats that were exposed to modified Cloisite 30B nanoclay via gavage in a dose equal to 1,000 mg/kg of body weight. EFSA experts [76] believe that bentonite and sepiolite are not absorbed in the gastrointestinal tract in any significant quantities.

Organic NC modifiers that can be dissociated from MMT both within a product and after being introduced into a body have their own toxic effects. In particular, Melin et al. [84] determined that quaternary ammonium bases caused grave disorders in reproductive health of mice. There was an experiment performed on rats; they were intragastrically exposed to didecyldimethyl-ammonium saccharinate via gavage in a dose equal to 2,000 mg/kg of body weight; the exposure resulted in death of all the experimental animals [54].

Only three research works focused on assessing clay nanocomposites toxicity. Thus, the authors of [64] indicated that NC composites didn't produce and toxic effects *in vivo* and were highly biologically compatible with polyurethane and chitosan. MMT nanocomposites / chitosan had more significant biological compatibility *in vivo* than pure chitosan [81]. Maisanaba et al. [85] exposed rats to substances that were extracted from a composite containing organically modified NC and poly-L-lactite and turned into a model drink; they didn't reveal any histopathologic or biochemical signs of toxicity.

Interaction with chemical toxicants. There are data in literature that allow assuming nanoclays being able to reduce toxicity of chemical toxicants when they are introduced together with them as the former can act as enterosorbents. Authors of some early works [35, 86] reported that it was quite efficient to add non-modified clays into forage for agricultural animals (chicken or pigs) in doses equal to 0.1–0.5 % of the overall ration in order to prevent adverse effects produced by aflatoxins from group B that could be found in forage. Simultaneously it was shown that NCs in the mentioned doses didn't deteriorate biological availability of vitamins B_2, and A, phosphor, and manganese although there was a slight decrease in biological availability of zinc when the dose reached 1 % (and it was higher than a recommended preventive one). Detoxication became especially efficient in case of aflatoxicosis when NCs were combined with such antioxidants as selenium, methionine, and vitamin E.

Afriyie-Gyawu et al. [39] examined clinical materials taken in regions that were endemic in terms of aflatoxicosis in people. They revealed that it was quite efficient to use NC NovaSil as an enterosorbent and it didn't result in any risks related to vitamins A and E and mineral substances becoming less biologically available. Data obtained from the work by Abdel-Wahhab et al. [34] indicate that organically modified NC is able to absorb aflatoxins, fumonizine, and zearalenone.

El-Nekeety et al. [87] exposed Sprague Dawley rats to fumonizine B_1 and/or zearalenone for 3 weeks; reference groups in their experiment were given only usual food or food with added MMT in a quantity equal to 0.5 % of the overall provided food. After that the authors determined nitrogenous and lipid metabolism in blood plasma, LP products in the liver and kidneys, antioxidant enzymes activity, levels of carcinoembryonic antigen (CEA), alpha-fetoprotein and IL-6, and morphological parameters of the liver and kidneys. All the determined parameters indicated that MMT produced favorable therapeutic effects. There were no signs of toxic effects on animals that were given only MMT in the above-mentioned dose.

Therefore, high activity of NCs as enterosorbents for various toxic substances provides certain opportunities for using NCs in treating and preventing various intoxications both among people and animals. It creates additional exposure to both native and modified NCs.

Antimicrobial activity. Some native clays were used to treat wounds in ancient times, long before their antimicrobial properties became known to medical science [88]. Antimicrobial effects produced by clays can occur due to both physical interactions with microbe cells and emissions of various chemical components. Physical antimicrobial effects are presumably determined by bacteria sticking to clay particles and, as a result, microorganisms fail to absorb necessary nutrients, they discharge metabolites, and their cellular walls are broken (Ferris et al., 1987, cited as per [5]). Wherein this clays rather produce bacteriostatic effects and not bactericidal ones [89].

Antimicrobial effects produced by NCs can be related to their ability to cause dysbiotic disorders in case they are introduced orally. Thus, Smirnova V.V. et al. [82] exposed Wistar rats to non-modified MMT and revealed lowering functional activity of symbiotic bifidobacteria in exposed animals together with a substantial (by 3 times) growth of yeast flora in the caecum.

When assessing probable chemical antibacterial effects produced by NCs, one should bear in mind that though aluminosilicate particles are poorly soluble in water, they can still release ions of metals (in particular aluminum or iron) in a biological medium where organic molecules are present; these released ions are able to produce antimicrobial effects [88]. Wang et al. [90] revealed antimicrobial effects produced on *S. aureus* and *E. coli* by exfoliated NCs and their forms modified with cation-active, anion-active and non-ionogenic SAS, as well as by nanocomposites with polyurethane.

Composite films based on *Salvia macrosiphon* sage seeds gluten and organically-modified Cloisite 15A nanoclay demonstrated their antimicrobial properties regarding *E.coli* and *S.aureus* in test on agar [91]. A composite formed from negatively charged bentonite NC layers and monomolecular chitosan layers turned out to have antimicrobial effects on *Pseudomonas syringe pv. tomato* bacteria and *Fusarium solani f. sp. Eumartii* mold fungus that caused agricultural products spoilage [92]. It is assumed that such nanocomposites can also be so called “elicitors”, that is, substances that non-specifically increase plants resistance to pests and pathogens.

Antimicrobial effects produced by clay minerals became widely used in clinical practices. Thus, patients suffering from diarrhea of various etiologies (viral infection, food allergies, spastic colitis, mucus colitis, and food poisoning) were given bentonite orally and it resulted in favorable clinical effects in 97 % cases [93]. “Diosmectite” adsorbent that consists of natural aluminosilicate-magnesium clay is widely used in clinical practices to treat diarrhea and irritable bowels syndrome. The medication was shown to normalize stool due to its ability to absorb toxins, bacteria, and viruses and to enhance barriers created by the intestine mucosa thus reducing inflammation and penetration of luminal antigens through mucus layers. It also prevented adsorption of bacteria, enterotoxins, viruses, and other potentially diarrhea-inducing substances on cell membranes [94]. “Diosmectite” is also recommended to prevent negative side effects produced by radiation and chemical therapy as well as to treat acquired immunodeficiency syndrome involving chronic diarrhea [95]. When bentonite was prescribed to patients in a dose equal to 3 g/day for 8 weeks, it resulted in less apparent signs of irritable bowels syndrome against placebo [40].

Clinical research also revealed probable side effects produced by clay-based medications. Diosmectine (smecta) caused constipations, though in rare cases, and the effect was eliminated after a dose had been adjusted; sometimes (better to say, rather rarely) there were allergic reactions (nettle-rash, rash, itch, and Quincke’s edema). Clays as enterosorbents have certain contraindications such as bowel obstruction, fructose intolerance, impaired glucose and galactose absorption, invertase-isomaltase insufficiency [95].

Therefore, plenty of data on antimicrobial effects produced by NCs and their composites not only substantiate their use in producing medical appliances and medications but also provide better insight into a probability that biological effects produced by NCs become

obvious due to their interaction with intestinal microbiocenosis components under oral introduction into a body.

Migration form packing materials. A most significant role in assessing probable health risks caused by NCs belongs to determining quantities in which these nanomaterials and their organic modifiers migrate from packing materials [96, 97]. We should note that contradictory results have been obtained in researching the issue. Schmidt et al. [98] didn't reveal any clay migration from poly-L-lactite in 95 % ethanol within detection limits; and Bott et al. [99] also didn't detect any laponite migrating from low-density polyethylene into SAS solution. Xia et al. [100] detected only trace clay quantities (3–6 μg/l) migrating from polypropylene and polyamide films into ethanol. According to theoretical calculations made by Simon et al. [101], particles with their diameter exceeding 1 nm should not at all migrate from polymer phases due to their significant viscosity. However it is not the case with particles that are located on an interphase surface or close to it as well as with a situation when a polymer is being destroyed. Probably it was the reason for Avella et al. [19] to determine an increased concentration of silicon in vegetables that contacted a nanocomposite. However, it is rather difficult to interpret these data as a packing material used in that work was biologically degradable. Farhoodi et al. [–102] revealed that aluminum and silicon could migrate from PET bottles in acid medium under 25 and 45 °C. Other researchers showed that both aluminum and silicon could migrate from nanocomposites into water and water-ethanol media [103].

Echegoyen et al. [96] examined how aluminum migrated from containers consisting of two various polyethylene composites with organically modified NCs. All the tests were performed according to the EU Regulation 10/2011/EU that involved using 10 % ethanol and 3 % acetic acid as model media under 70 ºC for 2 hours or under 40 ºC for 10 days. Under given conditions, Al migrated from samples in quantities varying from 2 to 51 ng from 1 cm^2 of their surface; the authors applied electronic microscopy and it allowed them to detect clay nano-plates in the model media. Their structure was additionally confirmed via energy dispersive spectroscopy. Xia et al. [100] examined migration of clay particles, Si, Al, and organic modifiers (quaternary ammonium bases) from polypropylene (PP) composites and polyamide 6 (PA6) with organically modified MMT into ethanol under 70 °C. It was shown that more clay particles migrated from PP films than from PA6 ones (0.15 and 0.10 mg/l respectively); it was probably due to weaker interaction between organically modified NC and the first polymer. Modifiers migrated in ethanol in quantities being equal to 3.5 mg/l for PP films and 16.2 mg/l for PA6 ones. Treatment with ethanol also led to changes in nanocomposite ultra-structure. Aluminum migration from polyethylene nanocomposite into 3 % acetic acid reached 5.16 μg/cm^3 [96]; aluminum migration from PET-based nanocomposite into the same medium amounted to 0.34 mg/kg, and silicon, 9.5 mg/kg [102]. Biopolymer films made from wheat gluten emitted up to 1 mg/kg aluminum and up to 4.5 mg/kg silicon into water, 3 % acetic acid, 15 % ethanol and vegetable oil [104].

NCs can migrate from packing materials not only under exposure to a medium that is characteristic for a food product but also due to weathering [105]. To assess this factor, model clay nanocomposites were treated with UV-radiation or ozone under 40 °C. Physical and chemical properties of nanocomposites changed rather slowly during the first 130 hours and clay migration was also slow; after that, materials degraded rapidly and completely. Built-in nanoclays made an initial composite more stable and durable; however, they resulted in its faster degradation under exposure to UV-radiation. Nanoparticles that migrated from nanocomposites under weathering were 2–8 nm in size. Their concentration grew as treatment period got longer.

Therefore, contradictory data on migration of NCs and their components from packing materials indicate it is necessary to apply an individual approach to assessing their safety taking into account what structure an applied nanocomposite has, under what conditions it is

applied, as well as properties of products that are packed in it.

Exposure and probable risks. People as a biological species have always been exposed to clays to a certain extent [106]; however, this exposure obviously has increased over the last two decades due to clay minerals being widely used in technology and medicine. Inhalation exposure accounts for the biggest part of the total one; intragastric and subcutaneous exposure follows [107, 108].

Occupational inhalation exposure usually occurs predominantly when minerals are extracted, in agriculture, and at aluminum-producing enterprises [5, 109]. This review doesn't include profound consideration of such exposure.

Data on oral exposure of people to MMT, kaolinite, and other clays are rather contradictory [4]. When clays are introduced into polymer materials, it can result to spontaneous exposure to them and it requires assessing migration of both microparticles and nano-structured clays from packing materials into food products [41]. Besides, NCs can penetrate the environment in considerable quantities during a product life cycle, for example, when wasted packaging and packing materials are incinerated at combustion plants [110]. Food additives and medications are other significant sources of oral exposure to clays in developed countries [111, 112]. Here we should mention bentonite, montmorrilonite, kaolinite, and palygorskite that are used as antacids and antidiarrhea medications [113]. Some clays are applied to prevent mycotoxicosis and aflatoxicosis in people in regions where endemic risks as per these health disorders are rather high [39, 114].

Bearing probable risks analysis in mind, we think that the most significant task here is to examine exposure of people to aluminosilicate food additives as they can be result in toxic aluminum being introduced into a human body. As per EFSA assessment, five aluminum-containing food additives, between them four should be treated as clays (acid sodium aluminophosphate E541; sodium aluminosilicate E554: potassium aluminosilicate E556; aluminosilicate-kaolin E559), are consumed by people from various age groups in quantities, recalculated as per aluminum, varying from 2.3 to 76.9 mg/kg per week on average for the whole population and from 7.4 to 145.9 mg/kg per week on average for 95 % population depending on a scenario [1]. According to an alternative scenario assuming that food products with aluminum-containing food additives in their structure are consumed in greater quantities, average consumption amounted to 18.6–156.2 mg/kg of body weight per week; consumption for a 95 % population sampling amounted to 5.3–286.8 mg/kg of body weight per week [115]. Therefore, aluminum that is contained in food additives is consumed by people from various age groups in quantities being considerably higher than its tolerable weekly intake from all the possible sources (TWI = 1 mg/kg of body weight) fixed by EFSA [, 116] as well as its provisional tolerable weekly intake (PTWI = 0–2.0 mg/kg of body weight) fixed by JECFA [1, 117].

Results obtained by other authors confirm that children, as a rule, consume aluminum with food in greater quantities per a unit of their body weight than adults though these data are a bit different from EFSA estimates. According to it, probable aluminum consumption by children aged 3–15 in France amounted to not less than 0.7 mg/kg of their body weight per week in 97.5 %. This quantity was equal to 2.3 mg/kg of body weight per week for pre-school children aged 1.5–4.5. In Great Britain in 1988 children aged 4–18 consumed aluminum in a quantity equal to 1.7 mg/kg of body weight. In Germany 10 % of children aged 5–8 consumed aluminum with food in a quantity being higher than 0.38 mg/kg of body weight per week. Potential consumption of aluminum contained in food products for infants by children aged 0–3, 4–6, 7–9, and 10–12 months amounts respectively to 0.1, 0.2, 0.43, and 0.78 mg/kg of body weight per week [115, 117]. Research performed in China allowed revealing that average aluminum consumption by children in Shenzhen amounted to 3.272 mg/kg of body weight per week which was even higher than PTWI = 2 mg/kg of body weight per week fixed for adults [118].

According to [119] aluminum contents in various products for children aged 0–12 months varied from 224 μg/l per day to 592 μg/l. Taking into account maximum recommended quantities in which these products can be consumed, aluminum consumption become unacceptably high for children older than 6 months.

There was a piece of research accomplished in Spain; it revealed that milk-based formulas for babies contained aluminum in quantities equal to 0.24–0.69 mg/l, and soya-based ones, 0.93 mg/l [120]. The obtained data gave grounds for additional assessment of aluminum consumption with such products and it amounted to 0.2–0.6 mg/kg of body weight per week for 3-mointh old children in case milk-based food products were used, and 0.75 mg/kg of body weight per week for soya-based products. Should these products be consumed in significant quantities, aluminum consumption grew to 0.3–0.9 mg/kg of body weight per week and 1.1 mg/kg of body weight accordingly [121]. These data on increased aluminum contents in food products for babies (milk products, biscuits, dried cereals, desserts, fish, fruit purees, mea, macaroni, crackers, and vegetables) were confirmed by numerous research works [113, 122, 123].

