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

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BASIC TRENDS IN BEHAVIORAL HEALTH RISKS

V.R. Kuchma, S.B. Sokolova

National Medical Research Center for Children's Health, 1 Bldg., 2 Lomonosov Avenue, Moscow, 119991, Russian Federation

A scope of research entitled Health Behaviour in School-Aged Children [HBSC] allowed to obtained data for further analysis and systematization; there were some basic trends in behavioral risks revealed as a result which were hazardous for health of schoolchildren in Russia aged 11, 13, and 15. We applied data taken from reports on international HBSC research conducted in 1993, 1997, 2001, 2005, 2009, and 2013. All the data were statistically processed with non-parametric analysis. We compared risk factors prevalence among children aged 11, 13, and 15; among boys and girls; over time dynamics; factors revealed in different countries. We detected both age and gender peculiarities in behavioral factors that influenced health; there were also discrepancies in their prevalence taken in dynamics over years and differences between children of the same age living in Russia and abroad. A lot of risky behavioral patterns are more widely spread among boys. Since 1993 there has been a growth in parameters related to risky behaviors among girls in Russia. Over the last ten years there have been positive trends in behavioral risk factors prevalence. However, when compared with their foreign counterparts, Russian teenagers perceive their school environment more negatively; they more frequently tend to estimate their health as "being poor"; they are less satisfied with their lives; they less frequently consume fruit and pay less attention to oral health; they tend to be physically inactive. Risky behavioral patterns such as smoking, alcohol use, or aggressive behaviour, are more widely spread among younger teenagers. When creating programs aimed at establishing healthy lifestyle, it is necessary to take into account age and gender differences, as well as trends related to behavioral risk factors which change over time and which can be objectively assessed with an international questionnaire entitled Health Behaviour in School-Aged Children.

***Key words**: children and teenagers hygiene, behavioral risk, non-parametric analysis, time dynamics, gender difference, healthy lifestyle, international questionnaire.*

Introduction

Health of coming generations still tends to deteriorate and requires immediate attention given to it by the overall society [1–7].

Health disorders occur due to risk factors that lead to greater probability of various diseases, their development, and adverse outcomes [8–9].

According to contemporary concepts, a person's health is a result of complicated convergence that comprises biological (physiological and genetic), behavioral, social, economic, and ecological factors. WHO experts spot out four groups of factors that determine one's health; they are heredity (20%), environmental factors (20%), quality and availability of medical aid (10%), and factors related to a person's lifestyle (50%). An extent to which various factors can influence functional state of a schoolchild's body depends on his or her age; thus, contribution made into it by physical activity amounts to 45.3% for junior schoolchildren (aged 7–11), and to 18.4%, for older ones (aged 12–18); social factors, to 19.7% and 9.6% respectively; educational loads, 10.2% and 35.8%: lifestyle factors, 9.8% and

Vladislav R. Kuchma – Corresponding Member of the RAS, Doctor of Medical Sciences, Professor, Deputy Director responsible for research, Head of the Scientific Research Institute for Children Health Protection and Hygiene "National Medical Research Center for Children Health" of the Russian Federation Public Healthcare Ministry (e-mail: info@niigd.ru; tel.: +7 (495) 917-48-31; ORCID: http://orcid.org/0000-0002-1410-5546).

Svetlana B. Sokolova – Candidate of Medical Sciences, Leading researcher (e-mail: s-s-b@mail.ru; tel.: +7 (495) 917-48-31; ORCID: https://orcid.org/0000-0001-5895-4577).

27.6%; learning conditions, 15.0% and 8.6%, respectively [10].

Lifestyle-related adverse factors tend to prevail significantly among contemporary schoolchildren and in future it is these factors that will determine mortality and morbidity (their levels and dynamics) among adult population [11–17].

As per data provided by WHO, 70% untimely death cases among adults are related to behavioral factors that first occurred during teenage years. Children's attitudes towards their own health tend to differ greatly from those of adults'. WHO experts state there are about 60 behavioral factors that influence teenagers' health [18]. International research entitled "Health Behaviour in School-Aged Children [HBSC]" enables obtaining the most comprehensive picture of teenagers' health and well-being. Research within HBSC project is accomplished once in four years. Its tool is a standard questionnaire developed by an international scientific-research network and its target groups are schoolchildren aged 11, 13, and 15. A minimal recommended sampling is equal to 1,500 schoolchildren for each age group. Questioning is usually performed by specially trained teachers, school nurses, and experts from scientific research institutes. Most countries and regions stratify their samplings in order to provide as wide geographic coverage as it's only possible.

HBSC project was first introduced in 1983 in five countries, and soon its participants established relations with WHO European Office. At present there are 44 countries in Europe and North America that participate in it. The project network includes 340 researchers who are employed at public healthcare universities and institutes. Since 1993, this research has been accomplished in Russia according to a unified procedure that allowed comparing results obtained for different groups of children as well as in different years.

A range of project priorities is constantly being enlarged as per their contents and aspects related to exploring health and behavioral issues as regards schoolchildren. First research works tended to focus on such factors as smoking, physical activity and certain psychosocial health aspects. Later on some other proprieties were added such as eating behaviour, proneness to injuries, sexual behavior, social differences, etc. Reports issued in 2005–2006 and 2009–2010 included more than 60 parameters divided into 4 basic groups: "social context" (family, peers, school); "health outcomes" (positive health, medically attended injuries, body weight); "health behaviours" (eating behaviour, oral health, physical activity and sedentary behaviour); "risk behaviours" (smoking, alcohol and cannabis use, sexual behaviour, fighting and bullying). In 2016 the last available report was published that contained results of the last questioning performed in 2013–2014 and included a number of new priorities, such as support by family and peers, most serious injury requiring medical treatment, migration, and cyberbulling.

Most results are systematized as per geographic regions (countries and regions in Europe and North America); age (11, 13 and 15); sex (boys or girls); social and economic status.

Our research goal was to analyze and systematize risk factors prevalence among Russian teenagers aged 11, 13 and 15 taken in time dynamics.

Data and methods

We took data from international reports issued within "Health Behaviour in School-Aged Children [HBSC]" project in 1993, 1997, 2001, 2005, 2009 and 2013, processed them statistically, performed comparative analysis and interpretation.

Research data were statistically processed with non-parametric analysis techniques. Data were accumulated, adjusted and systematized, and results visualized, with Microsoft Office Excel 2016. We performed statistical analysis with STATISTICA 13.3 package (developed by StatSoft Inc.) and Open Epi calculator (https://www.openepi.com).

Nominal and ordinal data were described with stating their % fractions.

Risk factors prevalence was compared between boys and girls; between 11-eyar and 15-year old teenagers; between different years; between different countries.

We compared nominal data with Pearson's χ^2 test that allowed assessing significance of discrepancies between actual number of outcomes or qualitative characteristics of a sampling that were included into each category, and a theoretical quantity that could be expected in examined groups, a zero hypothesis being valid. Results were considered to be statistically significant at $p \leq 0.05$.

To test a hypothesis on a possible statistic correlation between risk factors and age (or years), we applied χ^2 for a linear trend. In addition, we assessed whether an existing dependence could possibly deviate from a linear one. To do that, we deducted χ^2 for a linear trend from overall χ^2 value and compared an obtained difference with a critical χ^2 value for remaining degrees of freedom. If a difference was smaller than critical χ^2 value, it meant that data didn't deviate from a linear statistical correlation. To assess an amount of an effect, we applied Kramer V-test for nominal data (sex) (interpreted according to Rea & Parker recommendations) [19, 20].

To compare parameters obtained for Russian schoolchildren with those obtained for their counterparts from other countries, we divided countries that took part in HBSC research into three equal groups: favorable, intermediate, or unfavorable situation depending on a factor. A situation (favorable, intermediate, or unfavorable) prevailing in the RF in comparison with other countries from the list was established for each factor.

Results

Age-related peculiarities

Drastic changes occur in endocrine glands functioning in teenagers aged 11–15. It is a period when pubescence develops rapidly in girls and starts in boys. Usually there is a prepubertal jump in a teenager's growth combined with certain disharmonic changes as sex-related physical and mental features occur and develop. It is the most difficult stage in psychological development when a person's will, consciousness and morality start to shape. Frequently it is a rather dramatic re-assessment of all life values, attitudes towards oneself, parents, contemporaries, and society in general. So, extreme judgments and behavior are quite possible as well as striving for self-assertion and conflicts [14, 21].

At this age teenagers usually adopt their behavioral patterns and attitudes towards their health; these attitudes in future will influence their health, welfare, and life quality. To improve teenagers' health and secure their welfare, it is necessary to obtain epidemiologic data on age-related differences in teenagers' perceptions of their social environment and on prevalence of both health-improving factors and factors that cause health risks.

Teenagers tended to perceive their educational environment in a more negative way as they grew; thus, 27.5% 11-year old teenagers stated "they like school a lot", but their number dropped to 19.3% and 16.8% at 13 and 15 respectively (Table 1). Interaction and socializing between teenagers have changed considerably over recent years due to rapid development of social networks and other electronic mass media. A number of teenagers that every day communicated with their peers via electronic media communication (EMC) grew with age, from 46.8% to 53.3% respectively; it was revealed that every day 29.5% 11-year old teenagers communicated with their friends in social media; they figure amounted to 42.5% among 13-year old teenagers, and to 51.0%, among 15-year old ones; 21.0%, 32.0%, and 41.5% teenagers respectively communicated with their friends via text messages.

Negative trends determined by age-related changes can be seen in a way teenagers estimated their health in a period from 11 to 15: they started to think their health was poor more frequently (20.0% and 27.5% accordingly); they had multiple health complaints (32.6% and 35.4% accordingly). Number of teenagers who were satisfied with their life also dropped with age, from 82.0% among 11-year old, to 79.9%, among 13-year old, and to 78.4%, among those who are 15. 11-year old boys and girls tended to have overweight and obesity more frequently than those who were 15 (18.0% against 10.7%). However, it was 15-year old teenagers who thought they were too fat more frequently than 11-year old ones (15.9% against 14.6%) and who engaged in weight-reduction behaviour, 14.1% against

Table 1

Age-related peculiarities of health-influencing behavioral factors among Russian teenagers aged 11, 13 and 15

Parameters	Mean value (%)			N	$\chi^2_{d=2}$	p	$\chi^2_{l.t.\ d=1}$	p	$\chi^2 - \chi^2_{l.t.}$
	11	13	15						
Liking school a lot	27.5	19.3	16.8	29,562	448.9	<0.001	437.5	<0.001	11.4
Communicate daily via EC,	46.8	51.3	53.3	21,237	62.8	<0.001	52.8	<0.001	10.0
social networks, and with	29.5	42.5	51.0	4,274	123.7	<0.001	121.0	<0.001	2.7*[1]
text messages	21.0	32.0	41.5	4,274	125.0	<0.001	124.7	<0.001	0.3*
Fair or poor self-rated health	20.0	23.5	27.5	25,511	173.3	<0.001	173.1	<0.001	0.2*
Multiple health complaints	32.6	33.9	35.4	25,511	14.4	<0.001	14.3	<0.001	0.1*
High life satisfaction	82.0	79.9	78.4	25,511	35.5	<0.001	35.1	<0.001	0.4*
Overweight	18.0	12.5	10.7	17,680	146.8	<0.001	135.9	<0.001	10.9
Feeling too fat	14.6	15.6	15.9	25,511	5.9	<0.001	5.2	<0.001	0.7*
Engaged in weight-reduction behaviour	10.9	11.4	14.1	25,511	48.8	<0.001	41.9	<0.001	6.9
Injuries required medical intervention	46.0	44.0	40.1	25,511	61.7	<0.001	59.7	<0.001	2.0*
Eating breakfast every day	66.4	62.8	59.0	25,511	99.3	<0.001	99.3	<0.001	0.0*
Daily fruit consumption	34.4	32.1	27.4	25,511	100.9	<0.001	96.8	<0.001	4.1
Daily vegetables consumption	34.0	32.8	31.8	25,511	9.9	<0.001	9.8	<0.001	0.1*
60 minutes of MVPA daily	17.3	15.2	11.8	17,680	72.0	<0.001	70.9	<0.001	1.1*
Using a computer for 2 or more hours a day	34.0	43.2	52.2	17,680	401.8	<0.001	401.8	<0.001	0.0*
Weekly smoking	3.0	11.1	19.1	32,659	1448.4	<0.001	1448.4	<0.001	0.0*
Weekly alcohol consumption	5.1	11.4	18.1	32,659	903.5	<0.001	903.5	<0.001	0.0*
Having been drunk on 2 or more occasions	3.5	12.2	24.9	32,659	2178.2	<0.001	2151.4	<0.001	26.8*
Fighting three or more times in the last 12 months	18.9	16.9	12.6	17,881	68.9	<0.001	64.9	<0.001	4.0
Being a victim of bullying at school	23.0	18.8	11.3	17,881	222.1	<0.001	214.2	<0.001	7.9

[1] * значение разности меньше критического значения χ^2 (3,841) для d = 1

10.9% accordingly. Number of injuries that required medical intervention fell with age (46.0% among 11 year-old teenagers, 44.0%, among 13-year old, and 40.1%, among 15-year old ones).