We should note that aluminum-containing food additives are not included into the list of those that are permitted to be applied in products for children in conformity with the CU TR 029/2012 “Requirements to safety of additives to food, flavoring agents, and technological auxiliaries”, Codex Standard 192-1995 “General Standard for Food Additives” approved by Codex Alimentarius, EU Regulation No. 1333/2008 regarding use of food additives. Besides, EU Regulation No, 1333/2008 strictly forbids using aluminum-containing food additives in ingredients for children nutrition. Nevertheless, as analytic research indicates, aluminum is detected in food products for children; it can occur there due to “transition” from food raw materials (for example, from powdered milk) as well as due to migration from package and packing materials.

Available data on aluminum toxicity and quantities in which the element was consumed with food products gave grounds for excluding potassium aluminosilicate E555 and bentonite E 558 from the list of food additives given in the Codex Standard 192-1995 “General Standard for Food Additives”. However, an issue related to aluminum consumption with food additives probably exceeding maximum permissible levels still remains open. Therefore, experts from the RF proposed discussing the necessity to reconsider use of aluminum-containing food additives that were permitted for use in food industry at the 46 session of the FAO-WHO Experts Committee (CCFA46) held in Hong Cong on March 17-21 2014. CCFA46 voted for the proposal [124].

The last version of The EU Regulation No. 1333/2008 that was edited in 2019 excluded several types of clays from the list of food additives permitted for use in food industry; excluded clays were sodium aluminosilicate E554, potassium aluminosilicate E555, calcium aluminosilicate E556, bentonite E558, and aluminosilicate (Kaolin) E559 [125, 126].

Experts assessed aluminum consumption with nutrition by RF population and revealed that aluminum was consumed by all age groups in quantities substantially exceeding maximum permissible weekly levels that were equal to 0–2.0 mg/kg of body weight; aluminum consumption was 2–8 times higher than maximum permissible levels at the minimum calculated consumption of aluminum-containing food additives, and 30–95 times higher at the maximum calculated one. Given that, it seemed well-grounded to exclude certain food additives from the list of those permitted for use in food industries; excluded food additives were sodium aluminosilicate (E554), potassium aluminosilicate (E555), calcium aluminosilicate (E556), bentonite (E558), and aluminosilicates (kaolin) (E559)[2]. However, according to a deci-

[2] The draft decision by the EAEU Council. On making alterations No 2 into the Customs Union Technical Regulation “Requirements to safety of food additives, flavoring agents, and technological auxiliaries” (CU TR 029/2012). *The Eurasian Economic Union*. Available at: https://docs.eaeunion.org/pd/ru-ru/0103370/pd_17122018 (14.02.2020).

sion made by the workgroup on making alterations into the Customs Union Technical Regulations "On Requirements to safety of additives to food, flavoring agents, and technological auxiliaries", only four out of five food additives were excluded from the list of those permitted for use in food industry, namely E555, E556, E558, and E559.

Conclusion. NCs are nanomaterials made of aluminosilicate layers (plates) or, in some cases, nanotubes and nano-disks. NCs structure, in particular nanometer-thick weakly bonded layers in them, their ion-exchanging and sorptive properties, as well as poor gas permeability provide opportunities for their wide use in industry, agriculture, and medicine. Production of composite package and packing materials usually involves using hydrophobic organically modified NCs.

People can be orally exposed to NC particles due to their migration from packing materials into food products and drinks; when NCs are applied in medicine as enterosorbents; when they are contained in food additives or occur in residual quantities of technological auxiliaries; due to spontaneous contamination of agricultural raw materials and products with clays. Numerous researches that involved using model *in vitro* systems revealed cytotoxic effects produced by NCs on various cells as well as their potential mutagenic effects on microorganisms. As a rule, organically modified NCs tended to have greater cytotoxicity than their non-modified analogues. Effects produced by NCs on cells became apparent through oxidant stress, depletion of reduced glutathione resources, breaks in cell ultrastructure (mitochondria swelling, changes in endoplasmic reticulum), proteome and metabolome shifts, cellular necrosis and lysis. NC toxicity can probably have a mechanism that involves generating reactive oxygen forms at interphase boundaries as well as membranetropic effects produced by cation-active SAS that migrated from organically modified clays. And here, as a rule, experts tend to neglect another potentially significant mechanism that involves migration of toxic aluminum ions from NCs. Effective NC concentration in different *in vitro* systems varies from 0.001 to 1 mg/ml; it is extremely doubtful that such NC concentration could occur in organs or tissues under systemic exposure (obviously, excluding cases when NC clays are accumulated in lung alveoli under chronic inhalation exposure).

Researches on NCs toxicity *in vivo* yielded some contradictory results. NCs didn't produce apparent acute toxic effects on rodents as it was indicated practically in each research work. Results obtained via sub-acute and subchronic experiments that lasted up to 196 and single clinical observations were not so unambiguous. As opposed to original NCs, their organic modifiers were highly toxic when introduced into animals in their free form; however, a possibility that they could migrate from organically modified clay *in vivo* or *in vitro* still remains disputable. Despite their low toxicity, NCs are apparently able to release such microelements as silicon and aluminum *in vivo* and these microelements are partially biologically available. Many NCs produce antimicrobial effects and it indicates that dysbiotic disorders can possibly result from their oral introduction.

Apart from toxic effects, NCs can also exert various non-toxic impacts on a body related to enterosorption. Such impacts include protection from adverse effects produced by aflatoxins and, probably, other chemical contaminants in food products. According to available data, orally introduced NCs don't exert any significant influence on provision with vitamins; however, in certain cases they are able to reduce provision with microelements (such as zinc).

There are still significant gaps in assessing NCs toxicity and related risks; they should be filled via experimenting. Thus, effects produced by NCs in systems *in vivo* are characterized within insufficient dose ranges and it doesn't allow assuming there are no effects that paradoxically occur not under exposure to high doses but to low ones (as it was repeatedly shown in experiments with carbon nanotubes). Not enough attention has been given to local effects produced by NCs in the gastrointestinal tract as per such parameters as morphological changes in intestine mucosa, micro-

biocenosis, and cytokine production by lymphoid tissue in the intestines. Influence exerted by NCs on microelement state of a body hasn't been studied at all regarding a wide range of parameters (excluding such "marker" elements as silicon and aluminum).

Contribution made by NCs contained in packing materials into overall population exposure to toxic aluminum should be thoroughly assessed due to already hazardous situation resulting from clay minerals being introduced into a body with food additives. Assessment of aluminum consumption with food by RF population revealed it was necessary exclude potassium aluminosilicate (E555), calcium aluminosilicate (E556), bentonite (E558), and aluminosilicates (kaolin) (E559) from the list of food additives permitted for use in food industry.

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STRATIFICATION OF PRETERM BIRTH RISK: PECULIARITIES

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According to the official statistics, in Perm region a number of preterm births (PB) has declined by 1.5% over the last 10 years; however, PB is still a leading cause for neonatal mortality. Despite a lot of already performed research and studies that are being performed at the moment, there is still no clear understanding what etiological factors cause PB. The paper contains a literature review of research that focused on PB risk factors. Certain factors are generally recognized and included into «Preterm birth» clinical report (2013), but literature data analysis allowed revealing additional PB risk factors. Cervical incompetence (CI) is a generally recognized PB risk factor. In 2018 clinical recommendations were published; they contained a list of CI risk factors and some of them coincided with PB risk factors (bad habits, extreme anthropometric parameters, peculiarities in obstetric and gynecologic case history, certain extra-genital diseases, multiple pregnancy, application of assisted reproductive technologies, complicated pregnancy); some others are considered to cause only CI (application of anti-tumor hormonal preparations, CI in family case history, abnormal development and disorders in the structure of female genital organs, surgeries on ovaries in case history). We performed comparative analysis of PB and CI risk factors and it helped us substantiate a necessity to create a unified list of PB risk factors as it would allow optimizing not only procedures applied to rank female patients but also accomplishing relevant PB prevention.

Key words: *risk factors, preterm birth, cervical incompetence, clinical report, клинические recommendations, procedures for female patients ranking, preterm birth prevention, literature review, comparative analysis.*

Research works that focus on preterm birth (PB) are performed quite continually and are still vital due to PB remaining the leading cause for neonatal deaths. We should note that over the last 10 years number of PB has reduced considerably. For example, according to data provided by obstetric hospitals, in Perm region preterm birth accounted for 7.5 % in 2006 but the share declined to 6 % by 2017. In 2017 in Perm preterm births cases accounted for only 4.7 % of the total number of births [1]. However, the most important thing is not a decrease in PB as it is but a change in delivery terms and a decrease in number of too early PB. This task is also being solved successfully despite new live birth criteria that were adopted in 2012; one of them is a pregnancy term being 22 weeks and more[1]. We analyzed official statistic data and revealed that there was a decrease in number of too early PB (22–27 weeks) in Perm region and they accounted for exceptionally low 0.5 % in 2017 against 1.6 % in 2006 [1]. All the achieved successes are undoubtedly due to new approaches implemented into everyday practice by both neona-

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tologists and obstetricians and gynecologists; these new approaches are regulated by federal and regional regulatory documents.

Despite a great number of research works accomplished in the past and being accomplished at the moment, etiological factor that causes PB hasn't still been assuredly identified; several factors are known to play their roles in pathogenesis. They are activation of a mother's and/or fetus' hypothalamo-pituitary-adrenal axis; thrombophilia; uterine muscle overstretching caused by fetal hydrops, multiple pregnancy, or uterine malformations; local or systemic inflammation; ischemia; rejection of a transplanted fetus; etc. [2, 3]. It is extremely difficult to comparatively analyze results obtained by different authors due to different design of their works (prospect and retrospect studies; "case – control" studies; groups under comparison being created as per various risk factors, various PB types, and fetus mass; differences related to criteria that are included into a research work etc.) and due to different statistical analysis techniques applied in them (descriptive statistics; comparison between groups as per either absolute or relative values; parametric and non-parametric analysis; comparison based on calculating odds ratio (OR), relative risk (RR), x^2 criterion; regression analysis; etc.). Despite all the difficulties related to comparative analysis, we took an effort to estimate to what extent various factors influencing PB have indeed been examined so far.

Age. Chances that a woman might have PB depend on her age. Thus, PB chances grow insignificantly among women aged younger than 30 (OR 1.2–1.59 [4, 5]; RR 1.0–1.7 [6]). PB probability grows after a woman reaches 35 (OR 1.64–2.7); the worst forecast is for women older than 40. Beta J. et al. note that women who are 45 years old run approximately 2 times higher PB risks that those who are just 20 [7].

But still, researchers tend to have different opinions on probable influence exerted by a woman's young age on PB risks. Some experts state that PB risk grows for women who are younger than 18 (by 2.2 %, $\chi^2 = 7.7$; $p < 0.01$ [8]); other believe that this increase in PB risks occurs when a woman is younger than 20 (OR 2.144 [4]; by 9 % [9]; RR 2.5 [6]). There are also data that there is no dependence between young age and PB frequency [10]. Our colleagues don't confirm data on isolated impacts exerted by a woman's age on PB probability in their works [11].

We should note that the clinic protocol which is valid at present considers a woman's age younger than 18 and older than 34 to be a risk factor that causes PB[2].

Several researchers consider a woman **being an Afro-American** a risk factor that leads to a 2-time increase in PB frequency [7, 12]. But other researchers disprove this fact. And it is disputable that this factor can have any significance for our country, so at present it is not included into the clinical protocol[2].

Social factors. There are several works where it is noted that **low socioeconomic status** is a risk factor that can cause PB; the same data can be found in the clinical protocol. However, there are no precise criteria for assigning pregnant women into this risk category[2]. Given that, it doesn't seem possible to apply this factor in everyday medical practice when a pregnant woman attends regular medical check-ups. The same goes for assessing impacts exerted by **stress**. Gravett M.G. et al noted that when a pregnancy is full-term, expression of placental adrenocorticotropic hormone is induced by fetal hypothalamo-pituitary-adrenal axis; and when a mother has a stress (either a physical or mental one), it is induced by stress mediators including hydrocortisone and adrenal and thus resulting in preterm expression of

[2] Preterm birth. Clinical recommendations (treatment protocol): The Letter by the RF Public Healthcare Ministry dated December 17, 2013 No. 15-4/10/2-9480 / approved by V.N. Serov, the President of the RF Obstetricians and Gynecologists Association, 2013, 20 p.

placental adrenocorticotropic hormone. It stimulates placental estrogen and prostaglandin synthesis and ultimately causes PB [13]. This risk factor is also included into the clinical protocol but an obstetrician-gynecologist can't determine whether his patient has a stress in her life without specific tests which at present aren't regulated by any documents[2]. In our opinion, should these risk factors be included, it could only make a ranking system more complicated and prevent from organizing efficient prophylaxis.

Next, we are going to dwell on certain social factors that were given some attention in literature but are not included into the protocol; still, their influence on PB risk is being examined at the moment. Thus, for example, there are data that **unemployed** women run higher PB risks (RR 4.0 [6]), 1.7 times higher risks [9]), and female students and clerks run similar risks (RR 1.0 and 1.3 accordingly. Casas M. et al analyzed 13 research works accomplished in Europe and revealed that PB risks was insignificantly lower for employed women (OR 0.86) but still depended on their occupation. Thus, for example, PB risk was on the contrary higher for women employed at food-making enterprises (OR 1.5) [14].

According to Yu.A. Semyonov et al., **education** is a significant factor as it influences a woman's behavior: how often and regularly she attends a doctor, how precisely she adheres to doctor's recommendations, and how persistent she is in pursuing healthy lifestyle which is necessary for pregnant women. Women with secondary education were met almost two times more frequently than women with higher education among those who had preterm birth. These results are also confirmed by a research work accomplished by N.Yu. Katkova et al.; they showed that absence of higher education resulted in greater PB risks (OR 4.64).

Yu.A. Semyonov et al. believe that **marital status and age of coitarche** also have their influence. **Single women** run 2 times higher PB risks; women who had **their first sexual intercourse** when they were younger than 16 run 3 times higher PB risks. However, it is the only research work available to us where we managed to find some data on influence exerted by age of coitarche [9]. Dyadichkina et al. didn't reveal any relation between marital status and PB probability [5].

Several authors state that PB depends on **quality of prenatal observation**; there is a 2.4 times growth in PB [15]; inadequate observation, OR 2.87–3.2; no observation at all, OR 3.0–5.19 [16].

Data on impacts exerted by physical loads are rather contradictory. Excessive **physical loads** during pregnancy as well as hypodynamia can influence PB frequency. The problem is that there are no common objective parameters of physical activity that can be applied when studying this risk factor for pregnant women [17, 18].

Bad habits. According to the clinical protocol, if a mother has bad habits (alcohol, nicotine, or drug dependence), it is a risk factor that can cause PB[2].

Researchers adhere to practically the same opinion regarding **smoking** as PB risk factor (OR 2.33–5.57 [3, 5, 11]; risk is 5 times higher [9]). Beta J. et al. noted that smoking resulted in a higher risk of PB up to 34 weeks (OR 1.81) [7].

Some authors believe that **alcohol intake** is also a risk factor that causes PB but there are no more precise results due to a small number of observations and absence of common objective criteria (how much alcohol is consumed, how often it is taken etc.) [5, 9, 19]. In everyday medical practices doctors can rely only on "yes / no" ranking, that is, whether a women drinks alcohol during pregnancy or not.

Anthropometric parameters. Despite a lot of talks on obesity being a real epidemic nowadays, anthropometric parameters are not included into a list of risk factors that can cause PB[2].

A lot of researchers revealed that a mother having low height influenced PB risks [6, 7, 20], but N.Yu. Katkova et al. didn't reveal any dependence between a woman's height and PB frequency [3].

Body mass deficiency leads to a growth in PB risk depending on how apparent it is; thus, BMI 17–18.5 kg/m^2 means RR equal to 0.8–1.22; BMI 16–17 kg/m^2, RR 0.7–1.41; BMI lower than 16 kg/m^2, RR 1.61–1.9 [6, 21].

Overweight and obesity results in 2.4 times higher PB risks [9], OR amounts up to 2.08 [5, 6, 22, 23].

Obstetrician and gynecological case history. A lot of authors think that **preterm birth in case history** plays the most significant role [3, 5, 6, 9, 12, 16, 24], and should there be two or more PB, it causes even greater risks. This factor can be found in the clinical protocol and is the most important one for determining how to treat a patient; it regulates preventive prescription of micronized progesterone and it fully corresponds to instructions attached to medications[3]. In this relation there have been large-scale studies over the last 5 years; they focused on examining efficiency of micronized progesterone medications prescribed to prevent PB and miscarriages [25, 26].

The clinical protocol also has data on such risk factors as one or more miscarriages at later terms and two or more medical abortions[3]. Let us consider these factors in greater detail. Procedures for medical abortion have changes considerably over the last 10 years and nowadays we have two clinical protocols that regulate medical abortion at early and later terms[3, 4]. Given that, we should revise our attitude towards abortion being a PB risk factor and clarify that it depends on a procedure applied to make an abortion.

When analyzing literature data, we noted that an issue related to dependence between **abortions in previous pregnancy and PB** remained disputable. Some authors believe that even one abortion or endometrectomy results in higher PB probability, especially if it is the previous pregnancy that was aborted [6, 15, 24]. The worst forecast is given in case of abortion in the previous pregnancy which was also the first one (RR 3.0 [6]) and two or more intrauterine interventions (OR 5.3 [3]). However, other authors note that it is only spontaneous miscarriage that matters (OR 2.84 [5]), and that induced abortions, ectopic and anembryonic pregnancies in case history don't result in greater PB risks [5, 9, 16].

J. beta et al. give the most detail description of influences exerted by obstetrician case history in PB risks, but they focus only on pregnancies that ended up on 16–30th week and 31–36th week, one or two abortions, and absence or occurrence of birth in due time (OR from 2.33 to 18.73 depending on a combination of events) [7].

Cone biopsy or uterine neck amputation that are mentioned in the clinical protocol are also considered PB risk factors[2].

All the factors that we are going to describe below are not included into the clinical protocol; still, we think it is advisable to discuss them as influence exerted by them is being studied at the moment. PB probability is related to a cesarean operation in previous births (OR up to 2.2); it grows with each subsequent operation and with stillbirth and / or early neonatal death in case history [6, 9, 11, 12].

A small break between pregnancies also results in insignificant PB risk growth; should it be less shorter than 6 months, OR amounts to 1.71 [27]); 6–11 months, OR 1.2 [27]); shorter than 18 months, OR 1.37 [11]); and a break more than 60 months results in OR varying from 1.1 to 1.5 depending on PB term [12]. **A great number of births** (more than 4) is also included into the clinical protocol as PB risk factor.

Polycystic ovary syndrome, infertility, chronic inflammation in small pelvis organs,

[3] Medical abortion. Clinical recommendations (treatment protocol): The Letter by the RF Public Healthcare Ministry dated October 15, 2015 No. 15-4/10/2-6120 / approved by V.N. Serov, the President of the RF Obstetricians and Gynecologists Association, 2015, 35 p.

[4] Induced abortion at late terms due to anomalies in fetus development. Clinical recommendations (treatment protocol): The Letter by the RF Public Healthcare Ministry dated December 04, 2018 No. 15-4/10/2-7839 / approved by V.N. Serov, the President of the RF Obstetricians and Gynecologists Association, 2018, 43 p.

and uterine myoma are also considered adverse factors; however, they require further research and their influences have not been given sufficient proof [3, 5, 6, 9, 24].

Somatic pathology that is included into the clinical protocol is pancreatic diabetes in its grave form and grave extragenital pathology. However, there are some research works where it is stressed that greater PB probability is caused not only by pancreatic diabetes in case history before pregnancy but especially when it is combined with smoking (RR = 5.99) [28].

Arterial hypertension before pregnancy leads to higher PB risks depending on PB type and term and should be taken into account as it is a proven risk factor that causes preeclampsia [5, 12].

There are no unambiguous data on impacts exerted by anemia. Some authors state that it increases PB risks (RR 3.0 [6]), OR 1.2–1.8 depending on PB type [12]). At the same time, Ahumada et al. didn't confirm that such dependence existed [16].

It was revealed that PB was related to autoimmune diseases, such as rheumatoid arthritis (OR 2.1), Crohn's disease (OR 1.87), psoriasis (OR 1.88) [29], and systemic lupus erythematosus (OR 2.57–8.66 depending on how active a disease was) [30].

Few research works focus on impacts exerted by diffuse endemic goiter (OR 2.29) [5], gastrointestinal tract diseases (1.6 times higher risk [9]), mitral valve prolapse (OR 2.35 [5]). It should be noted that N. Yu. Katkova et al. didn't reveal significant influence exerted by somatic pathology on PB risk [3].

Current pregnancy and its course. If a pregnancy is due to assisted reproductive technologies (ART), than there are great risks of complications including PB; a significant role here belongs to gynecological pathology that resulted in infertility [7]. For example, according to S.V. Rishuk et al., each 4–5th pregnancy that is due to in vitro fertilization ends up in preterm birth [31].

Multiple pregnancy also ends up in preterm birth more frequently (OR 2.4) [16, 32]. The worst forecast is for multiple pregnancies that are due to ART as PB probability for them varies from 80 to 100 % [33, 34].

Both these factors (ART and multiple pregnancy) are included into the clinical protocoal[2].

Placental pathology. According to several authors, **placenta incompetence** authentically frequently occurs in women with preterm births in case history and it can be due to a common pathogenetic mechanism (RR 2.6 [24]; OR 14.5 [3]). At the same time Dyadichkina et al. didn't confirm such data [5]. We should note that placenta incompetence in itself as well as ways how to prevent and treat this pregnancy-related complication are to be discussed more profoundly. However, such a complication as **fetal hydrops** which is caused by functional disorders in fetus-placental complex is a commonly recognized PB risk factor[2]. According to the clinical protocol, only pathological placenta location and premature detachment of a normally located placenta are PB risk factors[2].

Patients that suffer from **preeclampsia** run well-proven higher PB risk (OR 1.9 [16]; 4.43 [35]; 6.9–89.7 depending on PB type [12]). But the clinical protocol that is valid at the moment doesn't list preeclampsia among risk factors that can cause PB in a current pregnancy; probably, this disease should be assigned into a category that includes grave extragenital pathologies[2].

Infectious diseases during pregnancy induce a whole cascade of systemic inflammatory reactions and it is a pathologic section in PB mechanism [3]. There are data on impacts exerted by acute viral infections [5, 24], urinary infections including inapparent bacteriuria [36, 37]; cervical-vaginal infections [5, 9, 24].

Parodentium diseases, together with all the above mentioned infections, are also considered to be risk factors as pathogenic flora from parodentium can occur in placenta tissues [38]. N.N. Trigolos et al. confirmed that oral cavity infections occurred 5 times more frequently among women with PB [39], and Gesase N. noted that PB occur more frequently

among women with parodentium infections, OR 2.32 [40].

The clinical protocol stresses that any **uterine bleedings** during a current pregnancy are a risk factor that can cause PB[2], and it is indisputable. On the contrary, a **threatened miscarriage** during the first half of a pregnancy can still be discussed. Several domestic researchers proved that a threatened miscarriage resulted in lower chances that a pregnancy would end up in due time (OR 2.45 [5]; x^2 = 5.41 [41]; RR 2.4 [24]), and a threatened miscarriage in the second trimester caused higher PB risks than the same threat in the first one (RR 3.8 [24]; by 62.7 % [41]). However, precise criteria for diagnosing "a threatened miscarriage" were determined in 2016 only in the clinical recommendations entitled "Miscarriage at early stages of a pregnancy: diagnostics and treatment procedures"[5]. A threatened miscarriage involves scanty bloody discharge from genital tracts. It allows stating that it is uterine bleedings during a current pregnancy that should be investigated in research works as it will make for data obtained by different authors being quite comparable.

A group of Chinese researchers performed meta-analysis of research works that focused on **vitamin D deficiency**; they revealed that when vitamin D concentrations in blood dropped lower than 20 ng/ml, PB risks grew (OR 1.29) [42]. Flood-Nichols S.K. et al. noted that there was no statistically significant discrepancy between various groups as per PB frequency when vitamin D concentrations in blood were lower than 30 ng/ml [43]. It was also confirmed by L. Yang et al. as they didn't reveal any dependence between BP and vitamin D deficiency [44]. Nowadays medical experts are trying to develop a project of clinical recommendations on how to prevent and treat vitamin D deficiency before pregnancy, during it, and after childbirth as well.

Any relation between **zinc deficiency** and PB probability is also to be discussed as there is no unambiguous evidence on the matter [45].

Surgeries and injuries are also factors that can cause PB and it is indisputable[2].

We should give special attention to **preterm cervical maturation or cervical incompetence** as it is a commonly recognized PB risk factor (OR 2.45 [5]; RR 39.8 for uterine neck length being 25 mm and shorter in the 2nd trimester [46]). In 2018 clinical recommendations on cervical incompetence were published; according to them, there are predisposing factors that can cause this pathology[6].

Domestic researchers mention other risk factors that cause cervical incompetence in their works. N.A. Linchenko et al. stated that a risk group as per cervical incompetence probability included women with overweight, obesity, 3 or more pregnancies, miscarriages at later terms, with two or more intrauterine interventions in case history, with chronic inflammations in small pelvis organs, surgeries on ovaries, and infertility in case history [47]. M.M. Padrul et al. determined only one risk factor that resulted in discrepancies between groups with cervical incompetence and without it; it was spontaneous late miscarriage or PB in case history (x^2 = 5.04) [48]. Yu.D. Kaplan et al. revealed several risk factors that could cause late spontaneous miscarriages and spontaneous PB among women with treated cervical incompetence. They were combined late spontaneous miscarriages and spontaneous PB in case history and a threatened miscarriage during a current pregnancy, a higher contribution being made by this threat during the 2nd trimester [49]. V.I. Chernyaeva et al. highlighted the fol-

[5] Miscarriage at early stages of a pregnancy: diagnostics and treatment procedures. Clinical recommendations (treatment protocol): The Letter by the RF Public Healthcare Ministry dated June 07, 2016 No. 15-4/10/2-34820 / approved by V.N. Serov, the President of the RF Obstetricians and Gynecologists Association, 2016, 33 p.

[6] On clinical recommendations (treatment protocol) "Cervical incompetence": The Letter by the RF Public Healthcare Ministry dated December 28, 2018 No. 15-4/10/2-7991. *KonsultantPlus*. Available at: http://www.consultant.ru/document/cons_doc_LAW_320915/ (22.09.2019).

lowing risk factors in case history that could cause cervical incompetence: late abortions and PB; two or more intrauterine interventions; surgeries on uterine neck. They also spotted out risk factors that were related to a current pregnancy; they were a threatened miscarriage; acute respiratory infections; acute pyelonephritis; acute vulvovaginitis; preeclampsia [50]. Taking into account available literature data, it seems advisable to compare existing research works on risk factors that cause PB and cervical incompetence (Table). As we can see from the Table, risk factors causing PB and cervical incompetence coincide partly; some of them, however, cause only cervical incompetence. The situation doesn't seem adequate to us and allows us to include risk factors that cause cervical incompetence into the list of factors that cause PB.

Risk factors that cause preterm birth and cervical incompetence (CI)

Risk factor	Preterm birth		CI
	+	–	+
Senior age Young age	Beta[7], Wang[4], Dyadichkina[5], Azbukina[6], **Report** Laryusheva[8], Wang[4], Semyonov[9], Azbukina[6], **Report**	Wong[11], Katkova[3] Sujan[10]	
Low social and economic status	Katkova[3], **Report**		
Stress	Gravett[13], **Report**		
Unemployed women	Azbukina[6], Semyonov[9], Casas[14]		
Without higher education	Katkova[3], Semyonov[9]		
Single	Semyonov[9]	Dyadichkina[5]	
Age of coitarche	Semyonov[9]		
Prenatal observation quality	Leneuve-Dorilas[15], Ahumada-Barrios[16]		
Physical loads	Portela[17]	Silva[18]	
Smoking	Wong[11], Dyadichkina[5], Katkova[3], Semyonov[9], Beta[7], **Report**		
Alcohol intake	Green[19], Semyonov[9], Dyadichkina[5], **Report**		
Intake of anti-tumor hormonal preparations			**Report**
African American	Jelliffe-Pawlowski[12], Beta[7]	Wong[11]	
CI in the closest female relatives			**Report**
Low body height	Azbukina[6], Beta[7], Morisaki[20]	Katkova[3]	
Body mass deficiency	Azbukina[6], Girsen[21]		
Overweight and obesity	Semyonov[9], Dyadichkina[5], Azbukina[6], Katkova[3], Ju[22], Dudenhausen[23]		Linchenko[47], **Report**
3 or more pregnancies	Nabeeva[24]		Linchenko[47]
More than 4 births	**Report**		
Preterm birth in case history	Ahumada-Barrios[16], Jelliffe-Pawlowski[12], Dyadichkina[5], Katkova[3], Nabeeva[24], Azbukina[6], Semyonov[9], **Report**		Padrul'[48], Черняева[50], **Report**
Abortion at early stages	Leneuve-Dorilas[15], Azbukina[6], Nabeeva[24]	Semyonov[9], Dyadichkina[5]	
Spontaneous miscarriage at early stages	Dyadichkina[5]		