Number of teenagers who ate breakfast, fruit and vegetables every day also tended to decrease with age while prevalence of low physical activity (less than 60 minutes of moderate-to-vigorous physical activity (MVPA) per day), on the contrary grew and a share of teenagers who had daily physical activity also went down (17.3% among 11-year old, 15.2%, among 13-year old, and 11.8%, among 15-year old teenagers). There was also a growth in number of teenagers who used a computer for 2 or more hours a day, 34.0%, 43.2%, and 52.2% respectively.

Behavioral patterns that could cause health risk (such as smoking or alcohol use) prevailed greater among 15-year old school children against 11-year old ones. With age, more teenagers smoked daily (3.0% among 11-year old, 11.1%, among 13-year old, and 198.1% among 15-year old ones) and drank alcohol every day (5.1%, 11.4%, and 18.1% accordingly). Number of those teenagers who have been drunk on 2 or more occasions also grew with age (3.5%, 12.2%, and 24.9% respectively).

Number of teenagers who have been involved in a physical fight at least three times in the last 12 months tended to decrease with age (18.9 %, 16.9%, and 12.6% accordingly) as well as number of teenagers who have been bullied at school (23.0 %, 18.8%, and 11.3% accordingly).

Gender differences

Gender differences are usually related to psychological traits and behavioral patterns that are formed within a culture on the basis of differences between sexes. Men and women tend to have their specific gender roles in a society. Culture makes for these or those differences in behavioral patterns between men and women becoming apparent as well as differences related to their social roles, responsibilities, and obligations. Gender inequality index introduced by the UN allows evaluating a correlation between gender inequality and health of population in various countries and regions. It reveals that population in countries where social inequality is higher tend to have poor health as regards both sexes; in other words, gender inequality causes damage to health of both young men and women [22].

To create efficient and targeted activities aimed at improving health and preventing diseases, it is necessary to have an insight into gender differences and similarities.

Girls had positive school experience more frequently than boys (Table 2) as they more often stated they "liked school a lot" (22.8% against 19.6%) and they had good or very good perceived school performance (56.3% against 51.1%). However, girls stated that they felt pressured by schoolwork more frequently than boys (32.4% and 29.4% accordingly).

Girls communicated with their friends via EC, with text messages, or contacted them in social media more frequently than boys.

Table 2

Gender peculiarities of health-influencing behavioral factors among Russian teenagers aged 11, 13, and 15

Parameters	Mean value (%)		N	$\chi^2_{d=1}$	p	f-test	Correlation
	boys	girls					
Liking school a lot	19.6	22.8	30,280	46.1	<0.001	0.04	insignificant
Good or very good perceived school performance	51.1	56.3	26,229	70.1	<0.001	0.05	insignificant
Feeling pressured by schoolwork	29.4	32.4	26,229	27.6	<0.001	0.03	insignificant
Communicate daily via EMC	42.7	58.3	21,513	525.3	<0.001	0.16	weak
social media	35.3	46.7	4,716	55.1	<0.001	0.11	weak
text messages	24.3	38.7	4,716	108.8	<0.001	0.15	weak
Fair or poor self-rated health	18.4	28.9	26,229	73.3	<0.001	0.05	insignificant
Multiple health complaints	27.3	40.6	26,229	514.0	<0.001	0.14	weak
High life satisfaction	82.1	78.8	26,229	46.2	<0.001	0.04	insignificant
Injuries required medical intervention	49.1	37.7	26,229	346.2	<0.001	0.12	weak
Overweight	17.9	9.6	18,128	268.8	<0.001	0.12	weak
Feeling too fat	11.8	18.9	26,229	254.9	<0.001	0.09	insignificant
Engaged in weight-reduction behaviour	6.9	17.3	26,229	680.9	<0.001	0.16	weak
Eating breakfast every day	66.3	59.1	26,229	147.2	<0.001	0.08	insignificant
Oral health	47.0	64.9	34,339	1114.0	<0.001	0.18	weak
60 minutes of MVPA daily	18.4	11.1	18,128	195.0	<0.001	0.10	weak
Playing games on a computer or games console for 2 or more hours a day	53.7	32.4	18,128	832.7	<0.001	0.21	weak
Weekly smoking	12.7	9.4	34,339	96.4	<0.001	0.05	insignificant
Weekly alcohol consumption	13.7	9.4	34,339	154.9	<0.001	0.07	insignificant
Having been drunk on 2 or more occasions	15.5	13.4	34,339	29.3	<0.001	0.03	insignificant
Fighting three or more times in the last 12 months	25.0	7.3	26,229	1565.0	<0.001	0.24	weak
Bulling others at school	20.6	12.8	26,229	292.1	<0.001	0.11	weak

More girls than boys estimated their health as poor (28.9% against 18.4% accordingly); girls tended to complain about their health more frequently (40.6% against 27.3%) and were less satisfied with their life (78.8% against 82.1% accordingly).

Injuries that required medical intervention more frequently occurred among boys than girls (49.1% against 37.7 % respectively).

More boys than girls tended to have overweight or obesity (17.9% against 9.6% accordingly) but girls stated they was too fat (18.9% against 11.8%) and that they engaged in weight-reduction behaviour (17.3% against 6.9%) more frequently than boys.

A number of those who had breakfast every day was higher among boys than girls 66.3% against 59.1%). Girls stated that they brushed their teeth regularly (more than once a day) more frequently than boys (64.9% against 47.0%).

Boys were more physically active than girls (18.4% against 11.1%) but at the same time they much more frequently admitted playing games on a computer or games console for 2 or more hours a day (53.7% against 32.4%).

There were distinctive gender differences among Russian teenagers as regards behavioral patterns that were hazardous for their health as boys were more prone to risky behavior than girls.

Smoking, alcohol use, and drunkenness were more widely spread among 15-year old boys. Over the last decade there has been some leveling in traditional gender differences and gender convergence as regards certain risky behavioral patterns among 11 and 13-year old boys and girls as more 11- and 13-year old girls started to smoke, alcohol or cannabis use.

Boys took active part in fights (25.0 % against 7.3%) and have bulled other at school (20.6% against 12.8%) more often than girls.

Changes in prevalence of health-influencing behavioral factors taken in dynamics over several years

Most researchers are more interested in data that allow making some judgments on changes that happen over tine in their regions and countries; data on such changes helps them assess impacts exerted by health-improving activities in their country or a region.

There were some positive trends observed in 2013–2014 in comparison with previous years (Table 3); thus, we detected a growth in number of teenagers who liked school (15.5% in 2001 against 27.5% in 2013), who reported good or very good perceived school performance (32.2% in 2005 against 28.0% in 2013); there was a decrease in number of children who thought their health was poor (31.7% in 2001 against 17.0% in 2013) and who complained about their health (37.0% in 2005 against 32.7 in 2013); only 76.3% were satisfied with their life in 2001 but in 2013 their number increased up to 82.2%.

But still, we detected certain negative trends in 2013–2014 in comparison with 2005–2006. Thus, there was a growth in traumatism among 15-year old girls (35.3% in 2005 against 39.7% in 2013); more teenagers had overweight and obesity (9.8% in 2005 against 19.7% in 2013) and thought they were “too fat” (10.8% in 2005 against 26.3% in 2013).

There are also some other positive trends as regards health behaviour such as an increase in number of schoolchildren who eat fruit every day (26.8% in 2001 against 35.7% in 2013) and decrease in number of those who consumed soft drinks daily (26.2% in 2005 against 10.8% in 2013) and who watched TV for 2 or more hours a day (73.0% in 2005 against 60.0% in 2013).

In 2013–2014 fewer schoolchildren had breakfast on weekdays than in previous years (61.2% in 2005 against 59.3% in 2013) or brushed their teeth more than once a day (63.7% in 1997 against 57.3% in 2013). There was a considerable growth in number of children who used a computer for 2 or more hours a day at a PC (19.8% in 2005 against 57.7% in 2013).

We detected some positive trends as regards risky behavior; thus a number of children who smoked every week went down in 2013 in comparison with 2013 (14.7% against 8.7% accordingly); fewer teenagers drank alcohol every week (16.7% in 2005 against 6.2% in 2013); there was also a decrease in number of teenagers who had been drunk 2 or

Table 3

Trends in health-influencing behavioral factors taken in dynamics over years among Russian teenagers aged 11, 13, and 15

Parameters	Mean value (%)						N	χ^2	d	p	$\chi^2_{\text{l.t.}}$ d=1	p	$\chi^2_2 - \chi^2_{\text{l.t.}}$	Linear trend revealed
	1993	1997	2001	2005	2009	2013								
Liking school a lot	–	19.3	15.5	19.3	24.0	27.5	30,274	306.5	3	<0.001	306.0	<0.001	0.5	2001–2013
Feeling pressured by schoolwork	–	–	30.8	32.2	31.2	28.0	26,223	25.0	2	<0.001	22.9	<0.001	2.1	2005–2013
Fair or poor self-rated health	–	–	31.7	26.3	19.7	17.0	26,223	438.7	3	<0.001	429.9	<0.001	8.8	–
Multiple health complaints	–	–	31.7	37.0	34.5	32.7	26,223	26.0	2	<0.001	25.8	<0.001	0.2	2005–2013
High life satisfaction	–	–	76.3	79.5	82.3	82.2	26,223	70.5	2	<0.001	70.4	<0.001	0.1	2001–2009
Overweight	–	–	–	9.8	11.7	19.7	18,122	267.4	2	<0.001	237.2	<0.001	30.2	–
Feeling too fat	–	–	9.3	10.8	15.0	26.3	26,223	816.4	3	<0.001	692.7	<0.001	123.7	–
Injuries required medical intervention*[2]	–	–	36.3	35.3	39.3	39.7	13,902	17.6	2	<0.001	15.0	<0.001	2.6	2005–2013
Eating breakfast every day	–	–	68.7	61.2	61.7	59.3	26,223	154.5	3	<0.001	111.8	<0.001	42.7	–
Daily fruit consumption	–	–	26.8	29.3	33.3	35.7	26,223	135.6	3	<0.001	134.1	<0.001	1.5	2001–2013
Daily consumption of soft drink	–	–	21.7	26.2	23.0	10.8	26,223	433.7	2	<0.001	390.0	<0.001	43.7	–
Oral health	38.3	63.7	61.3	60.7	57.3	54.3	34,333	107.4	4	<0.001	101.1	<0.001	6.3	1997–2013
Watching TV for 2 or more hours a day	–	–	–	73.0	67.0	60.0	18,122	234.7	2	<0.001	234.3	<0.001	0.4	2005–2013
Using a computer for 2 or more hours a day	–	–	–	19.8	51.8	57.7	18,122	2,324.1	2	<0.001	2,063.6	<0.001	260.5	–
Weekly smoking	10.1	11.4	12.4	14.7	9.2	8.7	34,333	61.2 145.4	3 2	<0.001 <0.001	58.9 123.1	<0.001 <0.001	2.3 22.3	1993–2005– 2005–2013
Weekly alcohol consumption	9.8	13.5	14.7	16.7	8.3	6.2	34,333	106.5 396.9	3 2	<0.001 <0.001	102.1 363.2	<0.001 <0.001	4.3 33.7	1993–2005– 2005–2013
Having been drunk on 2 or more occasions	8.0	13.3	19.3	20.5	13.3	6.7	34,333	376.4 467.7	3 2	<0.001 <0.001	345.2 467.5	<0.001 <0.001	31.2 0.2	–1993–2005 2005–2013
Fighting three or more times in the last 12 months	–	–	18.3	18.3	15.2	12.7	26,223	75.2	2	<0.001	74.9	<0.001	0.3	2005–2013
Being a victim of bullying at school	–	–	17.7	16.3	17.8	18.8	26,223	13.8	2	<0.001	13.7	<0.001	0.1	2005–2013
Bulling others at school *[2]	–	–	11.3	13.0	13.0	13.3	13,902	8.6	3	0.03	6.1	0.013	2.5	2001–2013

[2] Data are given for girls.

more times in their life (20.5% in 2005 against 6.7% in 2013) and in a number of fights with teenagers' participation (18.3% in 2005 against 12.7% in 2013).