Risk factor	Preterm birth		CI
	+	–	+
Spontaneous miscarriage at later stages	Beta[7], **Report**		Linchenko[47], Padrul'[48], Chernyaeva[50], **Report**
Spontaneous miscarriages at later stages + preterm birth in case history			Kaplan[49]
2 or more intrauterine interventions	Katkova[3], **Report**		Linchenko[47], Chernyaeva[48], **Report**
Cesarean section in previous births	Wong[11], Jelliffe-Pawlowski[12]		
Perinatal losses	Azbukina[6], Semyonov[9]		
A short break between pregnancies Long breaks between pregnancies	Shachar[27], Wong[11] Jelliffe-Pawlowski[12]		
Hormonal disorders	Azbukina[6]		
Polycystic ovary syndrome	Dyadichkina[5]		**Report**
Hyperandrogenism			**Report**
Lack of progesterone			**Report**
Genital infantilism			**Report**
Uterine malformations			**Report**
Infertility	Dyadichkina[5]		Linchenko[47]
Inflammatory diseases in small pelvis organs	Nabeeva[24], Semyonov[9]	Dyadichkina[5], Katkova[3]	Linchenko[47], **Report**
Surgeries on ovaries			Linchenko[47]
Uterine myoma	Nabeeva[24]		
Surgeries on uterine neck	**Report**		Chernyaeva[50], **Report**
Congenital shortening of uterine neck			**Report**
Uncured cervical raptures			**Report**
Somatic pathology	**Report**	Katkova[3]	
Arterial hypertension before pregnancy	Dyadichkina[5], Jelliffe-Pawlowski[12], Semyonov[9]		
Pancreatic diabetes before pregnancy	Jelliffe-Pawlowski[12], Borsari[28]		
Thyroid gland diseases	Dyadichkina[5]		
2 and 3 degree anemia	Azbukina[6], Jelliffe-Pawlowski[12]	Ahumada-Barrios[16]	**Report**
Autoimmune diseases and collagenosis	Bandoli[29], Skorpen[30]		**Report**
Mitral valve prolapse	Dyadichkina[5]		
Digestive tract diseases	Semyonov[9]		
Assisted reproductive technologies	Beta[7], R[31], **Report**		
Multiple pregnancy	Ahumada-Barrios[16], Arkhipov32], **Report**		**Report**
Assisted reproductive technologies + multiple pregnancy	Egorova[33], Perepelitsa[34]		
Pathological placenta location	Nabeeva[24], **Report**		
Placenta incompetence	Nabeeva[24], Katkova[3]	Dyadichkina[5]	
Hydramnios	**Report**		**Report**

Risk factor	Preterm birth		CI
	+	–	+
Preeclampsia	Ahumada-Barrios[16], Davies[35], Jelliffe-Pawlowski[12]		Chernyaeva[50]
Threat of miscarriage in the 1st trimester	Dyadichkina[5], Cherepakhin[41], Nabeeva[24], **Report**		Kaplan[49], Chernyaeva[50]
Threat of miscarriage in the 2nd trimester	Cherepakhin[41], Nabeeva[24], **Report**		Kaplan[49], Chernyaeva[50]
Infectious diseases	Katkova[3], Nabeeva[24], Dyadichkina[5], Smail[36], Lai[37], Semyonov[9], **Report**		Chernyaeva[50], **Report**
Parodentium diseases	Akhil'gova[38], Trigolos[39], Gesase[40], **Report**		
Vitamin D deficiency	Qin[42]	Flood-Nichols[43], Yang[44]	
Zinc deficiency	Wilson[45]		
Cervical incompetence	Dyadichkina[5], Nabeeva[24], Wulff[46], **Report**		

Therefore, in spite of multiple research works that focus on PB etiology, the issue still remains extremely vital. Should a complete list of PB risk factor be created, it will allow optimizing not only a system for ranking patients as per their risk groups, but also performing adequate PB prevention. Prevention activities are first of all aimed at reducing a number of extremely early pre-term births and at improving neonatal outcomes. This goal can be achieved due to organizing a qualitative system for predicting obstetrician complications including pre-term birth.

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IRRATIONAL LIGHTING AS A HEALTH RISK OCCURRING IN THE ARCTIC

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We assessed health risks for operators who had to live in mobile houses in the Arctic regions. Inadequate lighting is a most significant factor related to housing conditions that can cause various pathologies resulting in decreasing working capacity. We revised data on impacts exerted by luminous and LED lighting on operators and it allowed us to determine reasons for "aftereffects" produced by LED lighting regarding an increase in latency in No. 95 pattern electroretinogram (PERG); this latency characterizes a situation with ganglionic cells in the visual analyzer. We put forward a hypothesis that lower "inhibition" efficiency was caused by absorption of blue light within 380–450 nanometers range, and an increase in PERG P50 amplitude was caused by an additional increase in Na^+, Ca^+ ions flows when ChR2 protein absorbed excessive 470 nm blue light against a blue light dose in a luminous lamp spectrum.

We showed that there were practically no changes in operators' health after they had been exposed to dynamic LED lighting; however, all the participants in the experiment had a W-like splitting in P100 peak in visually induced cortical potentials as a response to stimuli with different angle sizes. When ganglionic cells are exposed to blue lighting, interaction between their degrading mitochondria and astrocytes becomes very important. LED lighting results in damage to mitochondria in ganglionic cells. Mitochondria are moved to the optic nerve head to be utilized where they are absorbed by astrocytes and eliminated with their lysosome. Should a speed of degrading mitochondria inflow exceed a speed at which they are utilized, it will cause mechanic strains in fibers of the optic nerve head due to "mitochondria jam"; this, in its turn, can lead to long-term disorders in the optic nerve head and glaucoma occurrence.

We formulated recommendations for the State Standard 23274-84 "Mobile houses. Electrical appliances. Overall technical conditions" and advised applying semi-conductor white light sources in them as they had a biologically adequate irradiation spectrum.

Key words: *mobile houses, LED lighting, blue light, optic nerve, mitochondrion, astrocyte, glaucoma, biologically adequate irradiation spectrum.*

The Arctic zone is naturally essential for the Russian Federation; development of the region is truly vital bearing in mind not only future prospects but also outstanding economic tasks the country is to solve at present. The Arctic zone is an essential border outpost where our northern borders are protected. Today, there is a base built by the RF Defense Ministry on Zemlya Aleksandry, an island belonging to the Zemlya Frantsa-Iosifa archipelago. The base is called «The Arctic trefoil». Overall, the Defense Ministry is planning to build 6 military settlements, 13 airfields, a land aviation range, and 10 technical facilities for radar stations and targeting points in the Arctic zone. These bases are planned to provide placement for several thousand military personnel; these people will have to spend most part of a year in the region during the polar night and live with artificial light only with its spectrum not being similar to that of sunlight.

Poor lighting quality is known to be able to cause a shift in a circadian phase, especially when it is combined with sleep disorders at night. Poor lighting during a day can also cause somnolence. A human body requires both periods with bright lighting and periods of darkness during a day in order to maintain an optimal daily rhythm. Should this rhythm be disharmonized, it can result in both physiological changes and health disorders and, consequently, lower working capacity and intellectual abilities.

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Some say, that Diogenes, an ancient Greek philosopher, was able to develop his talents due to living in a tub. Dozen thousand people working in the Arctic zone have been living in comfortable and warm metallic «tubs», an all-metal cylinder-like unified block; sometimes they felt themselves being «contemporary Diogeneses…» [1].

Life-giving light makes a tub a true home for a person and exerts favorable influence on his or her health and creative abilities.

An idea to live in well-lit cylinder-like houses turned out to be quite feasible and practical. It found its implementation in the arctic zones, space, and sea depths. A cylinder-like shape provides high security during transportation in areas without good roads, minimal quantities of metal required to make it, minimal labor and other costs as well.

TSUB-2M housing module has become widely spread as it is equipped with all necessary communal utilities and can provide accommodation for 4 people who work on shifts in the arctic regions and other remote areas. TSUB-2M houses are also used in the army where they serve as accommodation for military personnel in unpopulated areas. All-metal complete unified blocks for military personnel have an advantage related to their readiness to provide accommodation immediately as they are equipped with all necessary appliances, such as folding sleeping berths, tables, and sanitary units.

All the above mentioned peculiarities allowed providing maximum possible comfort for people living in TSUB-2M blocks in northern regions with filament lamps being their only light source.

Figure 1 shows a layout of a modern mobile building that can provide accommodation for 8 people; there are two basic models, «CABA A8» 8x2.4 or «CABA A8» 8x2.8 [2].

To light TSUB blocks, different lighting sources were applied, starting from filament lamps and on to luminous and energy-saving lamps; they were then replaced by LED lighting. In 2018 a state standard GOST 23274-84[1] was approved; its authors made provisions for used of LED lighting. Item 3.4.4 in the said document fixes that «to provide electrical lighting inside a building, one, as a rule, should use lighting appliances with luminous and LED lighting sources. It is also permissible to use filament lamps in case it is impossible to use the above mentioned lighting sources». But it raises an issue whether it is truly advisable and permissible to apply too bright LED lighting sources in rooms with low ceilings as per GOST 22853-86[2]; according to the said document, dimensions of container housing should correspond to values given in Table 1.

Figure 1. A possible TCUB layout for 8 people equipped with two-tier sleeping berths and energy-saving lamps

It is very important to estimate whether luminous and LED tubes are photobiologically safe in case of mobile houses with low ceilings (2,200 mm height); they should correspond to IEC 62471:2013[3] standard. When a room height is equal to 2,200 mm, lamps are too close to human eyes. And when photo-safety is estimated, it is very important to assess dependence between basic luminance of a lighting source and a distance between it and human eyes. Such dependences were obtained for a 200 mm distance and further on to 1,000 mm distance in works by F. Leccese et al. [3]; the results are given on Figures 2 and 3.

[1] GOST 23274-84 Mobile buildings (inventory buildings). Electrical units. Overall technical conditions. *GOSTs database*. Available at: https://allgosts.ru/91/140/gost_23274-84 (12.04.2019) (in Russian).

[2] GOST 22853-86. Mobile buildings (inventory buildings). Overall technical conditions. *KODEKS: an electronic fund for legal and reference documentation.* Available at: http://docs.cntd.ru/document/gost-22853-86 (12.04.2019) (in Russian).

[3] GOST IEC 62471-2013. Photobiological safety of lamps and lamp systems. *Normaks. The system of standards*. Available at: http://www.normacs.ru/Doclist/doc/12080.html (12.04.2019) (in Russian).

Table 1

A building	Width	Length				Height*
		3,000	6,000	9,000	12,000	
Towed** with non-detachable running gear	2,500	+	+	+	–	Not less than 2,200
Transported*** and towed with detachable running gear	3,000	–	+	+	+	The same 2,400
	3,000	+	+	+	+	

Note:

* means height of a room inside a block-container is a distance taken directly from the floor to the ceiling. Should a ceiling be not rectilinear, height is taken as per an average value between the maximum and the minimum one;

** towed buildings are those equipped with their own non-detachable or detachable running gear;

*** transported building are those that are not equipped with a running gear (block-containers).

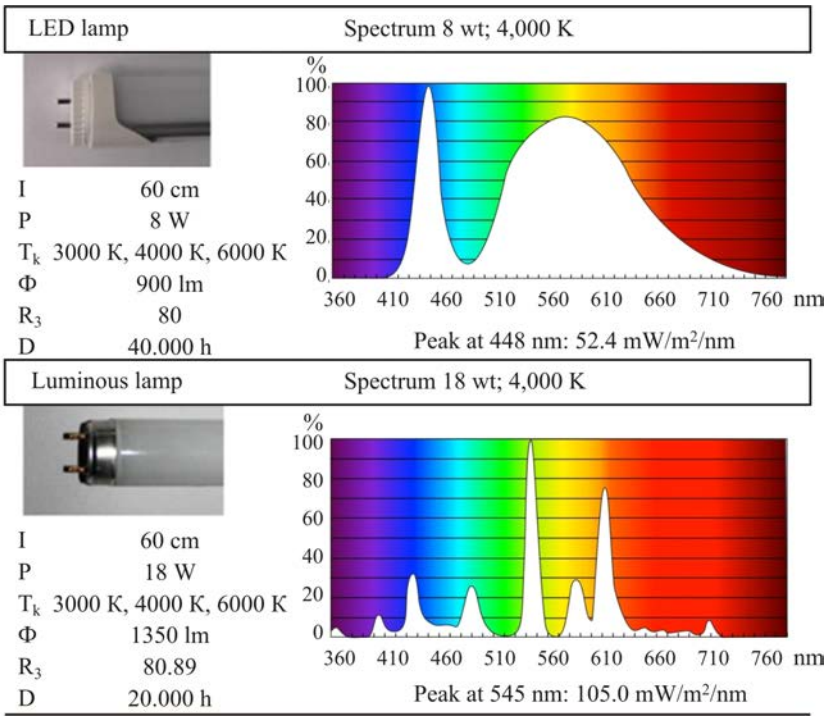

Figure 2. Overall features of a Led Tube lamp and a luminous lamp

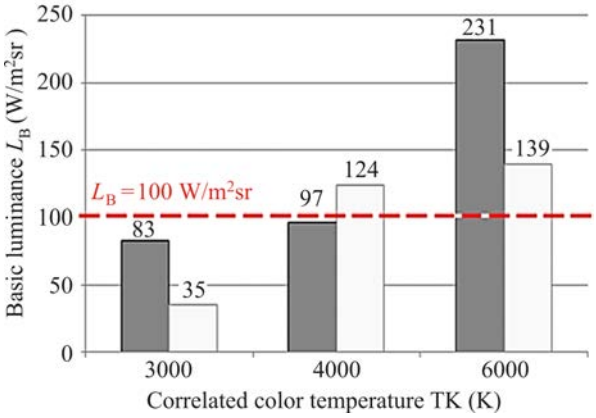

Figure 3. Basic luminance L_B within blue light range: a comparison between LED (dark grey) and luminous lamps (light grey) with different color temperatures at a distance equal to 0.20 meters

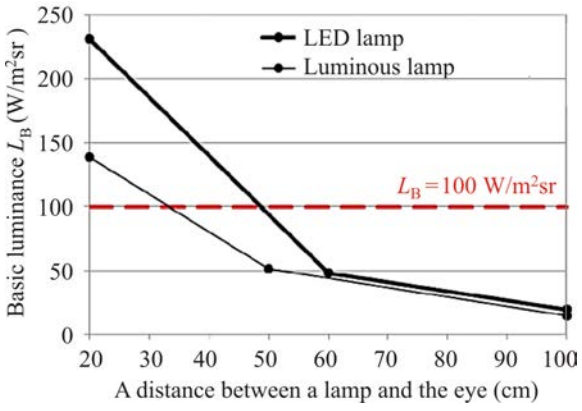

Figure 4. Dependence between basic luminance L_B of LED tubes and luminous lamps with color temperature 6,000K and a distance to the eye [4]

Figure 4 shows dependence between weighted luminance $\mathbf{L_B}$ of blue light and a distance between a lighting source and the eye.

When a person's height is 180–190 cm and a ceiling is 210–220 cm (2,100–2,200 mm), a minimal permissible distance between the eyes and a LED lamp that is photobiologically safe should be not less than 50 cm. Such TSUB dimensions are comparable to dwelling units at space stations and cabins aboard a ship. L.V. Bazyleva et al. [5] and V.N. Bolekhan et al. [6] state that working out optimal luminance regimes and giving scientific grounds for a possibility of their long-term use in closed objects is a vital practical task not only for the space brunch but also for other spheres of human activities.

Nowadays it is well known that irrational lighting is a most significant factor related to dwelling as it can lead to development of various pathologies accompanied with reduced working capacities[4].

A blue component (440–460 nm) has the greatest photochemical activity regarding the retinal pigment epithelium. This component prevails in irradiation spectra of modern white LEDs; therefore, many researchers consider LED lamps being potentially hazardous [7]. Overwhelming majority of literature data indicating that LED lighting is photochemically dangerous have been obtained via experiments on animals.

Thus, experiments performed on Japanese quails (Coturnix japonica) revealed that exposure to light from blue LEDs resulted in changes in the choroid as well as photo-induced changes in sub-cellular structures of the retinal pigment epithelium. Those experiments also showed that moderate blue LED lighting (440–460 nm) led to 1.5 times greater loads on the retina metabolism in young animals (against filament lamps); those loads resulted in accelerated ageing of the retina and reduced functional activity of the blood-retinal barrier structures [8].

Results of those experiments confirm a hypothesis that a blue part in the spectrum has more apparent ability to do photochemical damage to the retina than yellow-green and red ones.

However, some foreign and Russian experts [8] believe that results obtained via experiments on animals can't be directly extrapolated on people due to apparent morphofucntional differences of their visual analyzers as well as due to laboratory conditions not corresponding to the natural lighting environment people live in.

There have been few experiments involving human participants and they mostly focused on examining melatonin levels during nighttime. The administration of the RF Defense Ministry Central Research Institute were the first to raise an issue related to obligatory certification of LED lighting sources as per medical standards. But it turned out to be impossible to fulfill such a task in 2007 at the expense of the RF Defense Ministry. In 2008 the US Department of Defense set a task to perform a research work entitled SB082-055 «A Spectrally Dynamic Berth Light for Active Circadian Cycle Management» [9]. The costs on this research amounted to $98,990.00. The research clearly indicated that working capacities of military personnel serving on a US Navy ship changed considerably under short-term exposure to blue part in the light spectrum [10, 11].

LED lighting sources used on US Navy ships were developed by Energy Focus Company that signed a governmental contract worth $ 1,600,000,000.00. Roger Buelow, the leading researcher at the company, noted that «The repeated DARPA research revealed how a daily rhythm developed under influence exerted by improved lighting provided for the Navy. Those lighting sources regulated their spectrum during a day in order to improve sleep and performance and it is especially important regarding military personnel who are to be ready to operate round the clock» [12].

A thesis for the Candidate of Medical Sciences by A.E. Smoleevskii[4] is one of the latest works focusing on impacts exerted by

[4] A.E Smoleevskii. Mental performance of an operator working under LED lighting with different spectral and energetic properties: a thesis for Candidate of Medical Sciences degree. Moscow, GNTS RF-IBMP Publ., 2018, 133 p. (in Russian).

LEDs on mental performance of an operator that turned out to be quite interesting for us.

The research was performed in the RF State Scientific Center – RAS Institute for Medical and Biological Issues in 2014–2015; it was a part of a long-term complex experimental research program entitled «Tests of LEDs for pressurized chambers that are to be used in space crafts and assessment of psychophysiological effects produced by such use». The research was accomplished on samplings made up of healthy male volunteers; there were two stages in it involving permanent and dynamic lighting during 12 days.

Background parameters of mental performance and psycho-emotional state were estimated under luminous lighting with its colority being equal to 4,000K («neutral white» light). Lamps were located in a chamber with a limited volume in such a way that they could create lighting levels corresponding to the existing standards[5] and were comparable to lighting levels equal to 200 luxes created by experimental LED lamps. After-effects: produced by LED light were also assessed under luminous lighting.

The first stage involved assessing psychophysiological effects produced by permanent LED lighting created by board lighting sources CCD301, CCD305, and CCD307. CCD301 lighting sources were installed in toilets and passages; CCD305 and CCD307 lighting sources, in dwelling and medical units. Correlated color temperature (CCT) of the lighting system amounted to 4,000K, and luminance was equal to 200 luxes. Hence operational spectral and energetic properties of CCD311 lighting sources changed within a wide range, mental performance of operators was assessed at two CCT values, 4,000–5,000K and 8,000±800K[4].

Unfortunately, the thesis doesn't contain any data on spectral and energetic properties of CCD301, CCD305, CCD307, and CCD311 LED lamps; there are only data on LEDs applied in lighting sources manufactured by CREE and Seoul Semiconductor companies. To hygienically assess spectral properties of applied lighting sources, we have to rely on spectra of LEDs produced by CREE (Figure 5).

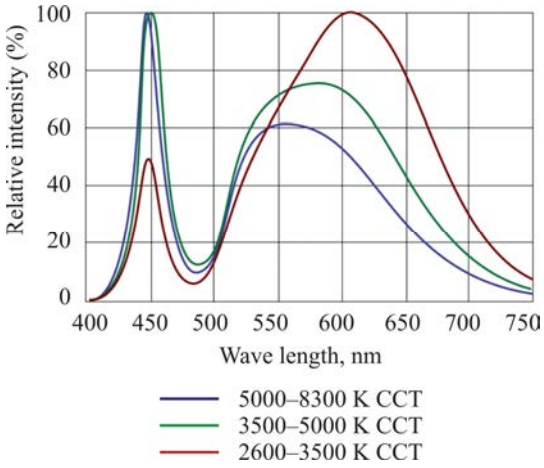

Figure 5. Spectra of LEDs produced by CREE

We can assume that spectra of applied LED lamps had little difference from the LED lamps produced by the said companies provided that «NII Mikropriborov» Scientific Center LLC that produced those LED lamps didn't apply any specific procedures aimed at adjusting LED spectra.

A spectrum of a LED lamp produced by CREE Company and shown in Figure 5 allows assuming that:

– this spectrum has an increased dose of blue light against hygienically safe sunlight under the same luminance;

– the maximum in the spectrum is at 450 nm blue light and it exerts negative impacts on the retina (on synapses in the dendritic field of ganglionic cells in the retina optic channel);

– blue light that produces its effects on ganglionic cells in the eye is equal to 60% of the maximum blue light value that is 450 nm; so, effects produced on the hormonal system (melatonin and hydrocortisone concentrations) and comfortable sleep are not so apparent;

– bearing the eye physiology in mind, we should remember that it is ganglionic cells and their dendritic field that are the first to perceive blue light. Excessive blue light dose ex-

[5] GOST R 50804-95. The dwelling environment of an astronaut in a manned space craft. Overall medical and technical requirementsю *KODEKS: an electronic fund for legal and reference documentation*. Available at: http://docs.cntd.ru/document/gost-r-50804-95 (12.04.2019) (in Russian).

erts its impacts on performance of ganglionic cells that perceive signals from other photosensitive retina cells reacting to light stimuli;

– there is a clear dip at 480 nm in the spectrum. Photon flow of 480 nm light-blue light is responsible for managing the pupil diameter and holding it closed. A dilated pupil exposed to LED light can't limit light flow with excessive blue light dose.

Authors of the research work [13] revealed dependence between the pupil diameter and luminance for LEDs with their spectrum given in Figure 5 (Figure 6).

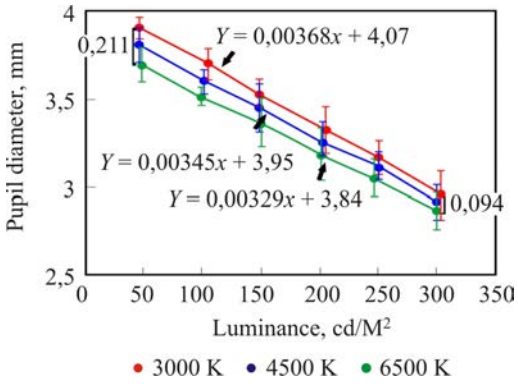

Figure 6. Dependence between the pupil diameter and luminance [13]

When overall illumination is equal to 200 luxes, luminance from a white sheet of paper is equal to less than 100 cd/m^2. It means that the pupil diameter will be greater than 3.5 mm. When the eye is exposed to such illumination and irradiation spectra, we can assume there is an elevated risk of «after-effects» in accommodative apparatus functioning, stochastic effects in retinal cells functioning, and changes in mental state of a person caused by excessive doses of blue light (from 4,500K to 8,000±800K).

Table 2 contains aggregated data on mental state of operators exposed to LED lighting with different lighting regimes. The examination was performed as per «Profile of mood» procedure, a Russian analogue of «Profile of mood states» questionnaire which is widely used in isolation and space-related experiments[4].

The given data clearly indicate that LED lighting exerts significant influence on mental state of some operators and it can result in negative consequences in small teams working in closed spaces. It is truly important to understand significance of these results for keeping a favorable working atmosphere among people fulfilling challenging tasks under grave conditions in the arctic zones or in sealed cabins (a space craft).

As per results obtained via completed examinations, the author pointed out that there was a decrease in certain functional parameters:

– relative accommodation reserves (by 14.3% as per median);

– aggregated field of vision border between blue and green color (by 6.3% and 9.1% as per median accordingly) without any subjective symptoms occurrence. However, there was a decrease in critical light flicker frequency in 25% operators after exposure to LED lighting (Operator No. 3 by 4 arbitrary units, up to 42/42 after 38/38, Operator No. 6 by 3 arbitrary units, up to 45/45 after 42/42)[4].

It is interesting to examine a pattern-ERG (PERG) that characterizes functions of the direct sight (P50) and ganglionic cells in the retina (N95) [14], Pattern-electroretinogram (PERG) is the most sensitive test to determine ganglionic cells function. When a person suffers from primary open-angle glaucoma (POAG), there is primary damage to ganglionic cells [15]. The work [15] contains data on (P50) and (N95) values in patients with primary glaucoma. The research was accomplished in a consulting polyclinic of the Republic Clinical eye Hospital in Kazan and involved examining 21 patients suffering from POAG. Aggregated results of the first measurements are given in Table 3.

A form of PERG signal response was standardized by ISCTV (International Society for Clinical Electrophysiology of Vision)[6]. A standard response is given In Figure 7 that shows P(50) and N95 levels characteristic for primary glaucoma.

[6] Standards, Guidelines and Extended Protocols. *International Society for Clinical Electrophysiology of Vision*. Available at: https://iscev.wildapricot.org/standards/ (12.04.2019) (in Russian).

Table 2

Mental performance of operators working under LED lighting

Parameter scales	Number of operators with changes in their mental performance (changes in parameters fixed in arbitrary units) in comparison with reference groups (working under luminous lighting)	
	Mental performance under LED lighting with permanent spectral and energetic properties (permanent LED lighting 4,000K)	Profile of moods specific for an operator under LED lighting with changing spectral and energetic properties (dynamic LED lighting, 4,000–5,000K and 8,000±800K)
"Anger – hostility"	Increased by 3 a.u. in 2 operators Decreased by 2–4 a.u. in 3 operators No changes in 5 operators	Increased by 10–20 a.u. (by 2–3 times) in 5 operators Decreased by 2–3 a.u. in 2 operators No changes in 1 operator
"Depression – gloom"	Decreased by 1–3 a.u. in 2 operators Increased by 1–3 a.u. in 2 operators No changes in 6 operators	Decreased by 3–6 a.u. in 2 operators Increased by 1–3 a.u. in 3 operators No changes in 3 operators
"Fatigue – inertia"	Increased by 2–9 a.u. in 4 operators Decreased by 1–3 a.u. in 4 operators No changes in 2 operators	Increased by 2–9 a.u. in 3 operators Decreased by 2–4 a.u. in 3 operators No changes in 2 operators
"Vivacity – activity"	Decreased by 2–8 a.u. in 6 operators Increased by 1–6 a.u. in 3 operators No changes in 1 operator	Decreased by 2–8 a.u. in 3 operators Increased by 2–5 a.u. in 2 operators No changes in 3 operators
"Stress – anxiety"	Increased by 1–4 a.u. in 4 operators Decreased by 1–7 a.u. in 4 operators No changes in 2 operators	Increased by 5–12 a.u. in 4 operators Decreased by 1–3 a.u. in 2 operators No changes in 2 operators
"Confusion – uncertainty	Increased by 3 a.u. in 1 operator Decreased by 1–2 a.u. in 5 operators No changes in 4 operators	Increased by 1–10 a.u. in 4 operators Decreased by 1–2 a.u. in 3 operators No changes in 1 operator
Integral mood parameters – "Overall changes in mood"	Increased by 5–25% in 5 operators Decreased by 15–20% in 4 operators No changes in 1 operator	Increased by 30–230% in 5 operators Decreased by 5–30% in 3 operators No operators without changes

Table 3

PERG parameters in patients with primary glaucoma

PERG parameters	Prior to treatment
P(50) Latency ms	$74^{+}_{-}3.3$
N(95) Latency ms	$118^{+}_{-}4.2$

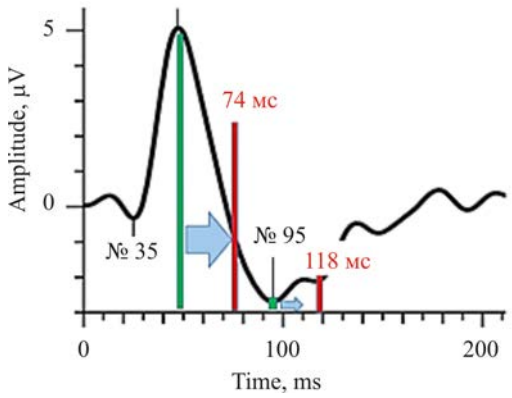

Figure 7. A standard response with given P(50) and N95 values characteristic for primary glaucoma [16, 17]

V.V. Egorov et al. give normal PERG responses in their work [18] (Figure 8).

This response pattern contains two basic components, a positive one approximately at 50–55 ms (P50) and negative one approximately at 85–90 ms (N95).

Latency parameter is the most informative in our case. It should be noted that latency reserve in a standard PERG N95 parameter is rather small and is equal to 23 ms; it is quite comparable to that of P(50) parameter which is equal to 24 ms. These reserve can be spent over years in patients' life.

Table 4 contains aggregated results obtained via PRG examination from the research work [18].