But still there were some negative trends in 2013–2014; thus there was a increase in number of teenagers who have been bullied at school (16.3% in 2005 against 18.8% in 2013) and in number of girls who have bullied other children at school (11.3% in 2001 against 13.3% in 2013).

Differences in parameters revealed in the RF and other countries

There are always differences in health and their social determinants revealed between different countries. Since HBSC was first introduced, it has become quite possible to reveal peculiarities related to health and lifestyle within a context determined by political and economical changes. Health parameters detected for children in the RF are worse than those detected for children in other countries.

Russian children aged 11 and 13 stated they liked school or thought their school performance were good less frequently than their peers in other countries; most teenagers aged 11, 13, and 15 didn't think their classmates were kind and helpful; they preferred to communicate with their friends via EMC more frequently than personally (table 4).

Russian schoolchildren more frequently thought their health was poor and were less satisfied with their life; injuries required medical intervention prevailed among 11- and 15-year-old teenagers. However, Russian teenagers suffered from overweight and obesity less frequently than their foreign peers; they also didn't think they were "too fat" and engaged in weight-reduction behaviour as often as schoolchildren in other countries.

Fewer Russian teenagers ate fruit every day (11- and 13-year-old ones), brushed their teeth more than once a day (11- and 15-year-old ones), or had sufficient physical activity

Table 4

Situation in the RF in comparison with other countries that take part in HBSC

Parameter	Situation in the RF against other countries	Age groups or years
Liking school a lot	unfavorable	11,13
Good or very good perceived school performance	unfavorable	11,13
Classmates are kind and helpful	unfavorable	11–15
Communicate daily via EMC	unfavorable	11–15
Fair or poor self-rated health	unfavorable	11–15
High life satisfaction	unfavorable	11–15
Injuries required medical intervention	unfavorable	11,15
Overweight	favorable	13,15
Feeling too fat	favorable	11–15
Engaged in weight-reduction behaviour	intermediate	11–15
Eating breakfast every day	intermediate	11–15
Daily fruit consumption	unfavorable	11,13
Daily sweets consumption	unfavorable	2013; 11,13
Daily consumption of soft drink	favorable	2013; 11,13
Oral health	unfavorable	2009–2013; 11–15
60 minutes of MVPA daily	unfavorable	11
Using a computer for 2 or more hours a day	unfavorable	2013; 11–15
Playing games on a computer or games console for 2 or more hours a day	unfavorable	11
Weekly smoking	unfavorable	11,13
Weekly alcohol consumption (beer, wine, or alcopops)	unfavorable	13
Having been drunk on 2 or more occasions	favorable	15
Fighting three or more times in the last 12 months	unfavorable	11–15
Being a victim of bullying at school	unfavorable	11–15
Bulling others at school	unfavorable	11–15

(11-year-old ones) than their foreign peers. They also ate sweets (11- and 13-year-old teenagers) and used a computer and watched TV more often than schoolchildren in other countries.

Risky behavior patterns were more widely spread among younger Russian teenagers; thus 11-and 13-year-old teenagers smoked more than their foreign peers; 13-year-old ones use alcohol (beer, wine, or alcopops) more frequently. Russian schoolchildren were much more aggressive than their peers from European or North American countries as it was confirmed by greater prevalence of fights and bulling among Russian teenagers.

Conclusions

Any activities aimed at improving health and providing welfare of teenage boys and girls should be developed taking into account differences between age groups. Greater efforts should be made to create stimuli to pursue healthy lifestyle among younger teenagers. A school is the most relevant place to implement such activates as it allows combining knowledge and skills of teachers and medical personnel[3] [23, 24].

Detected differences indicate that health-improving and preventing strategies should be developed separately for boys and girls. A lot of risky behavioral patterns are more widely spread among boys; therefore, preventive activities should be more focused on them. It is necessary to be very attentive to any potential increase in risky behavioral patterns among girls that can be caused by gender convergence.

Over the last decade some positive trends have been revealed as regards prevalence of behavioral risk factors; however, when compared with their peers from other countries, Russian teenagers have more negative attitudes towards their school environment, more frequently think their health is poor, they consume less fruit, pay less attention to oral health, and are not so physically active. Risky behavioral patterns (smoking, alcohol use, and aggression) are more widely spread among younger Russian teenagers than their foreign peers.

When creating programs aimed at stimulating healthy lifestyle, one should take into account age and gender differences, as well as trends related to behavioral risk factors that can change over time and that can be objectively assessed with "Health Behaviour in School-Aged Children" questionnaire.

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SCIENTIFIC AND METHODOLOGICAL ASPECTS OF LABPRATORY SUPPORT AIMED AT PROVIDING CHEMICAL SAFETY DURING INTERNAITONAL MASS EVENTS

A.Yu. Popova[1], N.V. Zaitseva[2], G.V. Karpushchenko[3]

[1]Russian Medical Academy of Postgraduate Education, 2/1 Barrikadnaya Str., Moscow, 123995, Russian Federation
[2]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation
[3]Hygiene and Epidemiology Center in Rostov region, 67 7th line Str., Rostov-on-Don, 344019, Russian Federation

International legal acts that regulate organizing and carrying out various mass events in our country impose an obligation on the RF to provide safety, including sanitary-epidemiologic ones, for their guests and participants. Tight schedules and short time span are peculiarities related to such events and, when it comes to hygienic aspects, it determines short-term exposure of guests and participants to environmental factors. Nowadays in Russia substantial experience has been accumulated in providing hygienic safety for population during international mass events.

Our research goal was to determine priority tasks in laboratory and information-analytical support for Rospotrebnadzor activities aimed at solving issues related hygienic safety of international mass events at various stages in preparation to them and during them depending on their scale and social significance.

This paper focuses on determining a necessity to work out a model for laboratory support aimed at providing chemical safety during international mass events as regards assessing health risks for participants and guests attending such events; the model is to involve implementing laboratory control over biological markers. The authors determine basic principles of the state policy in the sphere of providing hygienic safety, reasons for threats and health risks occurrence during international mass events. The present research allowed to reveal it was advisory to implement a system for biological control over markers related to impacts exerted by various chemicals on personnel involved into preparation and organization of international mass events.

Key words: *international event, mass event, hygienic safety, laboratory support, information and analytical support, sanitary-epidemiologic welfare, state regulation, safe environment.*

According to Clause 15, Part 4, of the RF Constitution, generally accepted principles and norms of the international law as well as international treaties signed by the RF are an integral part of the legal system in the country. If an international treaty signed by the RF fixes rules that are different from those fixed in the national legislation, than it is these rules that are to be applied.

International legal acts that determine organization of various global mass events on the RF territory commit The Russian Federation to provide their safety, including sanitary-epidemiologic welfare of their guests and participants [1].

World practice in organizing and holding global mass events[1] proves that greater attention is being paid now to issues related to hygienic safety provision [1–5].

Anna Yu. Popova – Doctor of Medicine, Professor, Head of Department of organization of sanitary and epidemiological service (e-mail: rmapo@rmapo.ru, tel.: 8 (499) 458-95-63; ORCID: https://orcid.org/0000-0002-4315-5307).

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

Garri V. Karpushchenko – Candidate of Medical Sciences, Chief Physician (e-mail: master@donses.ru; tel.: +7 (863) 251-04-92); https://orcid.org/0000-0003-4672-8753).

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At present there is an integrated system that has been created in the RF and significant experience has been accumulated in the sphere of providing hygienic safety of the population, including global mass events [3–5, 7, 9]. Hygienic safety is determined by state authorities being well-prepared to prevent threats and risks caused by chemical or physical impacts and related to a global mass event[1] [10].

There are some basic principles of the state policy aimed at providing hygienic safety of the population. They are:

– a person's right to live in a safe environment;

– absence of unacceptable health risks related to conditions and factors of a global mass event;

– adherence to generally accepted international legal norms in the sphere of providing hygienic safety;

– coordinated interaction and mutual responsibility borne by all the structures that are to provide hygienic safety of a global mass event being held;

– providing information about risks to executive authorities and officials who are responsible for making managerial decisions;

– relevant state regulation of threats and risks for hygienic safety ;

– setting tasks solution to which will enable determining conceptual and methodical grounds for laboratory and informational-analytical support necessary to provide hygienic safety during glopal mass events [1].

Our research goal was to determine priority tasks which were to be solved to provide necessary laboratory and information-analytical support for Rospotrebnadzor's bodies and their activities aimed at securing hygienic safety of global mass events at different stages including preparation to them depending on their scale and social significance.

Hygienic safety includes a chemical and a physical aspect and their combination is an integral system but it is scientifically reasonable to explore them separately.

According to the RF President Order No. 202 dated May 09, 2017 all the enterprises that were located on territories where FIFA 2018 World Cup events were taking place and that dealt with hazardous productions or applied chemical substances or toxicants had to suspend their activities until these event were over[2].

Experience accumulated in the sphere of providing chemical safety of the population, including global mass events, allowed adjusting «The Basics of the state policy in chemical safety sphere approved by the RF President on November 1, 2013». The RF President Order No. 97 dated March 11, 2019, approved «The Basics of the RF state policy in the sphere of providing chemical and biological safety up to 2025 and beyond»[3].

The above-mentioned legal act fixes the following primary threats:

– spread and (or) application of chemical weapons, terrorists attacks that involve use of potentially hazardous chemicals;

– growing globalization of the world trade and persisting probability that potentially hazardous chemicals or products made out of them can be imported onto the RF territory;

– use of technologies that can't provide proper chemical safety;

[2] The RF President Order No. 202 dated May 09, 2017. On peculiarities related to implementing reinforced security measures for a period during which FIFA 2018 World Cup and FIFA 2017 Confederation Cup events are to take place in the RF [Web-source] // KonsultantPlus. – URL: http:www.consultant.ru/cons_doc (date of visit May 20, 2019).

[3] The RF President Order No. 97 dated March 11, 2019 [Web-source] // KonsultantPlus. – URL: http:www.consultant.ru/cons_doc (date of visit April 18, 2019).

– growing number of organizations that manufacture chemical products, substantial growth in volumes of chemical wastes, absence of efficient technologies regarding chemically dangerous wastes processing and reclamation of contaminated territories;

– accidents at chemical objects caused by critical wear and tear of equipment, technological processes becoming more complicated, and production personnel not being sufficiently qualified;

– a great number of potentially hazardous chemical objects that are either no longer exploited or their technical and technological resources are close to their limits or completely exhausted as well as territories that have been contaminated due to economic activities;

– development and manufacture of completely new chemicals that produce their effects on people and the environment but these effects haven't been studied in depth;

– wide use of highly toxic chemicals and accumulation of hazardous chemically stable compounds in the environment.

To eliminate or minimize all the above-mentioned threats as much as it was only possible was one of the basic tasks during global mass events that were held in the RF; to solve it, it was necessary to determine primary causes of threats and health risks for those who visited a global mass event or participated in it [9].

We highlighted the following primary causes for threats and health risks among the overall pool:

– activities performed by economic entities that belonged to 1–4 hazard category as per possible damage to health in case obligatory requirements fixed in the sanitary legislation were violated;

– terroristic threats;

– high population density within a limited space;

– high probability of vast crowds and them coming out of control in case a chemical agent occurs;

– limited possibility to provide control due to participants of an event being diverse;

– limited number of points where access to vital resources could be provided;

– logistics and transportation schemes failure;

– ecologically unstable technogenic objects and hazardous environmental objects that are located in the close proximity to a place where a global mass event is held.

Each threat out of the above-mentioned ones required the following:

– substantiating an algorithm that included actions and implementation mechanisms to be applied during multi-sectoral and multi-level interaction between Rospotrebnadzor's bodies and organizations and other executive authorities that took part in organizing a global mass event;

– implementing a set of risk-oriented control and surveillance activities concerning economic entities that belonged to 1–4 hazard categories as per potential health risks; results of this implementation were to be applied to divide a territory on which an event was organized into specific zones;

– creating a list of priority health risk factors that were subject to obligatory dynamic and (or) operative laboratory control at all stages during a global mass event;

– optimizing points and programs of social-hygienic monitoring taking into account number of people who participated in an event and ran a risk of exposure to chemical factors;

– indicating threats and quantitatively assessing current and predicted risks for sanitary-epidemiologic welfare at all stages during preparation and organization of an event as well as event itself;

– providing information support and functioning of a multi-level system for information exchange.

All the above-mentioned tasks were successfully completed when local control and surveillance activities were performed during global mass events that were organized and held in the RF. It is confirmed by sanitary-epidemiologic welfare at such events being highly estimated by experts worldwide.

But still there were some difficulties, mostly concerning laboratory support for hygienic safety provision due to absence of unified approaches to solving issues related to screening laboratory research and tests [8].

Global mass events have a peculiarity, that is, they happen during a rather short period of time and from a hygienic point of view it means that guests and participants of such events are under short-term exposure to environmental factors. And as crowds of people gather in a very limited space, it imposes greater obligations to provide hygienic safety on an area where such an event takes place.

Insufficient laboratory control over chemical factors can cause threats to health of people who visit an event or participate in it; probability of such threats should be determined depending on a scale or geopolitical significant of a global mass event.

Clause 32 of the Federal Law issued on March 30, 1999 No. 52-FZ obliges an economic entity to perform industrial control, including laboratory research and tests, in order to make its activities safe and (or) harmless for people and the environment[4].

Experts performed retrospective analysis of issues concerning laboratory support for hygienic safety provision that occurred during global mass events held in Russia. The analysis revealed a variety of factors exerting negative influence on quality and good timing of laboratory control, lowering its efficiency, and increasing expenses required to conduct it.

Problems exerting negative influence on laboratory support provided for global mass events were ranked and it enabled determining the most significant ones; thus, a leading factor was insufficient information-analytical support and a poorly structured information system that made it difficult to exchange information between different subjects in the process.

It is becoming even more a pressing issue to develop a basic concept of information-analytical support for laboratory control as terroristic threats that can possibly involve chemicals are growing and there can be newly created substances used in them that have not been stated openly and therefore can hardly be identified in environmental objects.

Laboratory control over chemical factors performed during preparation and organization of global mass events that had been already held revealed a low share of samples with adverse chemicals in concentrations higher than MPC. This can either be due to organizers and constructors having adhered to all the technological standards during preparation to and an event itself or due to insufficient laboratory control. Sometimes it is actually impossible to perform laboratory control during critical periods of preparation or an event itself and it is a grave issue that needs to be resolved.

Personnel employed at construction sites or dealing with repairing or preparing objects

[4] On sanitary-epidemiologic welfare of the population: The Federal Law No. 52-FZ issued on March 30, 1999 [Web-source] // KonsultantPlus. –URL: http:www.consultant.ru/cons_doc (date of visit May 14, 2019).

for a global mass event are exposed to all occurring chemical factors and exposure period for them is longer than a period of time during which an actual event takes place.

In this respect it seems advisable to implement a system for biological control over markers of impacts exerted by various chemicals on personnel involved into preparations to global mass events.

Results of the said biological monitoring will substantially increase authenticity of assessed health risks for guests and participants of a global mass event. Health risk assessment methodology that is officially recommended by global scientific society and officially applied in the RF suggests assessing a multi-factor risk as a sum of one-factor risks [7]. A suggested procedure for biological control provides a comprehensive solution to all the set tasks.

If this system is implemented, it will also allow making objects safer as regards possible terroristic threats involving chemicals; should any biological markers of effects produced by potentially hazardous chemicals be revealed among personnel involved in preparation and organization of an event, relevant authorities will have additional opportunities to perform prevention activities.

This model for laboratory control can be tested in 2020 when several global mass events are to take place in Russia such as UEFA Euro 2020 events in Saint Petersburg, AIBA World Boxing Championship in Yekaterinburg and Ufa in 2019, etc.

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METHODICAL APPROACHES TO MEDIUM-TERM PLANNING AND ASSESSING HEALTH RISK MANAGEMENT EFFICIENCY IN A MUNICIPAL ENTITY (BY THE EXAMPLE OF AN INDUSTRIALLY DEVELOPED CITY)

Yu.Ya. Barmin[1], V.B. Gurvich[2], S.V. Kuz'min[3], O.L. Malykh[4], T.M. Tsepilova[2], A.A. Shevchik[2], S.V. Yarushin[2]

[1]Administration of the Federal Supervision Service for Consumer's Rights Protection and Human Wellbeing in the Sverdlovsk Region, 86 Oktyabr'skoi revolyutsii Str., Nizhnii Tagil, 622036, Russian Federation
[2]Ekaterinburg Medical Research Center for Prophylaxis and Health Care in Industrial Workers, 30 Popova Str., Ekaterinburg, 620014, Russian Federation
[3]Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, 18/5 and 7 Vadkovskiy pereulok, Moscow, 127994, Russian Federation
[4]Hygienic and Epidemiological Center in in Sverdlovsk region, 3 Otdel'nyi pereulok, Ekaterinburg, 620078, Russian Federation

A system of activities aimed at managing health risks caused by environmental factors has been created and successfully implemented in Sverdlovsk region. The system covers risks detected as per social-hygienic monitoring results. Priority tasks and measures that should allow risk management experts to provide sanitary-epidemiologic safety and to manage health risks are annually substantiated basing on systemic analysis and risk assessment.

Health risk management system in municipal entities is a key component in a unified regional system for providing sanitary-epidemiologic welfare and protecting population health taking into account peculiarities related to a sanitary-epidemiologic situation in a specific municipal entity. Such risk management systems should be created and developed basing on unified goals, tasks, and parameters for assessing activities performed by all the participants in risk management processes as well as on applying a unified information and analytical database containing social and hygienic monitoring data as such a database can ensure appropriate management of risks and threats to population health. The paper dwells on methodical approaches, basic results, and scientific and practical experience in medium-term planning and assessing efficiency of activities performed to manage population health risks in an industrially developed city in Sverdlovsk region. The authors also give recommendations and set tasks which are to be solved in order to create and develop municipal systems for managing population health risks.

Key words: *population health risk management, municipal entity, planning, efficiency assessment, epidemiologic welfare, social-hygienic monitoring, health protection, Sverdlovsk region.*

Population health risk management is a multi-level system that comprises various risk management subjects such as regional and municipal authorities, economic entities, state surveillance and municipal control bodies, and population; risk management techniques such as risk prevention, decrease, compensation, risk communication and informing about risks; risk management objects such as risk factors, territories, surveillance objects, and population groups. Its functioning is aimed at achieving socially significant results, namely, providing

Yurii Ya. Barmin – Head (e-mail: barma@e1.ru; tel.: +7 (343) 525-45-70; ORCID: https://orcid.org/0000-0002-2766-6676).

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

Sergei V. Kuz'min – Head of the Board, Doctor of Medical Sciences, Professor (e-mail: kuzmin_sv@rospotrebnadzor.ru; tel.: +7 (499) 973-26-90; ORCID: http: //orcid.org/0000-0002-9119-7974).

Ol'ga L. Malykh – Candidate of Medical Sciences, Head of the department (e-mail: malykh_ol@66.rospotrebnadzor.ru; tel.: +7 (343) 362-86-15, +7 (902) 263-57-27; ORCID: https: //orcid.org/0000-0001-8394-627X).

Tat'yana M. Tsepilova – Junior researcher (e-mail: zaikinatm@inbox.ru; tel.: +7 (343) 312-87-56; ORCID: https: //orcid.org/0000-0002-1678-3180).

Anastasiya A. Shevchik – Researcher (e-mail: shevchik@ymrc.ru; tel.: +7 (343) 312-87-56; ORCID: https: //orcid.org/0000-0002-4380-1575).

Sergei V. Yarushin – Head of the laboratory (e-mail: sergeyy@ymrc.ru; tel.: +7 (343) 312-87-56; ORCID: http: //orcid.org/0000-0001-8215-9944).

sanitary-epidemiologic welfare and protecting population health.

Health risk management system in a municipal entity is a key component in a unified regional system for providing sanitary-epidemiologic welfare and protecting population health in RF regions. Its goal is to solve priority problems in a region taking into account a specific sanitary-epidemiologic situation in a municipal entity. Such a system should be created basing on unified goals, tasks, and parameters for assessing efficiency of activities for all risk management subjects as well as on applying a unified information and analytical database containing social and hygienic monitoring data collected in a specific RF region. Social and hygienic monitoring is to provide adequate and timely management of risks and threads for population health.

Urgency to create and develop health risk management systems in municipal entities is determined by a necessity to create and apply approaches, methods, and technologies that allow solving two interrelated tasks [1]:

– to optimize budgetary and program-targeted middle-term planning of activities performed by local authorities in a municipal entity and aimed at providing sanitary-epidemiologic welfare of population;

– to predict economic efficiency and productivity of activities performed by local authorities in a municipal entity and aimed at achieving key medical and demographic parameters that are socially significant for municipal entity development (increase in life expectancy, natural population growth, decrease in overall mortality and mortality among employable population, decrease in overall morbidity and morbidity among children).

There was a scientific and practical work performed in 2015–2017 by Yekaterinburg Medical Scientific Center for Prevention and Health Protection for Workers Employed at Industrial Enterprises together with Rospotrebnadzor's Regional Office in Sverdlovsk region and Sverdlovsk Regional Center for Hygiene and Epidemiology. The work dwelled on creating and testing above-mentioned methodological approaches; it had been requested by municipal authorities of an industrially developed city in Sverdlovsk region in order to help them improve demographic, ecological, and sanitary-epidemiologic situation in it.

Also we applied results of some other research that mostly focused on testing and implementing health risk assessment and management methodology (including issues related to risk assessment, management, and communication as well as monitoring and control) and research in human social biology accomplished under supervision and with participating of RAS Academician B.T. Velichkovskiy.

Data and methods. Techniques applied for planning and analyzing health risk management activities performed by local authorities in a municipal entity are based on multi-criteria assessment that usually comprises a set of parameters describing sanitary-epidemiologic welfare and population health. We applied unified scenario-based approaches to planning and economic tools for assessing efficiency and productivity of health risk management activities based on «cost – effectiveness» and «costs – benefits» techniques recommended by MG 5.1.0030-11 «Methodical guidelines on economic evaluation and substantiation of decisions on health risk management under exposure to environmental factors»[1].

Health risk management activities aimed at providing sanitary-epidemiologic welfare and improving medical and demographic situation in a municipal entity were analyzed, assessed, substantiated, predicated and planned for a middle term within social and hygienic monitoring performed in a specific RF region.

An algorithm for middle-term planning of health risk management activities in a municipal entity is given in Figure 1. Its performance comprises interrelated stages.

[1] MG 5.1.0030-11. Methodical guidelines on economic evaluation and substantiation of decisions on health risk management under exposure to environmental factors [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/1200088393 (date of visit January 03, 2019).

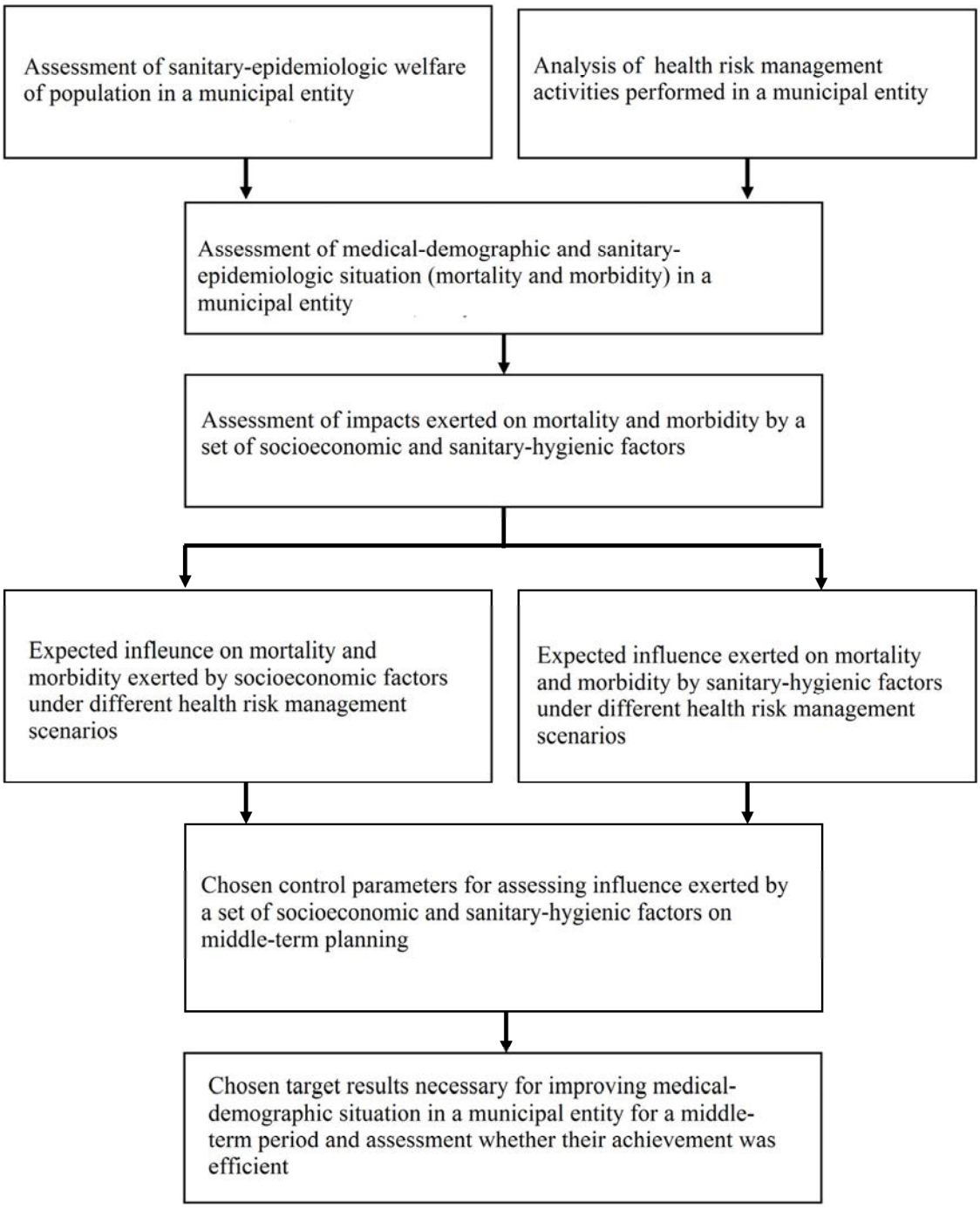

Figure 1. An algorithm for middle-term planning and assessing efficiency of health risk management activities in a municipal entity

Stage 1 involves analyzing medical-demographic situation, sanitary-epidemiologic welfare and performed health risk management activities in a municipal entity.