The given data show that latency time grew from 2 ms to 19–28 ms as per N95 parameters under LED lighting with color temperature being higher than 4,000K but lower

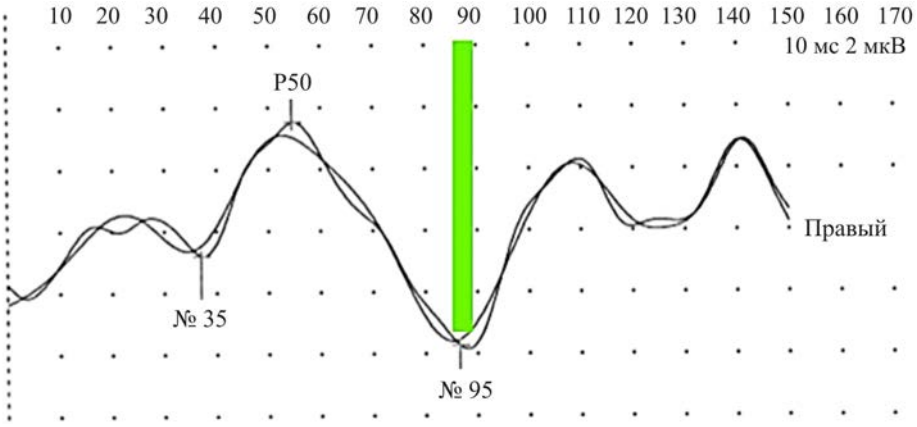

Figure 8. Standard ERG pattern [18]

Table 4

Aggregated results of PERG examination

Operator	Eye	Exposure stage	Cells sizes **0.8 º**		Cells sizes **0.3 º**	
			P50 T(ms)	**№95 T(ms)**	**P50 T(ms)**	**№95 T(ms)**
1	right eye	before	48.3	81.7	55.7	98.3
		after	53.9	101.4	55.7	105.7
	left eye	before	52.8	96.5	50.4	95.5
		after	52.1	108.1	55.3	105.3
2	right eye	before	50.4	88.4	50	92.3
		after	50.4	108.1	58.1	111.8
	left eye	before	51.1	87.7	54.6	92.6
		after	50.7	108.1	56	98.3
3	right eye	before	53.9	108.5	54.2	97.9
		after	57.1	98.3	50	89.5
	left eye	before	52.5	89.8	54.6	96.9
		after	55.3	117.3	60	98.3
4	right eye	before	53.2	92.6	53.5	96.9
		after	52.5	108.8	53.9	98.3
	left eye	before	49.3	92.6	53.5	97.6
		after	52.1	104.6	51.4	87.7

than 8,800K. This «after-effect» persisted in measurements performed by experts from the Clinical Eyesight Physiology Department at Helmholtz' Moscow Scientific Research Institute for Eye Diseases of the RF Public Healthcare Ministry[4].

Examining biological effects produced on a person by light remains a vital issue in lighting hygiene. Experiments performed at cellular, biological, and psychophysiological levels allowed proving that artificial and natural light with the same intensity **were still not biologically adequate**; this inadequacy persisted when illuminance from artificial sources grew [19].

This fact indicates that it is necessary to accomplish more profound research on studying stable functions of hydrogen, sodium, and chlorine ATPase in a scheme of sodium-calcium-chlorine transport within cells (their dendrites and mitochondria) [20] under exposure to excessive dose of 450 nm blue light.

A change in ions concentration leads to changes in water flows from cellular membranes and changes in their pH [21]. Light exerts substantial impacts on transfer of the said

ions as it influences proteins in relevant receptors. R.J. Sizemore et al. [22] and V. Shevchenko [23] state that such proteins as *halorhodopsin, archaerhodopsin, and channel rhodopsin* are classic optogenetic instruments (Figure 9). Halorhodopsin and archaerhodopsin are used to deactivate neurons and block a nerve impulse transfer. Under exposure to light halorhodopsin transfers negatively charged chlorine ions into a cell thus hyperpolarizing a neutron. Archaerhodopsin pumps protons (positive charges) out of a cell and it naturally also hyperpolarizes a neutron. Both halorhodopsin and archaerhodopsin are so called "pumps". Should there be an ion around that they need (and there is almost always one), then, having absorbed a quant of light, these proteins actively transfer an ion from one side of a membrane to another.

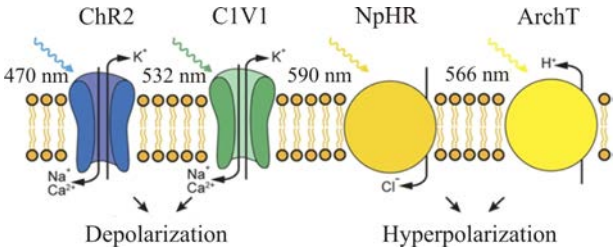

Figure 9. Principles of classic optogenetic instruments functioning: channel rhodopsin, halorhodopsin, and proton pump (archaerhodopsin)

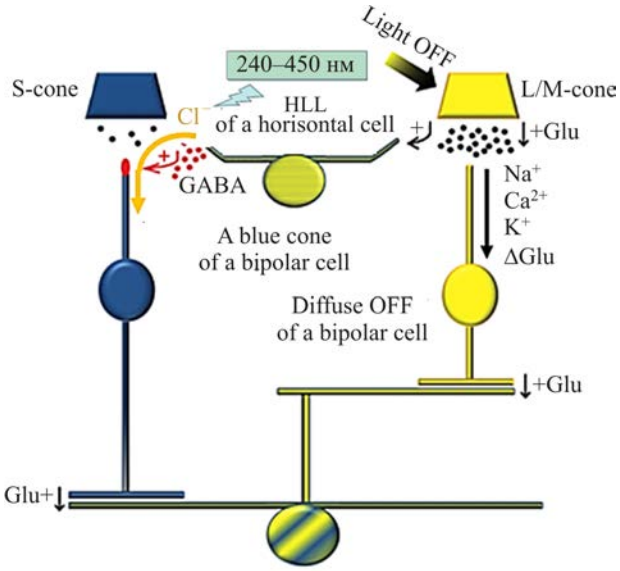

Figure 10. An overall scheme showing synapses placement between retina neurons [25] including a scheme for GABA and chlorine ions transfer as well as Glu and Na, Ca и K ions transfer

Blue light with its wave length equal to 470 nm stimulates ChR2 protein for Na^+ and Ca^+ ions transfer. And we should note here that chlorine absorbs light with its wave length ranging within 250–450 nm [24]. Chlorine ions together with gamma-aminobutyric acid (GABA) are agents that inhibit neuron conduction. Glutamine acid (Glu) is the basic excitatory mediator (about 40% of all neurons). Gamma-Amino Butyric acid (GABA) is the basic inhibitory mediator (also about 40% of all neurons). A disorder in this balance (as a rule, a decrease in inhibition) exerts negative impacts on multiple nerve processes. Proper CNS functioning is provided due to a fine balance between Glu and GABA that are neuromediators in the eye retina (Figure 10).

When chlorine ions absorb excessive blue light dose, a decrease in chlorine ions concentration appears, and, consequently,

$$2Cl^- - 2e^- \rightarrow Cl_2\uparrow,$$

excitement-inhibition efficiency goes down and responses to an excitatory stimulus are delayed.

Ion exchangers function on neurons and astrocytes membranes; they maintain a necessary gradient in concentrations of chemicals between intracellular and intercellular spaces in physiological conditions (potassium ions K+ and glutamate accumulate inside a cell, and calcium ions Ca2+, chlorine ions Cl–, and sodium ions Na+ accumulate outside it) [26, 27].

Astrocytes quantity is 20 times higher than neurons quantity. Astrocytes participate in hormone-induced restructure of synapses that perform endocrine functions [28].

Experts from University of California, San Diego School of Medicine, Johns Hopkins University School of Medicine, and Kennedy Krieger Institute revealed that certain neurons passed a function related to utilizing mitochondria (tiny intracellular energy stations) they no longer needed onto glia cells known as ***astrocytes***. Experts focused their attention on axons of retina ganglionic cells or neurons that transferred visual information from the eye to the brain [29].

Intra-neuronal ion stricture is a significant factor that predetermines brain functioning. There is evidence that abnormal homeostasis of CL-([CL-] i) cellular concentration together with Na+ и Ca2+ induces disorders in neurons excitability and nerve transmission thus making for occurrence of neurologic pathologies [30–33].

All neurodegenerative diseases have several common signs; they are a trend for progressing with slow functional degeneration; selective loss of specific neuron populations due to apoptosis; trans-synaptic degeneration (primary or secondary); common cell death mechanisms such as oxidative stress and ***glutamate toxicity***[7]. «Garbage» accumulation inside cells can result in neurodegenerative processes [29]. Cells in the eye retina have dendritic fields and we can assume that pathology of synapse dendrites is one of the earliest glaucoma signs. Ganglionic cells with changed dendrites have a weaker response to visual stimuli and it confirms that there is a direct relation between dendrite degeneration and visual dysfunction, and that synapses are the most vulnerable component susceptible to a degenerative process that results in cell death[7].

As opposed to electroencephalogram that shows brain cortex activity, visual induced cortical potentials (VICP) are an aggregated response from large neuron populations in the cortex that occurs due to a synchronic impulse flow induced by impacts exerted by an afferent irritant that comes to them. A difference in potentials that occurs between inter- and intracellular medium and is registered on a neutron membrane is determined by a difference in Na^+, K^+ and Cl^- ions concentration in extracellular medium and neuron protoplasm.

The author of the work[4] noted that there were practically no changes in VICP (visual induced cortical potentials) amplitudes after operators having spent some time exposed to dynamic LED lighting; however, all the participants in the experiment has a W-like split in P100 peak as a response to stimuli with different angle dimensions. There are no data in literature on similar changes in VICP morphology in healthy people spending some time in a pressurized object or being exposed to dynamic LED lighting, and it makes their assessment rather difficult. Probably, this result can be treated as evidence that dynamic LED lighting has border influence on functional state of the visual analyzer. In-depth study of the given phenomenon and clarifying its relation with functional activity of the retina and the brain will require a longer examination with much greater number of volunteers participating in it.

Mitochondria are organelles that perform a lot of significant functions including those related to providing cells with energy. Cells remove damaged mitochondria via a process that is called mitophagy. Mitophagy is a subelement in a process called autophagy; its aim is to deliver damaged organelles to liposomes for further degradation. Nevertheless, we have revealed that a great number of mitochondria leave neurons at a place called the optic nerve head in order to be degraded by lysosomes of the adjourning glia cells. This conclusion challenges an assumption that a cell necessarily degrades its own organelles.

The most important function performed by astrocytes is elimination of degrading mitochondria in ganglionic cells in order to maintain their viability and provide reliable and sustainable functioning when the eye is exposed to light loads. Absorption of ganglionic cells mitochondria was studied in detail in works by T.C. Burdett et al. [34], N.N. Osborne et al. [35], and S. La Fee [36]. This interaction between mitochondria and astrocytes is fundamentally important when it comes to impacts exerted on ganglionic cells by blue light.

The research performed by the author revealed that application of LED lighting based on «a blue crystal covered with yellow luminophor» LED technology could in future cause elevated risks of disorders in the visual analyzer functioning. In particular, the author

[7] L.A. Panyushkina. Clinical and morphological peculiarities of the visual tract in patients suffering from glaucoma and Alzheimer disease: a thesis for Candidate of Medical Sciences degree. Moscow, 2015, 106 p. (in Russian).

states that «If layouts and dimensions of a pressurized object allow long-term dwelling of several crew members in one room, then people should be provided with ***glasses with spectral lenses***. Such glasses allow not only facilitating adaptation of crew members to spectral and energetic properties of a lighting system but also reduce visual fatigue of an operator during work with VDU»[4]. The author also notes that «one should be cautions when using LED lighting with additional occurrence with a blue component in the irradiation spectrum of a lighting source. Long-term regular use of such lighting can result in morphofucntional changes in the visual analyzer (damage to the lens and eye retina); changes in mood (increased excitability, irritability, anger, and hostility); insomnia (difficulties in falling asleep, disorders in sleep structure and quality)»[4].

This conclusion is especially important hence personnel living in northern mobile settlements and military personnel serving on ships and submarines have to spend much longer time under such conditions than a 12-day experiment.

It should be noted that the author used lighting sources manufactured by foreign companies; in our opinion, they will be unavailable for use in northern military bases should there be an increase in tensions between Russia and other countries. However, in Russia experts employed at Rospotrebnadzor's Russian Scientific Research Institute for Railway Hygiene and «ELTAN» LLC have developed theoretical grounds for a concept of «LED lighting sources with a biologically adequate spectrum» that is shown in Figure 11 [37].

The developed technology has been patented and it secures the priority Russia has in creating energy-efficient LED lighting sources with white light and biologically adequate spectra. To reduce effects produced by low-intensity "bluish" LED light, it is necessary to make a white light spectrum of a LED lighting sources close to a luminous lamp spectrum or, even better, to the sunlight spectrum with hygienically safe color temperature that is equal to 3,000K.

This white light spectrum has no disadvantages that are characteristic for any standard white LED (a blue crystal covered with yellow luminophor) such as a significant dose of blue light and a dip at 480 nm with an insignificant dose of red light. This spectrum is uninterrupted and its structure corresponds to that of the sunlight spectrum with safe color temperature that is equal to 3,000K.

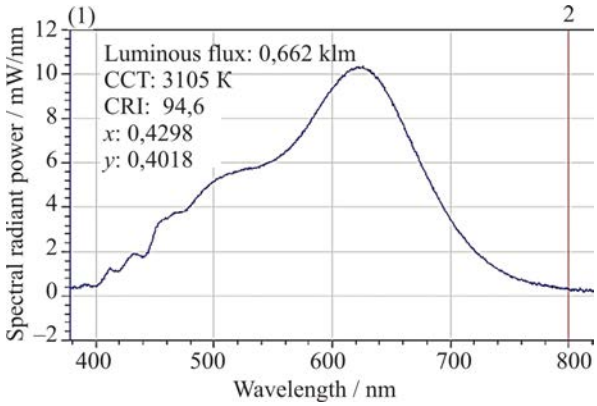

Figure 11. A light spectrum of updated retrofit lamps created by «ELTAN» LLC

We think that application of semi-conductor white light sources with biologically adequate spectra will provide:

– a decrease in negative influence exerted by light on the visual analyzed and the hormonal system;

– conditions for additional energy saving due to almost 2 times lower illuminance at workplaces with preserving the required labor productivity;

– stable light flow and required parameters of lighting system reliability.

Conclusions:

1. An original technology has been developed in Russia; it allows creating white LED lamps with biologically adequate light spectra. World priority of the technology is protected with a patent.

2. We recommend the authors of the GOST 23274-84 «Mobile buildings (inventory buildings). Electrical units. Overall technical conditions» to make amendments into unit 3.4.4 of the said document and state the following: «as a rule, lighting appliances with luminous lamps or white LED lighting sources with biologically adequate light spectra are to be used for electrical lighting of rooms inside mobile houses».

3. A choice between LEDs with permanent or dynamic spectral and energetic properties depends on specific conditions and tasks to be solved: whether it is necessary to optimize balance between CNS excitation and inhibition or to achieve more apparent activating effects on most parameters of psychoemotional state.

4. It is necessary to accomplish wider research on new lighting sources in relation to tasks in the sphere of labor medicine, military and communal hygiene, common and occupational pathology in order to preserve population health and strengthen defense capacities of the country.