An information and analytical database that is applied to assess sanitary-epidemiologic welfare in a municipal entity is based on regional information social-hygienic monitoring funds, annual reports "On sanitary-epidemiologic welfare of the population in a specific RF region", reports (or information bulletins) "On sanitary-epidemiologic welfare of the population in a municipal entity" or sanitary-epidemiologic profiles created for specific municipal entities over a recommended period not shorter than five years [2]. The research that

we consider here focused on analyzing sanitary-epidemiologic situation and its influence on basic medical-demographic parameters (life expectancy, mortality, morbidity, and natural population growth) performed with data collected in 2004-2014. That analysis included medical-demographic parameters (19 overall, including mortality and morbidity); 27 socioeconomic parameters; 29 sanitary-hygienic parameters; 6 socially significant parameters that allowed assessing whether health risk management activities were efficient.

Analysis of medical-demographic situation and sanitary-epidemiologic welfare revealed priority issues related to providing sanitary-epidemiologic welfare that were common for all municipal entities in any RF region as well as some issues that were specific for that particular municipal entity.

Efficiency of health risk management activities has been assessed in Sverdlovsk region since 2008 as per basic socially significant parameters (mortality, morbidity, natural population growth, and life expectancy) [1]; assessment results provided some additional data for analyzing and assessing situation in the chosen municipal entity. Analysis revealed existing problems related to functioning and development of health risk management system in the municipal entity; it also allowed comparative assessment of its efficiency against systems existing in other similar municipal entities in the given RF region.

Results obtained at Stage 1 in the middle-term planning algorithm give grounds for assessing influence exerted by environmental factors on medical and demographic parameters (mortality and morbidity) in a municipal entity.

Stage 2 involved assessing influence exerted by socioeconomic and sanitary-hygienic factors on medical and demographic parameters (mortality and morbidity) in a municipal entity.

At stage 2 in middle-term planning algorithm we analyzed dynamics of population mortality (overall one and among employable population) and morbidity (among overall population and among children), what causes led to mortality and morbidity cases, as well as dynamics and causes for annual growth (decrease) in these parameters over a period not shorter than 10 years [3]. We also analyzed dynamics of parameters that described socioeconomic factors (social welfare, social tension, and economic and industrial development) and changes in annual growth (decrease) in parameters of socioeconomic development in a municipal entity over e period of time not shorter than five years.

We analyzed changes (annual growth or decrease) in parameters related to sanitary-hygienic factors (ambient air quality, soil and drinking water quality, quality and safety of food products, learning and educational conditions for children, working conditions, incidence with infectious and parasitic diseases, injuries and intoxications); also, as deep as it was possible, we analyzed lifestyle parameters (smoking, alcohol intake, drug addiction, physical activities, etc.) [4].

We assessed impacts exerted on mortality and morbidity by a set of socioeconomic and sanitary-hygienic factors mostly with health risk assessment methodology and human social biology techniques[2] [5–8]. We also applied statistic and mathematical techniques for assessing health risks, expert evaluations and other techniques recommended by the RF State Standard GOST R ISO/MEK 31010-2011 «Risk management. Risk assessment techniques»[3].

So, we assessed impacts exerted by socioeconomic and sanitary-hygienic factors on medical and demographic parameters (mortality and morbidity) in a municipal entity and it allowed substantiating and creating statistic and (or) other quantitative or semi-quantitative models for assessing such impacts with a possibility to predict them depending on changes in parameters related to socioeconomic and sanitary-hygienic environmental factors [1].

[2] G 2.1.10.1920-04. Guidelines on assessment of population health risk under exposure to chemicals which pollute environment [Web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/1200037399 (date of visit January 04, 2019).

[3] GOST R. ISO/MEK 31010-2011. Risk management, Risk assessment techniques [Web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/gost-r-iso-mek-31010-2011 (date of visit January 04, 2019).

Stage 3 involved predicting effects that could be produced by socioeconomic and sanitary-hygienic factors on mortality and morbidity under different health risk management scenarios in a municipal entity.

When predicting impacts exerted by environmental factors on population health, we created several scenarios for managing population health risks. Basically, we considered an optimistic, realistic, and a pessimistic scenario. A specific health risk management scenario is a set of activities aimed at solving priority issues related to medical and demographic situation and sanitary-epidemiologic welfare in a municipal entity; these activities are coordinated as per their place, time, funding, as well as personnel, organizational, and regulatory-methodical provision necessary for their implementation [9]. A middle-term health risk management scenario should be implemented over a period of time not shorter than 5 years.

Middle-term prediction of impacts exerted by socioeconomic factors on mortality and morbidity among population took into account both data on predicted socioeconomic development of a specific municipal entity as per all the parameters that described social welfare, social tension, and economic and industrial development, and the same data on a region as a whole where that municipal entity was located. When creating health risk management scenarios depending on changes in socioeconomic factors, we applied results and opportunities granted by cyclic economic development models; in particular, we applied Kitchin and Juglar cycles for middle-term planning to create an optimistic, realistic, and pessimistic scenario [10].

Middle-term prediction of impacts exerted by sanitary-hygienic factors on mortality and morbidity among population took into account predicted changes in ambient air quality, soils and drinking water quality, learning and educational conditions for children, working conditions, incidence with infectious and parasitic diseases, injuries and intoxications, as well as overall development of health risk management system in a municipal entity [2]. When creating an optimistic, realistic, and pessimistic health risk management scenario depending on changes in sanitary-epidemiologic parameters, we applied evaluations given by experts who dealt with providing sanitary-epidemiologic welfare, ecological safety and health protection as per Delphi method and risk indexes technique[3].

After Stage 3 in the middle-term planning algorithm had been completed, we predicted sanitary-epidemiologic welfare and population health (as per population mortality and morbidity) depending on combined effects produced by socioeconomic and sanitary-hygienic factors as well as factors related to lifestyle.

Stage 4 involved creating a middle-term program for providing sanitary-epidemiologic welfare and health risk management in a municipal entity.

When creating the above-mentioned program, we considered all three variants based on an optimistic, realistic, and pessimistic health risk management scenario. To substantiate and correctly choose a program for providing sanitary-epidemiologic welfare and health risk management, we determined control parameters for assessing program implementation. We also outlined what target socially significant results could be applied to assess an expected effect which was a decrease in impacts exerted by socioeconomic and sanitary-hygienic factors on health as well as improved medical and demographic situation in a middle-term period of time as per all the priority problems revealed at Stages 1 and 2 in the algorithm for middle-term planning of activities aimed at managing health risks in a municipal entity [11].

A middle-term program aimed at providing sanitary-epidemiologic welfare and health risk management in a municipal entity was created for a period up to 2020. It outlined the following:

– a sphere significant for providing sanitary-epidemiologic welfare in which a suggested activity was to be performed;

– a task to be completed to provide solution to priority issues related to sanitary-epidemiologic welfare which could be achieved with a suggested activity;

– an activity itself, necessary funding and its sources, terms and personnel responsible for its implementation;

– control parameters and target results that can be applied to assess efficiency and productivity of an activity as well as people responsible for control over its implementation.

We substantiated a choice on a variant of middle-term program for approval and implementation by local authorities in a municipal entity and assessment how efficient its implementation was for an intermediate period up to 2020 in conformity with MG 5.1.0030-11[1]. We performed basic evaluations that described «costs – efficiency» and «costs – benefits» relations for various program scenarios. We also estimated minimal funds necessary for achieving target socially significant results via predicting prevented economic losses related to population health (decrease in losses of gross regional product).

Results. We analyzed effects produced by sanitary-hygienic situation on medical and demographic parameters in an industrially developed city within human social biology, in particular, taking into account considerable influence exerted by socio-economic factors (first of all, purchasing capacity and material motivation to work) on sanitary-hygienic factors and, accordingly, their cumulative effects produced on population health directly or via impacts exerted by sanitary-hygienic factors[4].

Over 2010–2014 first there was a stagnation and then a drastic deterioration of primary medical and demographic parameters both in Sverdlovsk region and, to a much greater extent, in the examined industrially developed city. Thus, population mortality among overall population reached 15.1 cases per 1,000 people in the city while in Sverdlovsk region it amounted to 14.0 cases (Figure 2). Mortality grew by more than 13.5% since 2012 in the city and it was considerably higher the same parameter in Sverdlovsk region as a whole (almost 2 times higher).

Mortality among employable population grew by 14% and reached 6.5 cases per 1,000 people while the same parameter amounted to 6.3 cases in Sverdlovsk region as a whole (Figure 3).

There was a drastic deterioration in natural population growth. Population continued to grow in Sverdlovsk region (natural population growth stabilized at 0.5 per 1,000 people), but natural population loss continued to increase in the industrially developed city in Sverdlovsk region.

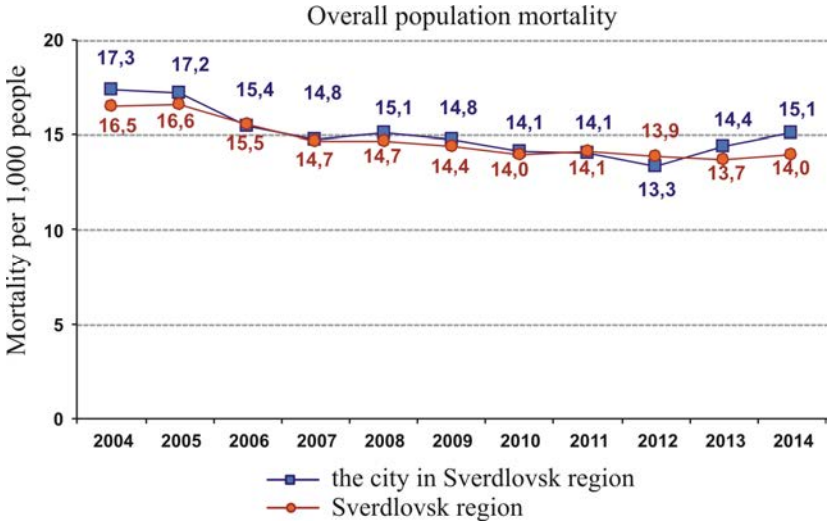

Figure 2. Dynamics of overall mortality in the city in Sverdlovsk region ad Sverdlovsk region as a whole (per 1,000 people) in 2004–2014

[4] V.B. Gurvich. Systemic approach to managing ecologically induced health risks on an example of aluminum-production enterprises: Doctor of medical sciences dissertation. – Saint Petersburg, 2008. – 345 p.