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NEW LEGISLATIVE, REGULATORY AND METHODOLOGICAL DOCUMENTS OF THE RUSSIAN FEDERATION IN THE FIELD OF HEALTH RISK ANALYSIS

01.01.2020–31.03.2020

The Decision by the Board of the Eurasian Economic Commission (EEC) dated December 17, 2019 No. 221. "On making alterations into the Decision by the Customs Union Commission dated September 23, 2011 No. 798"

The new edition contains list of standards, application of which will provide voluntary conformity with the requirements fixed in the Customs Union Technical Regulations (CU TR) "On safety of toys" (TR CU 008/2011). The list of standards containing rules and procedures for examination (tests) and measurements, including rules for sampling, was amended.

The Decision by the Board of the Eurasian Economic Commission (EEC) dated December 24, 2019 No. 236. "On the list of international and regional (interstate) standards... and the list of international and regional (interstate) standards... containing rules and procedures for examination (tests) and measurements, including rules for sampling..., necessary for applying and meeting the requirements of CU TR "On food safety" (CU TR 021/2011) and assessing conformity of objects which are subject to technical regulation"

Starting from July 1, 2020, the updated lists of standards required for applying and meeting the requirements of CU TR "On food safety" (CU TR 021/2011) have been applied. Paragraph 2 of the Decision by the Customs Union Commission No. 880 dated December 9, 2011, which approved similar lists of standards, shall be deemed invalid.

The Decision by the Eurasian Economic Commission (EEC) Council dated 08.08.2019 No. 115 "On making alterations into CU TR "On food safety" (CU TR 021/2011) published on the website 13.01.2020).

The requirements to food safety specified in the Regulations of the Customs Union "On food products safety" (CU TR 021/2011) have been enhanced. In particular, the grounds for non-admission to circulation and production (manufacturing) of food products of unprocessed food (edible) raw materials of animal origin have been established; the list of plants and products of their processing containing psychotropic, narcotic, potent or poisonous substances has been supplemented.

The Decision by the Board of the Eurasian Economic Commission (EEC) dated January 20, 2020 No. 12 "On the list of international and regional (interstate) standards... and the list of international and regional (interstate) standards containing the rules and procedures for examination (tests) and measurements, including rules for sampling, necessary for applying and meeting the requirements of CU TR "On packaging safety" (CU TR 005/2011) and assessing conformity of objects which are subject to technical regulation"

Starting from July 1, 2020, the updated lists of standards required for meeting and fulfillment of TU CR requirements "On Packaging Safety" (CU TR 005/2011) are applied. Paragraph 2 of the Decision by the Customs Union Commission No. 769 dated August 16, 2011 "On approval of CU TR "On packaging safety" on approving similar lists shall be deemed invalid. The Decision shall enter into force on July 1, 2020.

The Decision by the Eurasian Economic Commission (EEC) Council dated February 21, 2020 No. 18 "On making alterations into the Single List of Products, which are subject to mandatory requirements within the Customs Union"

The single list of products, which are subject to mandatory requirements within the Customs Union, has included nicotine-containing products, fillers for nicotine delivery systems, including nicotine-free systems.

Recommendation of the EEC Board No. 4 of 26.02.2020 "On methodical guidelines for establishment and substantiation of hygienic standards for the content of chemical impurities and biological agents in food products by human health risk criteria"

Instructions on establishing hygienic standards for chemical impurities and biological agents in food products have been prepared for EAEU Member States. When developing hygienic standards for the content of chemical impurities and biological agents in food products, it is recommended to use methodical guidelines on establishment and substantiation of hygienic standards for the content of chemical impurities and biological agents in food products according to human health risk criteria (see EAEU official website:

http://eec.eaeunion.org/ru/act/texnreg/depsanmer/regulation/Documents/My on establishment and substantiation of hygienic standards.pdf).

The Decision by the EEC Board dated January 28, 2020 No. 19. "On the list of international and regional (interstate) standards... and the list of international and regional (interstate) standards... containing rules and procedures for examination (tests) and measurements, including rules for sampling necessary for applying and meeting the requirements of CU TR "Technical Regulations for fruit and vegetable juice products" (CU TR 023/2011) and assessing conformity of objects which are subject to technical regulation"

Starting from January 1, 2021, the updated lists of standards necessary for implementation of the Customs Union Technical Regulation requirements "Technical Regulation on Fruit and Vegetable Juice Products" (CU TR 023/2011) are applied. Paragraph 2 of Decision by the Customs Union Commission of December 9, 2011 No. 882, which approved similar lists, shall be deemed invalid.

The Decision by the EEC Board dated March 03, 2020 No. 30 On the list of international and regional (interstate) standards... and the list of international and regional (interstate) standards... containing rules and procedures of examination (tests) and measurements, including rules for sampling, necessary for application and meeting the requirements of CU TR "On safety of personal protective equipment" (CU TR 019/2011) and assessing conformity of objects which are subject to technical regulation"

The lists of standards required for implementing and compliance with the requirements of CU TR "On safety of personal protective equipment" (CU TR 019/2011) have been updated. Paragraph 2 of the Decision by the Customs Union Commission No. 878 of December 9, 2011 on approval of the lists that regulate similar legal relations has been considered invalid. The decision shall enter into force upon 30 days from the date of official publication.

Federal Law No. 451-FZ of 27.12.2019 "On introducing amendments to the Federal Law "On special assessment of working conditions"

Starting from January 1, 2020, the control over entering information on Special Assessment of Working Conditions (SAofWC) into Federal Government Information System will be strengthened. It has been established that organizations are obliged to submit information to FGIS and to obtain an identification number for the forthcoming SAofWC, which is assigned automatically, and is included in the report on its implementation. Employees' comments and objections to the assessment results, submitted in writing, will be included in the report on implementing the Assessment. The content and procedure for transmitting information on SAofWC to the Ministry of Labour of Russia have been established. In case of revealing the facts of noncompliance with the established requirements, the activity of organization conducting SAofWC is to be suspended until violations are eliminated. The rules for using measurement methods, the composition of the harmful and/or hazardous factors measured for the production environment have been specified.

Federal Law No. 486-FZ of 27.12.2019 "On amendments to the Federal Act on compulsory social insurance against accidents at work and occupational diseases and to Article 2.3 of the Federal Act "On compulsory social insurance in the event of temporary disability and in connection with motherhood"

Starting from April 1, 2020, a mandatory pre-trial procedure for disputes related to assignment of insurance coverage for industrial accidents and occupational diseases is introduced. It has been established that the decision by the territorial body of the RF Federal Insurance Service on the assignment (refusal in assignment) of insurance coverage may be appealed against in court after appealing to a higher body of the insurer. The appeal shall be considered within ten working days from the date of its receipt. The superior body of the insurer shall be entitled to request the necessary information from the competent authorities and organizations. The applicant shall be notified of the decision within three working days from its acceptance date.

Decree of the Russian Federation President of 21.01.2020 No. 20 "On approving the Doctrine for food security of the Russian Federation"

The Russian Federation Doctrine for Food Security has been updated. The Doctrine reflects goals, objectives and main directions of the state social and economic policy in the field of ensuring food security of the Russian Federation, takes into account recommendations of the Food and Agriculture Organization of the United Nations on the maximum share of import and stocks of food resources, is the basis for the development of normative legal acts in the field of ensuring food security, development of agriculture and fishery. The Doctrine contains food security indices and indicators for assessing food security, specifies the existing risks and threats, and outlines the main tasks for implementing food security and mechanisms and organizational bases for ensuring it. The Decree of the RF President No. 120 of January 30, 2010, which approved a similar document of strategic planning in this area, has been declared invalid.

List of instructions on the issues of sanitary protection of water supply sources" (approved by the RF President Decree No. Pr-244 of 13.02.2020)

The RF Government has been instructed to strengthen legal regime of sanitary protection zones for drinking and domestic water sources. It is charged to make amendments to the legislation of the Russian Federation, providing: unification of norms, regulating land, water and other relations, arising at sanitary protection of drinking and domestic water supply sources; registration of water supply systems, water intake and other structures, placed in these zones, at establishing sanitary protection zones; differentiation of the sanitary protection zones legal regime for the sources of drinking and domestic water supply, zones (subzones) of these zones taking into account geographical, economic, ecological and other factors; determination of terms and conditions for establishing sanitary protection zones of drinking and domestic water sources.

Resolution of the RF Government No. 1859 of 26.12.2019 "On approving the rules for granting and distribution of subsidies from the federal budget to the budgets of the constituent entities of the Russian Federation for the purpose of co-financing expenditure obligations of the constituent entities of the Russian Federation arising from the implementation of regional projects that provide for the formation of commitment to a healthy lifestyle (HLS), and ensure the achievement of goals, indicators and results of the federal project "Formation of citizens motivation system for a healthy lifestyle, including healthy eating habits and abandonment of harmful habits"

Regions will be able to receive subsidies for implementation of regional projects to build commitment to HLS. The selection criteria for receiving subsidies are as follows: the existence in the region of socially oriented non-profit organizations and volunteer movements implementing measures to build citizens' commitment to a healthy lifestyle; the rate incidence of drug addiction disorders (mental and behavioral disorders related to the use of psychoactive substances), the value of which is higher than the national average; and the indicator "Share of citizens living a healthy lifestyle", the value of which is less than 6.5 percent.

Resolution of the RF Government of 24.12.2019 No. 1792 "On approving the requirements for the list of compensation measures aimed at improving air quality in each territory of experiment on quoting emissions based on air pollution aggregate calculations"

The requirements to the list of measures aimed at improving air quality in the cities participating in the experiment on quoting pollutants emissions (Bratsk, Krasnoyarsk, Lipetsk, Magnitogorsk, Mednogorsk, Nizhny Tagil, Novokuznetsk, Norilsk, Omsk, Chelyabinsk, Cherepovets and Chita) are established. The range of tasks for which the activities shall be directed is established.

Resolution of the RF Government of 24.12.2019 No. 1806 "On creation and operation of the Federal State Information System for monitoring atmospheric air quality in the urban districts of Bratsk, Krasnoyarsk, Lipetsk, Magnitogorsk, Mednogorsk, Nizhny Tagil, Novokuznetsk, Norilsk, Omsk, Chelyabinsk, Cherepovets and Chita"

A procedure has been established for the operation of the State Information System for monitoring atmospheric air quality in the cities participating in the experiment on quoting the pollutant emissions. The users of the system are public authorities, local self-government bodies, legal entities, individual entrepreneurs and citizens. Users' access to information is ensured through the use of the Unified System of Identification and Authentication.

Resolution of the RF Government No. 1956 dated 31.12.2019 "On approving the rules for identification of consumer goods industry items and peculiarities of implementing the State Information System for monitoring the turnover of goods subject to compulsory marking by means of identification"

Starting from January 1, 2021, the compulsory marking of the consumer goods industry items by means of identification will be introduced. Participants in the turnover of the consumer goods industry will be required, among other things: to get registered with the information system; to ensure readiness of their own soft- and hardware for information exchange with the information monitoring system; to test them; from January 1, 2021 – to enter data into the information system on marking of the consumer goods industry items, as well as on the introduction of consumer goods into circulation, their turnover and withdrawal from circulation.

Resolution of the RF Government dated 31.12.2019 No. 1957 "On approving the rules for marking perfume and eau de toilette items by means of identification and peculiarities of introducing the State Information System for monitoring the turnover of goods subject to compulsory marking by beans of identification with respect to perfume and eau de toilette"

Starting from October 1, 2020, applying identification means on perfumery products (perfumes and eau de toilette items) will be mandatory. If there are

any undistributed perfumery products, put into circulation as of October 1, 2020, the turnover parties have the right to sell them till September 30, 2021 without marking.

The rules shall determine: a) circle of parties in goods turnover and the procedure for their registration in the information system; b) procedure for information exchange in the system; c) procedure for goods registration in the information system; d) characteristics of identification means: structure and format of identification codes, identification codes and verification codes; e) procedure for providing identification codes; f) procedure for applying means of identification; g) procedure for providing data in the information system; h) procedure for making changes in the data contained in the monitoring information system, etc.

Resolution of the RF Government No. 31 of 22.01.2020 "On introducing amendments to the Resolution of the RF Government No. 407 of May 13, 2013"

The powers of Rosstandart and Rospotrebnadzor in exercising functions of surveillance to meet the requirements of a number of technical regulations have been differentiated.

Rosstandart is vested with the authority to exercise state control (surveillance) over compliance with the requirements: EAEU Technical Regulations (EAEU TR) "Requirements for liquefied hydrocarbon gases to be used as fuel" (EAEU TR 036/2016); "On safety of oil prepared for transportation and/or use" (EAEU TR 045/2017); "On safety of natural combustible gas prepared for transportation and/or use (EAEU TR 046/2018); "On restriction of using hazardous substances in electrical and radio electronics products" (EAEU TR 037/2016) with regard to electrical and radio electronics products, which are sold not for the needs of consumers.

Rospotrebnadzor is defined as a body exercising state control (surveillance) over compliance with EAEU TR requirements " On restriction of using hazardous substances in electrical and radio electronics products" (EAEU TR 037/2016) in respect of electrical and electronic products sold exclusively for personal, family, home and other needs not related to business activities.

Resolution of the RF Government No. 215 of 28.02.2020 "On introducing amendments to Clause 1 of the Resolution No. 836 of the RF Government dated June 29, 2019"

The experiment on marking by means of identification of certain types of dairy products has been extended until March 31, 2020. The experiment is held from July 15, 2019 in order to test the completeness and sufficiency of marking mechanisms by means of identification of certain types of dairy products to ensure countermeasures against illegal import, production and circulating certain types of dairy products, including of counterfeit, as well as to increase collection of customs and tax payments.

Resolution of the RF Government No. 216 of 29.02.2020 "On introducing amendments to the Resolution of the RF Government No. 860 of July 5, 2019"

Terms of introducing mandatory identification marking of footwear products have been extended. It is established that application of identification means on consumer packaging or on footwear products, or product label of footwear products is mandatory not from March 1, 2020, but from July 1, 2020. Start of entering information on marking footwear goods into the information monitoring system is postponed to July 1, 2020.

Resolution of the RF Government No. 66 of 31.01.2020 "On amending the list of diseases that threaten to People around Us"

Coronavirus infection (2019-nCoV) is included in the list of diseases posing danger for the wider public.

Order of Rospotrebnadzor No. 676 dated 02.09.2019 "On approving administrative policy for the provision of ... public service to assess quality compliance of socially-useful services by a socially oriented non-profit organization: to provide assistance in obtaining food in the place of temporary accommodation to persons recognized as refugees in accordance with the Federal Law № 4528-1 of February 19, 1993 "On Refugees"; to organize and conduct advisory, methodical, preventive and anti-epidemic activities to prevent the spread of HIV infection

The document defines, among other things, the content, sequence and timing of administrative procedures (actions), requirements to the procedure for their implementation, including its electronic form, an exhaustive list of documents required to provide a public service, an exhaustive list of grounds for refusal to provide a public service. It is provided that there are no grounds for refusal in accepting documents for rendering public service by the RF legislation. The period of providing a public service is 30 days.

Resolution of the RF Chief State Sanitary Doctor No. 20 of 05.12.2019 "On approving sanitary-epidemiological regulations and norms of SanPiN 2.1.7.3550-19 "Sanitary-epidemiological requirements to the maintenance of territories of municipalities"(registered with the Ministry of Justice of Russia, No. 56981 dated 25.12.2019)

Starting from January 1, 2020, the sanitary-epidemiological requirements to the maintenance of territories of municipalities (SanPiN 2.1.7.3550-19) come into force. The document establishes requirements for the accumulation, collection, transportation of production and consumption waste, consisting of solid municipal waste, including large waste, and liquid domestic waste. Its observance is mandatory for regional executive bodies, local authorities, citizens, individual entrepreneurs and legal entities, whose activities are related to the maintenance, servicing of municipal territories, as well as waste management in the territories of municipal entities. The requirements of Chapter V, SanPiN comes into effect on March 1, 2020.

Resolution of the RF Chief State Sanitary Doctor No. 2 of 24.01.2020 "On additional measures to prevent importation and spread of new Coronavirus infection caused by 2019-nCoV" (registered with the Ministry of Justice of Russia, No. 57269 of 24.01.2020)

A set of actions to control coronavirus infection has been approved. In the list of actions: strengthening the current disinfection programs in locations of people mass accumulation; training healthcare workers in the issues of clinics, diagnostics, treatment of a new coronavirus infection; if necessary, transfer of medical organizations to a strict anti-epidemic mode; making arrangements for reception of patients suspected to a new coronavirus infection disease in medical organizations; arranging a pick-up and delivery to Rospotrebnadzor' laboratory of a proper quality stuff from patients with a suspicion on a new coronavirus; establishing medical monitoring of students arriving from China; implementation of sanitary and quarantine control at checkpoints on RF state border in a reinforced regime.

Resolution of the RF Chief State Sanitary Doctor No. 3 of 31.01.2020 "On additional sanitary-and-epidemic (preventive) actions on prevention of import and spread of the new Coronavirus infection caused by 2019-nCoV" (registered in the Ministry of Justice of Russia by No. 57367 from 31.01.2020)

A set of measures to prevent coronavirus infection spread has been defined. In particular, the executive authorities of the Russian Federation subjects should: ensure preparation of places for continuous medical monitoring, taking into account the duration of such monitoring for 14 calendar days; organize measures to ensure an enhanced disinfection regime; provide continuous medical monitoring for 14 calendar days for Chinese citizens who have a residence permit in Russia, when they return from China. In case of symptoms that do not exclude a new coronavirus infection, to carry out their isolation and laboratory examination.

Resolution of the RF Chief State Sanitary Doctor No. 5 of 02.03.2020 "On additional measures to reduce risks of importation and spread of a new Coronavirus infection (2019-nCoV)" (registered with the Ministry of Justice of Russia, No. 57643 from 02.03.2020)

An additional set of mandatory measures to prevent spread of the new coronavirus infection (2019-nCoV) has been developed. The list includes, among other things: organization of healthcare organizations' activities with the priority of rendering primary medical aid at home to patients with respiratory symptoms, first of all to persons over 60 years of age, provision of separate reception (filter-boxes) for patients with signs of acute respiratory viral infections, community-acquired pneumonia; equipping ambulance crews, medical organizations with pulse-oximeters; departments of medical organizations – with non-invasive lungs ventilation devices; maintaining a minimum stock of antiviral drugs, including those recommended for treatment of the new coronavirus infection, disinfectants and personal protective equipment in medical organizations and pharmacy chains; medical supervision for a period of 14 calendar days of all citizens arriving from the Islamic Republic of Iran and the Republic of Korea at their place of residence. At manifestations of symptoms in them, which do not exclude the new coronavirus infection (2019-nCoV), to ensure their immediate isolation and hospitalization in medical units; providing a possibility for issuing sick leaves without visiting medical organization to the persons who have returned from territories where cases of the new coronavirus infection (2019-nCoV) are registered; strengthening of sanitary-quarantine control in the airports check points through the state border of the Russian Federation.

Citizens who have returned from territories where cases of new coronavirus infection (2019-nCoV) are registered, are recommended to: transfer data on a place and dates of their stay, and return, their contact information by the "hot line" arranged in the RF subject; at occurrence of the respiratory infection first signs – to remain at home (at a place of stay) and immediately to address for medical aid.

MR 2.4.0162-19. 2.4 "Hygiene of children and adolescents. Peculiarities of organizing nutrition for children suffering from diabetes mellitus and other diseases accompanied by dietary restrictions (in educational and health-improving organizations). Methodical recommendations"(approved by the RF Chief State Sanitary Doctor on 30.12.2019)

Methodical recommendations (guidelines) include, among others, the following: a) a list of industrial products that may contain hidden gluten; b) rec-

ommended food intake sets for organizing meals for children with diabetes; c) a set of operational charts for meals for children with diabetes.

The Letter by Rospotrebnadzor No. 02/2037-2020-32 dated 11.02.2020 "On sending interim recommendations for organization of observatory units"

Recommendations have been developed for organizing the operations of observatories to isolate conditionally healthy persons coming from the territories, which are epidemiologically unfavorable in terms of coronavirus. Only healthy people are to be placed in observatories, i.e. specially adapted institutions for isolation and medical supervision, for the period of 14 calendar days. People under observation are accommodated on a time-bound basis, in as small groups as possible, with measures to exclude communication with persons from other premises. Up to four persons (members of the same family or, with their consent, on the basis of gender) may be accommodated in one room.

Requirements have been established for the observatory premises, for the organization of its operations, for medical supervision and collection of material from the people under observation, for the purposes of biological safety, when working at the observatory, and for the collection and disinfection of medical waste.

The Letter by Rospotrebnadzor No. 02/2120-2020-32 of 13.02.2020 "On recommendations for cleaning and disinfection of motor vehicles"

Recommendations on disinfection to prevent coronavirus infection spread have been developed for persons providing services on transportation of passengers by road. The recommendations contain prophylactic measures in respect of drivers, regulate the procedure for preventive, focal (when a patient is detected) and final (after the patient is removed, and the cabin is free of people) disinfection of a vehicle, and provide the permitted disinfectants and the procedure for their application.

The Letter by Rospotrebnadzor No. 02/2230-2020-32 of 14.02.2020 "On preventive and disinfection measures in public catering organizations and food units of educational organizations"

A list of preventive recommendations to prohibiting the spread of coronavirus infection has been developed for catering establishments. The list includes, among others: observance of personal hygiene, non-admission to work of personnel with manifestations of acute respiratory infections (fever, cough, runny nose); provision of personnel with a stock of disposable masks, disinfectant wipes, skin antiseptics for hands, disinfectants; ventilation and wet cleaning with disinfectants; avoiding use of dishes with cracks, chips, broken edges, warped, damaged enamel.

It is recommended to equip public catering organizations and food establishments with modern dishwashers with disinfectant effect using treatment modes at temperatures not lower than 65° C for 90 minutes.

When a diseased are revealed after patient removal and the premises are vacated, final disinfection is carried out by specialized organizations.

The Letter by Rospotrebnadzor No. 02/3853-2020-27 dated 10.03.2020 "On measures to prevent new coronavirus infection (COVID-19)"

Guidelines have been developed for employers to prevent new coronavirus infection (COVID-19) spread among employees. Employers are recommended, in particular, to ensure: facilities of hand treatment with skin antiseptics or disinfectant wipes providing control over the compliance with this hygiene procedure; control of employees body temperature at the entrance to organization (company) and during the working day (according to indications), with mandatory suspension from work of persons with elevated body temperature and signs of infectious disease; proper cleaning of premises using virucidal disinfectants; disinfectants supply for at least five days for cleaning premises and hands-treatment, individual respiratory protection means (masks, respirators), in case persons with infectious disease signs are detected, to be available in organization.

Among other things, it is recommended to restrain any corporate events and employees participation in public events.

Order of the Ministry of Agriculture of Russia No. 634 of 19.11.2019 "On approving the form and procedure for using graphic image (mark) of a common pattern for organic products"

Starting from January 1, 2020, the procedure has been approved for placing a mark of organic products on goods. A manufacturer of organic products gets the right to use mark from the moment of entering to the Unified State Register of organic products manufacturers the information about the manufacturer, types of organic products he produces for the period, which does not exceed the validity period of the conformity certificate for organic products. Graphical image (mark) is a white sheet on a green background with the inscription ‘ORGANIC’ on top of the sheet and ‘ORGANIC’ – on bottom of the sheet. The product manufacturer has the right to place mark on packaging, consumer and/or transport packaging of organic products or on any information carriers attached thereto.

The Order of the Ministry of Natural Resources of Russia No. 794 dated 22.11.2019 "On approving methodical guidelines for determining the background level of air pollution"

Methodical recommendations for determining the background level of air pollution have been approved. The background is determined by sampling data from homogeneous series of observations at observation points over a five-year period. The primary data for determining the background is the results of measurements of one-time (20–30 minutes) concentrations of pollutants. The total sampling volume from a series of one-time concentrations at continuous observations is not less than 14 000 values, at discrete observations – not less than 800 values. The background value for residential settlements, where observations over the air pollution level are carried out annually during five years in different observation points under a reduced program, takes into account data of more than 200 observations per year.

The Order of the Ministry of Natural Resources of Russia No. 804 dated 27.11.2019 "On approving the method for determining emissions of pollutants into the air from mobile sources for consolidated calculations of air pollution"

The procedure has been established for calculating pollutant emissions into the air from mobile sources on the roads in summation. The results of full-scale in situ surveys of the composition by type of vehicles and traffic flows intensity, taking into account the category of roads and vehicle types, are used as input data for calculating maximum single and gross (annual) emissions. For the projected roads, the data on traffic structure and volume, according to the project documentation, are used.

Recommendations on the implementation of in situ surveys of traffic flow structure and intensity are given.

The Order of the Ministry of Natural Resources of Russia No. 811 dated 28.11.2019 "On approving requirements for measures to reduce emissions of pollutants into the air under unfavorable meteorological conditions"

Starting from 27 June 2020, the requirements for emission reduction measures during the adverse weather events (AWE) will be introduced. It is established that business entities shall develop measures to reduce emissions taking into account the hazard degree of the forecasted AWE for all sources of emissions at facilities with negative impact of I, II and III categories subject to rationing in the field of environmental protection. The recommended list of emission reduction measures is given.

The Order of the Ministry of Natural Resources of Russia No. 813 dated 29.11.2019 "On approving the rules for aggregate calculations of air pollution, including their updates"

The procedure has been defined for calculating ambient air pollution. Among other things, is has been established as follows: the procedure for collecting information for aggregate calculations; the procedure for organizing activities on aggregate calculations; the requirements for the content of the citywide emission data bank; the procedure for calculating pollutants concentrations in the ambient air; recommendations for assigning codes to road sections and other facilities.

The Order of the Ministry of Natural Resources of Russia No. 814 dated 29.11.2019 "On approving the rules for quoting emissions of contaminants (excluding radioactive substances) into ambient air"

The rules have been approved for establishing quotas on pollutants emission into ambient air. The following have been established: priorities in determining permissible contributions to the concentration of pollutants in the air; procedure for determining check-points for quoting emissions; mechanism for calculating permissible contributions to the concentration in quoting points; procedure for establishing quotas on emissions.

The Order by the Ministry of Health of Russia No. 1032n dated 13.12.2019 "On amendments to Annexes 1, 2 and 3 to the Order of the Ministry of Health and Social Development of the Russian Federation No. 302n of 12 April 2011 "On approving the lists of harmful and/or hazardous production factors and works for which preliminary and periodic medical examinations (screenings) are conducted, and the procedure for mandatory preliminary and periodic medical examinations (screenings) of employees engaged in heavy works and works in harmful and/or hazardous working conditions" (registered with the Ministry of Justice of Russia under No. 56976 dated 24.12.2019)

The procedure has been specified for identifying diseases that hinder the continuation of work related to the impact of harmful or hazardous production factors. It is allowed to take into account the results of examinations and/or preventive medical checkups performed earlier (not later than one year); a list of actions, which are necessarily performed during preliminary and periodic examinations, is introduced; it is allowed to provide information (with the consent of the examined person) on examination results to the Russian Federation Social Insurance Fund.

The frequency of compulsory medical examinations at occupational pathology center for employees engaged in heavy works and works in harmful or dangerous working conditions (Classes 3.1-3.4, Class 4) is once every five years. The same frequency of examinations is established for employees with persistent consequences of accidents at work.

The opportunity for compulsory periodic medical examinations by mobile medical teams is introduced for employees with a total work record of five years or more, if work site is located in the Far North and areas equal to them.

The Order by the Ministry of Health of Russia No. 8 of 15.01.2020 "On approving the strategy for the promotion of healthy lifestyle, prevention and control of non-communicable diseases for the period until 2025"

The Strategy provides: an analysis of the current state of healthy lifestyles issue, prevention and control of non-communicable diseases in the Russian Federation, the main goals, principles and objectives, the main directions for the implementation of tasks, prospects for promoting a healthy lifestyle, prevention and control of non-communicable diseases, mechanism for implementing the Strategy, the results of implementing the Strategy.

"Interim methodical guidelines. Prevention, diagnosis and treatment of a new Coronavirus infection (2019-nCoV). Version 2 (03.02.2020)" (approved by the Russian Ministry of Health, Rospotrebnadzor)

Interim recommendations for the diagnosis and treatment of a new coronavirus infection have been updated. The recommendations are intended for physicians at infectious disease treatment and prevention facilities, as well as intensive care physicians at the infectious disease hospital. The annexes contain, among other things, a list of possible medications for treating coronavirus infection in adults.

Letter by the Ministry of Education of Russia No. 05-PG-MP-20965 of 28.10.2019 "On the occupancy rate of groups of students in educational organizations"

The Ministry of Education of Russia reports that the recommendation norm setting the maximum occupancy rate for school classes of 25 people is excluded on the basis of SanPiN 2.4.2.2821-10. At the same time, according to the procedure for organization of educational activities under secondary vocational education programs (approved by the Order of the Ministry of Education and Science of Russia No. 464 of 14.06.2013), the number of students in a study group is not more than 25 people. Based on the specifics of the educational organization, training sessions and practice can be conducted with students groups of smaller numbers and individual students. It is also possible to combine groups of students when holding training sessions in the form of lectures.

The Letter by the Ministry of Natural Resources of Russia No. 12-47/22755 dated 20.09.2019 "On implementing industrial environmental control in the field of ambient air protection"

The Russian Ministry of Natural Resources has clarified the requirements for filling in the control schedules for stationary sources of pollutant emissions. When carrying out an industrial environmental control at a facility of NEI (Negative Environmental Impact) III Category, it is obligatory to include I, II hazard Class substances into the control schedule, as well as marker substances; in case the emissions of pollutants from a source, in terms of substances which are subject to state regulation measures, as well as marker substances, exceed 0.1 MPC of pollutants at the border of an enterprise, this source of emissions and contaminants emitted thereby should be included into the control schedule.

It is noted that adjustments to the industrial environmental control program are made when gross emissions from the enterprise change.