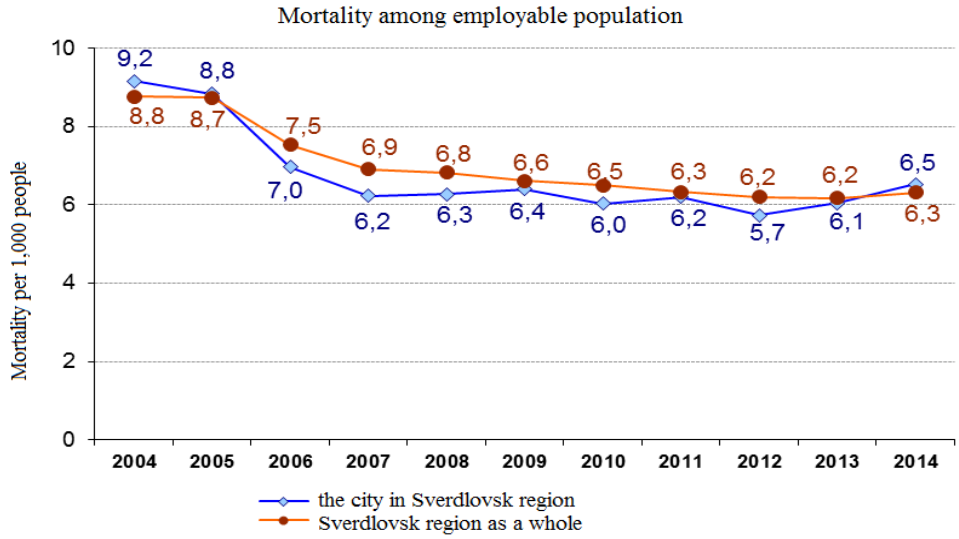

Figure 3. Dynamics of mortality among employable population in the city in Sverdlovsk region ad Sverdlovsk region as a whole (per 1,000 people) in 2004–2014

Table 1

Efficiency of health risk management activities in municipal entities located in Sverdlovsk region: comparative assessment, 2004–2014

No.	Municipal entity	Overall multi-criteria score	Rank place
1	Municipal entity No. 1	14	6
2	Municipal entity No. 2	23	4
3	Municipal entity No. 3	30	3
4	Municipal entity No. 4	22	5
5	Municipal entity No. 5	33	1
6	Municipal entity No. 6	31	2

Natural population loss amounted to –1.8 per 1,000 people in 2014 while it was 0.0 per 1,000 in 2012[5].

We comparatively assessed efficiency of health risk management activities in six municipal entities in Sverdlovsk region; the results are given in the Table where the examined industrial city is given as Municipal entity No. 1, other five entities being quite comparable to it. The higher total score as per multi-criteria assessment scale is the more efficient and productive is a health risk management system in a municipal entity.

Therefore, Municipal entity No. 1 has greater population loss and population mortality, both overall and among employable population, than in Sverdlovsk region in general and there is practically no trend for any improvement in these medical and demographic parameters. The system for managing sanitary-epidemiologic welfare and health risks that exists there can only be considered unsatisfactory and inefficient. This municipal entity takes the 6th rank place as per economic efficiency of health risk management activities among 6 municipal entities in Sverdlovsk region that are comparable in terms of population number, their economic and industrial development[6].

We applied Spearman correlation coefficients to assess impacts exerted by integral

[5] Human social biology. Experience in practical application: Methodical manual / B.T. Velichkovskiy, V.B. Gurvich, S.V, Yarushin, O.L. Malykh, E.A. Kuz'mina, T.M. Tsepilova, I.I. Oranskaya. – Yekaterinburg: Yekaterinburg Medical Scientific Center for Prevention and Health Protection for Workers Employed at Industrial Enterprises, 2017. – 81 p.

[6] The Concept for long-term social and economic development of the Russian Federation up to 2020 / Approved by the RF Government Order No. 1662-r Issued on November 17, 2008 [web-source] // KonsultantPlus. – URL: http://www.consultant.ru/document/cons_doc_LAW_82134/28c7f9e359e8af09d7244d8033c66928fa27e527/ (date of visit January 4, 2019).

Table 2

Pair Spearman correlation coefficients for socioeconomic and sanitary-hygienic factors and medical and demographic parameters in industrially developed city, 2004–2014

Parameters	Years	Spearman correlation coefficient	p-value
Overall population mortality			
An integral parameter for socioeconomic factors	11	0.76	0.007
An integral parameter for sanitary-hygienic factors	11	0.85	0.001
Overall population morbidity			
An integral parameter for socioeconomic factors	11	0.77	0.005
An integral parameter for sanitary-hygienic factors	11	0.89	0.000
Employable population mortality			
An integral parameter for socioeconomic factors	11	0.75	0.008
An integral parameter for sanitary-hygienic factors	11	0.73	0.011
Morbidity among children			
An integral parameter for socioeconomic factors	11	0.94	0.000
An integral parameter for sanitary-hygienic factors	11	0.90	0.000

socioeconomic and sanitary-hygienic parameters on population health in the examined city over 2004-2014; the results are given in Table 2.

All the obtained data are statistically significant and indicate there is significant influence exerted both by socioeconomic factors (Spearman correlation coefficient varies from 0.75 to 0.94) and by sanitary-hygienic factors[7] (Spearman correlation coefficient varies from 0.73 to 0.90) on primary medical and demographic parameters (overall population mortality, employable population mortality, overall population morbidity and morbidity among children). We applied created one-factor and multi-factor statistical models (based on integral parameters that described socioeconomic and sanitary-hygienic situation) to predict efficiency and productivity of planned scenario activities included into a program aimed at improving medical and demographic and sanitary-epidemiologic situation in the industrially developed city in Sverdlovsk region for e period up to 2020, together with experts evaluations as per Delphi method.

Data and information included into the analysis as well as prediction techniques were oriented at a period from 2015 to 2020. We performed our assessment as per an optimistic, realistic, and pessimistic health risk management scenario. When creating health risk management scenarios, we took socioeconomic development parameters in the industrially developed city in Sverdlovsk region as our basic ones; the parameters were assessed taking into account cyclic economic development models for Sverdlovsk region for a middle-term period. We applied Kitchin and Juglar cycles to create all three scenarios for socioeconomic development of a municipal entity. Socioeconomic factors that influenced health were ranked as per their impacts; we detected the following priority ones (in a descending order): social tension, quality and availability of medical aid, economic development, and social improvement [12].

We determined priority tasks that had to be solved in order to provide sanitary-epidemiologic welfare taking into account evaluations given by experts in Sverdlovsk region as per Delphi method and risk indexes technique.

We quantitatively assessed how significant those priorities were as well as suggested health risk management scenarios and it allowed us to predict population health losses prevented due to their implementation and, accordingly, recommended funding necessary

[7] On sanitary and epidemiologic welfare of the population in the Russian Federation in 2017: State Report. – M.: Federal Service for Surveillance over Consumer Rights Protection and Human Well-being, 2018. – 268 p.

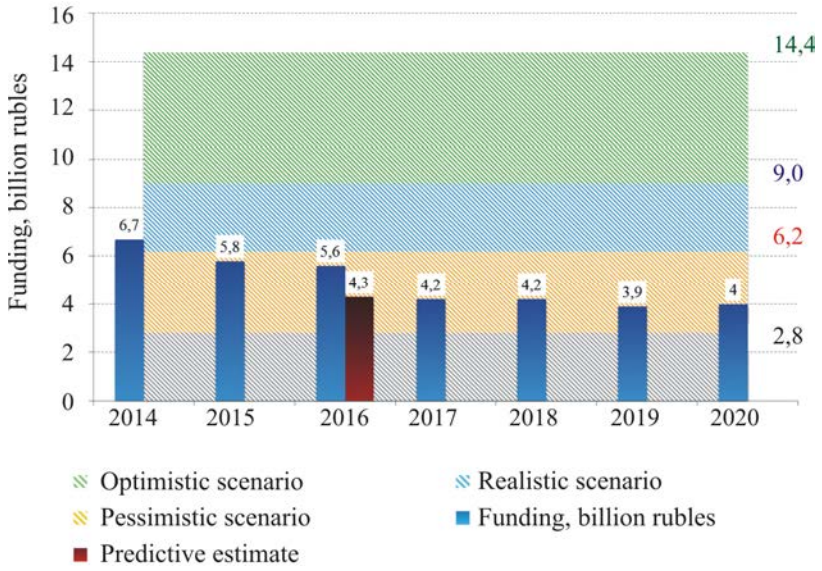

Figure 4. Funding provided for the Complex program for health risk management in the industrially developed city in Sverdlovsk region, predicted and actual funding, 2016

for it [13, 14]. These evaluations and prediction taken into account, we calculated expected socially significant results obtained due to implementation of the Complex health risk management program in 2018 (intermediate results assessment) and in 2020 (ultimate results assessment) as per three considered scenarios, optimistic, realistic, and pessimistic one.

Predictive estimate of necessary annual funding for the Complex program as per various health risks management scenarios and actual funding spent in 2015 and 2016 are shown in Figure 4.

We applied two economic criteria to assess different variants of the Complex program:

1. Criterion 1 («necessity»). It was applied to evaluate necessary funding for a set of activities as per different health risks management scenarios in a municipal entity (optimistic, realistic, and pessimistic one);

2. Criterion 2 («sufficiency»). We assessed structure of funds for a set of activities aimed at health risks management in a municipal entity as per priority tasks to be solved to provide sanitary epidemiologic welfare for population.

A variant of the Complex program that was actually implemented corresponds to a pessimistic scenario of socioeconomic development in the given industrially developed city in Sverdlovsk region. Planned funding varied from 4.3 billion rubles in 2016 to 4.0 billion rubles in 2020; actual funding reached 5.6 billion rubles.

Figures 5 and 6 show actual socially significant medical and demographic parameters (in 2015 and 2016); Figure 5 shows overall population mortality, Figure 6 shows mortality among employable population.

Changes in these parameters are the most significant for an industrially developed city as they determine overall medical and demographic situation and sanitary-epidemiologic welfare of population in a municipal entity [15, 16].

Under an optimistic health risk management scenario, overall population mortality can reach 11.6 cases per 1,000 people by 2020; under realistic one, 12.3 cases per 1,000 people; under pessimistic one, 14.2 cases per 1,000 people. But in 2016 actual mortality was equal to 14.9 cases per 1,000 people, and possible trends in changes in it are worse than pessimistic forecasts.

The same unfavorable trend remains for mortality among employable population. Under an optimistic health risk management scenario, mortality among employable population can reach 4.1 cases per 1,000 people by 2020; under realistic one, 4.6 cases per 1,000 people; under pessimistic one, 6.2 cases per 1,000 people. Actual mortality among employable population amounted to 6.9 cases per 1,000 people.

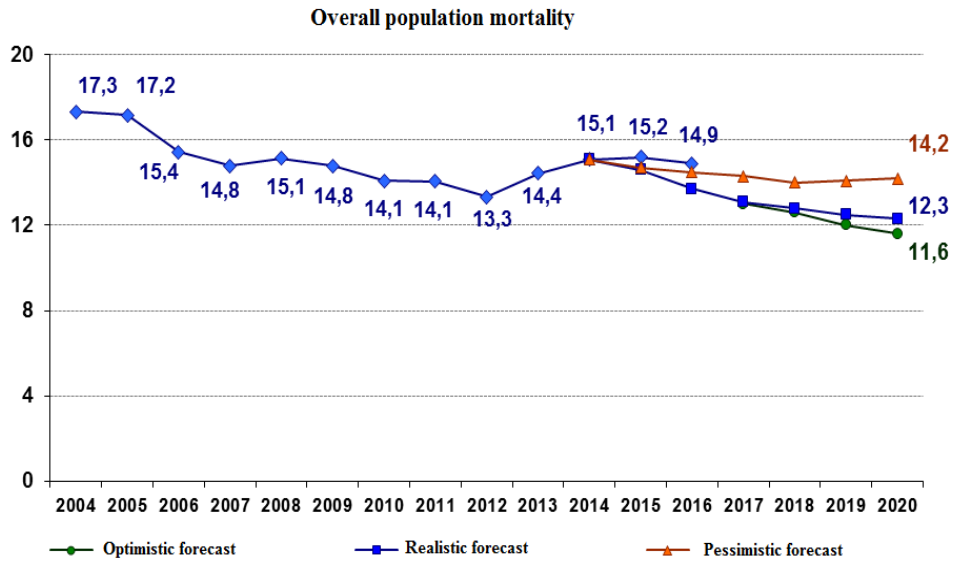

Figure 5. Actual overall population mortality up to 2016 and expected one up to 2020 in an industrially developed city In Sverdlovsk region (per 1,000 people)

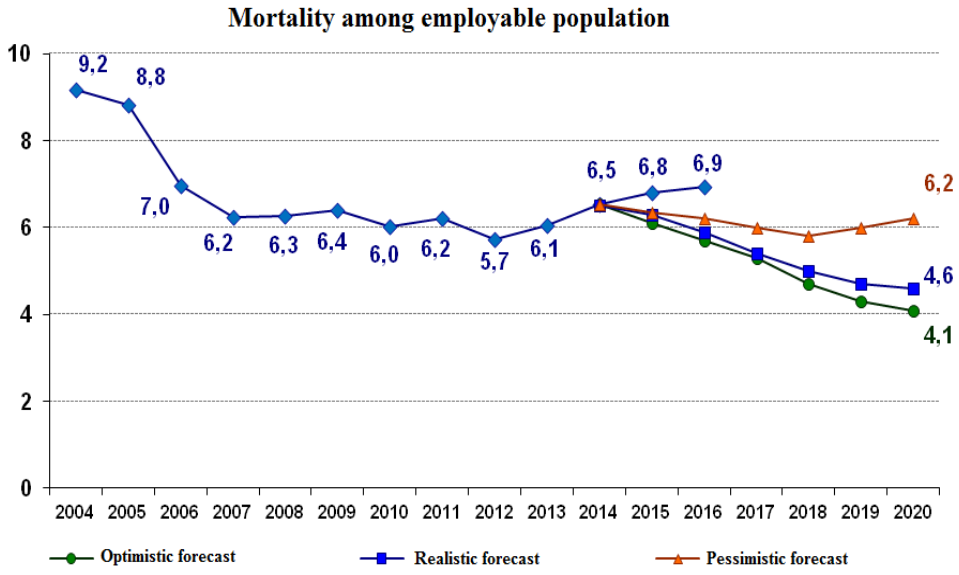

Figure 6. Actual mortality among employable population up to 2016 and expected one up to 2020 in an industrially developed city In Sverdlovsk region (per 1,000 people)

Any changes in mortality are rather inert (drastic ones are not possible) and it exerts its influence on en existing situation; another significant effect is produced by chosen priorities in funds allocated to solve key issues related to providing sanitary-epidemiologic welfare [17–19]. Figure 7 shows that there are discrepancies between recommended priorities based on predicted changes in impacts exerted by environmental factors on population health and priorities that were actually given financial support in 2015 and 2016.

Risk indexes as per specific tasks were determined for all 9 priorities in providing sanitary-epidemiologic welfare; they are given in % and correspond to the following priorities (in a descending order):

The 1st priority is poor quality of drinking water supply (risk index amounts to 32.4%, actual share of allocated funds amounts to 13.5%);

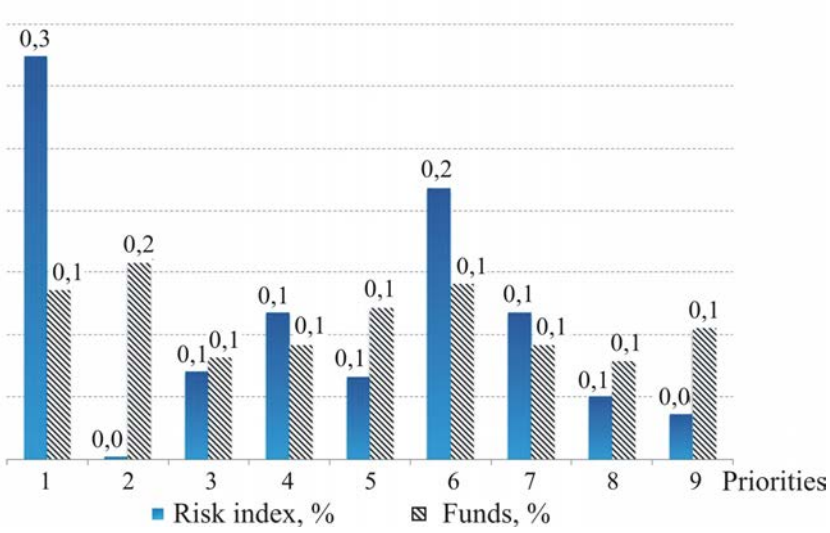

Figure 7. Priorities in providing sanitary-epidemiologic welfare recommended as per risk indexes and actual funds allocated for their implementation according to the Complex health risks management program

The 2nd priority is high mortality among employable population (risk index amounts to 21.8 %, actual share of allocated funds amounts to 14.0%);

The 3rd and 4th priorities are low quality nutrition and high prevalence of injuries and intoxications (risk index amounts to 11.7%, actual share of allocated funds amounts to 9.1%);

The 5th priority is ambient air and soil contamination (risk index amounts to 7.0%, actual share of allocated funds amounts to 5.0%);

The 6th priority are risk factors related to educational and learning conditions for children and teenagers (risk index amounts to 6.6%, actual share of allocated funds amounts to 12.1%);

The 7th priority is infectious and parasitic diseases (risk index amounts to 5.0%, actual share of allocated funds amounts to 7.9%)

The 8th priority is low efficiency of health risk management system (risk index amounts to 3.6 %, actual share of allocated funds amounts to 10.5%);

The 9th priority is adverse effects produced by physical factors, first of all, noise (risk index amounts to 0.2%, actual share of allocated funds amounts to 8.1%).

More significant priority tasks were given two times lower funds than they should have; on the contrary, less significant priorities were given more funds than actually required. Considerable financial resources that are available are not allocated efficiently and target results therefore can't be achieved.

Taking all research results and performed assessments into account, we recommended adjusting the Complex health risk management program in the given municipal entity in order to make funds [20] allocated for solving priority tasks closer to their recommended volumes starting from 2018 and to make sure target socially significant medical and demographic parameters are achieved by 2020.

Conclusions and recommendations.

1. Methodical approaches, algorithm, and middle-term planning procedures for health risk management in a municipal entity can be applied in order to:

– create and develop a health risk management system and techniques as support for making management decisions aimed at providing sanitary-epidemiologic welfare and health protection;

– implement health risk management techniques based on a systemic approach within activities aimed at providing sanitary-epidemiologic welfare and health protection taking into account effects produced by socioeconomic factors, sanitary-hygienic factors, as well as factors related to lifestyle;

– test health risk management techniques and technologies oriented at risk tolerability

(acceptability) concept together with hygienic standards created for environmental factors;

– plan and choose health risk management scenarios, a focus being shifted from technical requirements and possibilities related to social and economic potential to availability of resources necessary to achieve tolerable (acceptable) health risks in a middle-term period;

– perform economic evaluation, substantiate and select probable health risk management scenarios aimed at providing sanitary-epidemiologic welfare of the population within support systems for making managerial decisions;

– develop state social and hygienic monitoring basing on implementation of health risk management techniques and approaches in order to obtain and analyze the most complete, timely, and reliable information necessary to assess influence exerted by environmental factors on population health;

– make health risk management systems more efficient due to performing activities aimed at preventing, decreasing, and compensating risks outcomes and to applying scenario-based approach to their substantiation and implementation;

– implement measures aimed at raising awareness about factors related to environmental contamination that produce adverse effects on health and health risk management techniques, both among decisions-makers and population in general.

2. There are certain basic uncertainties and assumptions that are characteristic for assessing and predicting effects produced by socioeconomic and sanitary-hygienic factors on medical and demographic parameters; they should be taken into account when performing middle-term planning of health risk management activities and providing sanitary-epidemiologic welfare of the population. They are:

– whether data on population health (mortality and morbidity) in a municipal entity are complete and reliable;

– whether an existing situation is consistent with chosen scenarios of socioeconomic development in a municipal entity;

– whether models applied for predicting medical and demographic situation in a municipal entity are reliable;

– whether it is possible to accumulate financial resources from various sources to solve issues related to improvement of medical and demographic situation and sanitary-epidemiologic welfare provision.

3. We obtained significant results and gained substantial experience in creating and testing a municipal health risk management system; tested methodical and organizational approaches, methods and procedures for flexible middle-term planning and assessing efficiency and productivity of middle-term programs for providing sanitary-epidemiologic welfare and health risk management. These approaches, methods, and procedures turned out to have high potential for finding solutions to tasks related to social and economic development of municipal entities and can be considered suitable for any industrially developed city in any RF region.

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HYGIENIC ASSESSMENT OF POPULATION HEALTH RISKS CAUSED BY COMBINED ORAL INTRODUCTION OF HEAVY METALS

V.M. Boev, E.A. Kryazheva, D.N. Begun, E.L. Borshchuk, D.A. Kryazhev

The Orenburg State Medical University, 6 Sovetskaya Str., Orenburg, 460000, Russian Federation

There is a pressing issue related to combined oral introduction of heavy metals into a human body with drinking water and food products. It is caused by food products contamination, very high probability that metals migrate into water and plants from soils, ambient air, etc. The research goal was to hygienically assess combined oral introduction of heavy metals with drinking water and food products with subsequent population health risks assessment. The authors analyzed long-term data on structure and volumes of food products consumption and assessed population exposure under combined oral introduction of heavy metals (mercury, cadmium, arsenic, and lead) contained in drinking water and food products. Data were obtained from a regional information social-hygienic monitoring database and a regional office of the Federal Statistics Service. Both regional products and products delivered from other regions (or countries) were analyzed. It was detected that population in the region consumed food products per 1 person a year in a quantity which was by 93 kg higher than on average in the country. Calculated concentrations of the examined substances in food products and drinking water corresponded to hygienic standards. Dairy products had the first rank place as regards a contribution made into the overall exposure to lead, cadmium, and arsenic. The second and the third place belonged to vegetables and melons and grocery respectively. The first rank place as per a contribution made into the overall exposure to mercury belonged to vegetables and melons; the second place, to grocery; the third place, to dairy products. Non-carcinogenic risk assessment revealed that hazard quotients related to heavy metals contents in food products and drinking water were within acceptable risks limits. Total carcinogenic risk (TCR) was unacceptable (1.5E-03.). A number of additional oncologic diseases in the region could reach 557 cases (during 70 years) under the worst scenario.

Key words: *food products, drinking water, heavy metals, health risk assessment, non-carcinogenic risk, oncologic diseases, combined effect, oral introduction.*

Food products quality has become a pressing issue over the last few years as the Russian Federation is being integrated into the world economy, food exports are growing, agriculture is developing fast, and up-to-date technologies are being implemented into animal breeding and plant growing [1, 2]. The RF Government approved on "The Fundamentals of the RF State Policy concerning provision of population with healthy nutrition up to 2020" (dated October 25, 2010 No. 1873-p)[1], which determine priority trends in the state policy concerning provision of population with healthy nutrition in accordance with advanced medical requirements. And here it is truly vital to perform hygienic assessment of contaminants in food products and determine priority contaminants, and to rank food products

Viktor M. Boev – Doctor of Medical Sciences, Professor, Honored scientist of the RF, Honored worker of the higher education in the Russian Federation, Head of the Common and Communal Hygiene Department (e-mail: k_com.gig@orgma.ru; tel.: +7 (3532) 50-06-06 (ext. 320); ORCID: http://orcid.org/orcid.org/0000-0002-3684-1149).

Elena A. Kryazheva – a post-graduate student at the Common and Communal Hygiene Department (e-mail: kryazheva89@inbox.ru; tel.: +7(3532) 50-06-06 (ext. 320); ORCID: http://orcid.org/0000-0003-3527-2068).

Dmitrii N. Begun – Candidate of Medical Sciences, Associate Professor at the Public Health and Healthcare Department (e-mail: doctorbegun@yandex.ru; tel.: +7(3532) 50-06-06 (ext. 306); ORCID: https://orcid.org/0000-0002-8920-6675).

Evgenii L. Borshchuk – Doctor of Medical Sciences, Professor, Head of the Public Health and Healthcare Department (e-mail: be@orgma.ru; tel.: +7(3532) 50-06-06 (ext. 306); ORCID: https://orcid.org/0000-0002-3617-5908).

Dmitrii A. Kryazhev – Candidate of Medical Sciences, Associate Professor at the Common and Communal Hygiene Department (e-mail: kryazhev.87@inbox.ru; tel.: +7(3532) 50-06-06 (ext.320); ORCID: http://orcid.org/0000-0003-4592-3848).

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groups as per a contribution made by them into overall exposure[2] [3].

Food products quality and safety is determined, among other things, by absence of alien chemicals (contaminants) in them. Due to heavy metals being widely spread in the environment, it is essential to hygienically assess heavy metals contents, both in food products and drinking water [4–7]. When exposure to contaminants in food products and drinking water is determined, it allows not only to identify probable outcomes for population health but also to indirectly trace migration routes for examined alien chemicals in the environment [8–11]. Previous research that focused on hygienic assessment of heavy metals and microelements in environmental objects substantiates a biochemical province concept and makes it necessary to explore peculiarities and essence of inter-media migration taking into account regional peculiarities [12–15]. In this respect, if we assess a combined introduction of heavy metals with food products and drinking water, it will help us determine population health risks caused by oral introduction of heavy metals and reduce uncertainties [6, 16–19].

Our research goal was to assess population health risks caused by combined oral introduction of heavy metals with food products and drinking water.

Data and methods. Our research involved examining data on food products consumption by population provided by Orenburg regional office of the Russian Federal State Statistics Service; data obtained via research on food products which are subject to long-term social and hygienic monitoring (2008–2013); data taken from the State Reports "On sanitary and epidemiologic welfare of the population in Orenburg region over 2005–2013[3]. Overall, we examined more than 3,000 works on various food products, as well as structure and volumes of food products consumption. We performed a hygienic assessment as regards heavy metals contents in food products (cadmium, lead, mercury, and arsenic) and food products compliance with the requirements fixed in CU TR 021/2011 "On food products safety"[4]. Both local food products and those brought into Orenburg region from other places were assessed in the research; we also assessed food products grown by agricultural enterprises and by local population on their private farms.

Drinking water taken from centralized water supply systems in Orenburg was assessed as per 4 heavy metals contents according to SER 2.1.4.1074-01 "Drinking water. Hygienic requirements to quality of water from centralized drinking water supply systems. Quality control. Hygienic requirements to providing safety of hot water supply"[5] (with the latest amendments made on April 2, 2018).

Population health risks related to food products and drinking water were assessed in conformity with MG 2.3.7.2519-09 "Exposure determination and assessment of risks caused by effects produced on population by chemicals contaminants in food products"[6] and “Guidelines on assessment of population health risk under exposure to chemicals which pollute environment”[7]. When ranking food products as per their

[2] Working Principles for Risk Analysis for Food Safety for Application by Governments. CAC/GL 62-2007. – Rome: World Health Organization, 2007. – 41 p.

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[7] G 2.1.10.1920-04. Guidelines on assessment of population health risk under exposure to chemicals which pollute environment [Web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/1200037399 (date of visit January 15, 2019).

contribution into overall exposure, we calculated a fracture as per each examined substance with simultaneous rank determination. To calculate exposure, we took average long-term volumes of food products consumption, median values, and 90-th percentile. Carcinogenic risks were calculated as per 3 examined substances (cadmium, arsenic, and lead). Non-carcinogenic risks were assessed as per 4 examined substances (cadmium, arsenic, lead, and mercury). We processed the data statistically with Statistica R.10, "StatSoft Inc", and MS Office Excel-2010.

Results and discussion. Population exposure to heavy metals directly depends on food products contamination and quantities in which they are consumed. Comparative analysis of specific food products consumption (kg per capita) revealed that population in Orenburg region tended to consume more food products annually than in the country in general, both overall and as per specific food products groups; thus, overall annual food products consumption per capita was by 93 kg higher; vegetables and melons consumption, by 45 kg; milk and dairy products, by 60 kg; but at the same time, people living in Orenburg region consumed less sugar and confectionary, fruit, and potatoes (Table 1).

All the examined food products groups complied with the hygienic requirements fixed in the CU TR "On Food products safety"[4] as per heavy metals contents.

The next stage of our research was calculating median values and 90-th percentile ones in order to determine exposure as calculated average values could be overestimated or underestimated as compared with a median value (Table 2). We should note that calculated median values and 90-th percentile ones don't exceed levels fixed in the CU TR 021/2011[4]. We didn't detect any heavy metals in food products from "potatoes" group.

We compared all the obtained data with previous research and revealed that food products contamination with heavy metals tended to decrease as regards the examined food products groups. Lead contents went down by 1.4–1.6 times; cadmium, 1.5–2 times; mercury, 2–2.5 times; arsenic, 2.5–3 times [13].

We detected that milk and dairy products occupied the first rank place as per their contribution into overall exposure to lead, cadmium, and arsenic (Table 3). The second and the third rank places as per contributions made into overall exposure to lead, cadmium, and arsenic, belonged to vegetable and melons and grocery products respectively. Vegetables and melons also held the first rank place as per their contribution into overall exposure to mercury; the second place belonged to grocery products; the third place, to milk and dairy products. It is especially interesting to note that lead, arsenic, and cadmium are carcinogenic, and products that make the greatest contribution into overall exposure to them have the same rank places. When assessing the examined food products groups, we detected that it was exactly grocery products, vegetables

Table 1

Annual food products consumption per capita (kg/year)

Food products group	Orenburg region	The Russian Federation
Grocery	121.6±5.7	118.8±1.9
Vegetables and melons	**150±9.6***	105±1.9
Potatoes	**96.6±6.3***	109.6±2.3
Fruit and berries	53.0±2.3	59.6±1.6
Meat and meat products	69.3±3.1	65±1.8
Milk and dairy products	**307.7±8.5***	247±3.4
Eggs (items)	**310.7±7.6***	269±5.4
Fish and fish products	24.2±1.9	24.2±1.1
Sugar and confectionary	34.3±2.1	39.2±1.4
Vegetable oil and other fats	18.3±1.6	13.5±1.1

Note: validity of discrepancies between groups * – $p<0.05$.

Table 2

Contaminants concentrations in food products for exposure calculation (mg\kg, M±m)

Food products group		Lead	Arsenic	Cadmium	Mercury
Grocery	med	0.01±0.0012	0.01±0.0012	0.01±0.0011	0.005±0.0001
	90%	0.01±0.001	0.01±0.0011	0.014±±0.0001	0.005±0.0001
Vegetables and melons	med	0.01±0.0013	0.01±0.0019	0.01±±0.0001	0.005±0.0001
	90%	0.01±0.0017	0.01±0.0018	0.01±±0.0001	0.005±0.0001
Fruit and berries	med	0.01±0.0011	0.01±0.0011	0.01±0.001	0.005±0.0001
	90%	0.01±0.0012	0.01±0.0014	0.01±0.0011	0.005±0.0001
Meat and meat products	med	0	0	0	0
	90%	0.01±0.0013	0.01±0.0013	0.01±0.0001	0.005±0.0001
Milk and dairy products	med	0.01±0.0012	0.01±0.0012	0.01±0.0001	0.001±0.0001
	90%	0.01±0.0011	0.01±0.001	0.01±0.0001	0.005±0.0001
Fish and fish products	med	0.01±0.0011	0.024±0.001	0	0.012±0.001
	90%	0.07±0.0018	0.256±0.01	0.022±0.0001	0.05±0.001
Sugar and confectionary	med	0.01±0.0018	0.005±0.0001	0.01±0.0001	0.001±0.0001
	90%	0.01±0.0018	0.01±0.0014	0.01±0.0001	0.005±0.0001
Vegetable oil and other fats	med	0	0	0	0
	90%	0.01±0.0015	0	0	0

Table 3

Products rankled as per their contribution into overall exposure (%, rank)

Products	Lead	Arsenic	Cadmium	Mercury
Grocery	17.6(3)	17.2(3)	18.2(3)	27.0(2)
Vegetables and melons	21.7(2)	21.2(2)	22.5(2)	33.3(1)
Fruit and berries	7.7(4)	7.5(5)	8.0(4)	11.7(5)
Meat and meat products	0	0	0	0
Milk and dairy products	44.5(1)	43.5(1)	46.2(1)	13.6(3)
Fish and fish products	3.5(6)	8.2(4)	0	12.9(4)
Sugar and confectionary	5.0(5)	2.4 (6)	5.1(5)	1.5(6)

and melons, meat, milk and dairy products that were mainly produced or grown locally, in Orenburg region. We applied median values to rank products as per their contribution into overall exposure (Table 3).

We detected that more than 70 % of contribution made by food products contamination with lead, cadmium, mercury, and arsenic was determined by consumption of locally produced or grown food products.

It was especially interesting to examine products groups that made the greatest contribution into overall exposure. For more profound examination, grocery products were divided into the following categories: "bread", "bakery", "macaroni", "flour", "cereals", and "others". The lowest concentrations of lead (0.004 ± 0.0002 mg/kg; $p \leq 0.05$), arsenic (0.004 ± 0.0001 mg/kg; $p \leq 0.05$), cadmium (0.004 ± 0.0003 mg/kg; $p \leq 0.05$), and mercury (0.001 ± 0.0001 mg/kg; $p \leq 0.05$) were detected in "macaroni". The highest lead concentrations were detected in "flour" (0.009 ± 0.0006 mg/kg; $p \leq 0.05$) and "cereals" (0.008 ± 0.0003 mg/kg; $p \leq 0.05$). The highest arsenic concentrations were detected in "flour" (0.008 ± 0.0007 mg/kg; $p \leq 0.05$). The highest cadmium concentrations were also detected in "flour"

(0.009 ± 0.0006 mg/kg; p≤0.05) and "cereals" (0.009 ± 0.0005 mg/kg; p≤0.05). The highest mercury concentration was detected in "bread" (0.004 ± 0.0003 mg/kg; p≤0.05). Overall, we can conclude that "flour" and "cereals" are the most contaminated among grocery products, and they are mostly produced locally and from local raw materials (Figure).

Examined meat products were divided into the following categories: "sausages", "meat", "poultry", and "minced meat products" (Figure 2). We detected that "meat" was the least contaminated among all these products (lead concentration 0.001 ± 0.0005 mg/kg; p≤0.05; arsenic, 0.001 ± 0.0009 mg/kg; p≤0.05; cadmium, 0.001 ± 0.0005 mg/kg; p≤0.05; mercury, 0.0004 ± 0.00001 mg/kg; p≤0.05). The highest concentrations of lead (0.008 ± 0.0005 mg/kg; p≤0.05), arsenic (0.008 ± 0.0003 mg/kg; p≤0.05), cadmium (0,008 ± 0.0009 mg/kg; p≤0.05), mercury (0.004 ± 0.0001 mg/kg; p≤0,05) were detected in "minced meat products" which were basically frozen semi-finished products (cutlets, pelmeni, meatballs, and others), and most of them were brought to Orenburg form other regions while most "neat" and "poultry" was produced locally.

To examine dairy products more profoundly, we divided them into 3 categories: "milk", sour milk products", and "other milk products" (buttermilk, etc.). We detected that "sour milk products" were the most contaminated with lead (0.006 ± 0.0002 mg/kg), arsenic

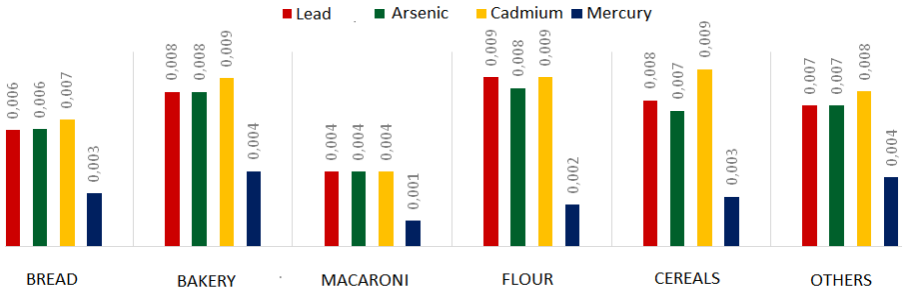

Figure 1. Grocery products contamination with heavy metals, mg/kg

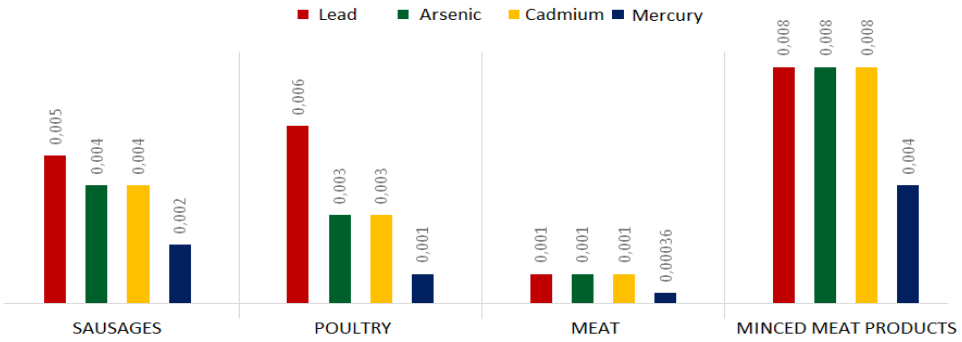

Figure 2. Meat products contamination with heavy metals, mg/kg

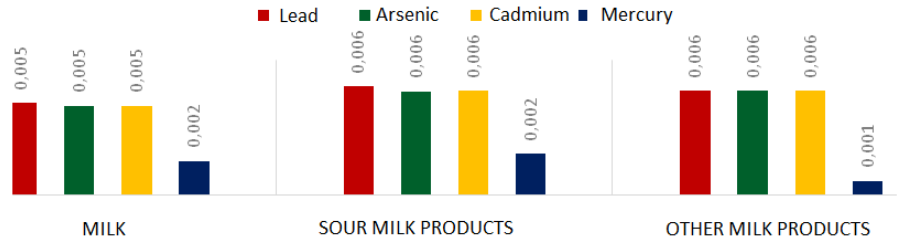

Figure 3. Dairy products contamination with heavy metals, mg/kg

(0.006 ± 0.0001 mg/kg), and cadmium (0.006 ± ± 0.0006 mg/kg) (Figure 3). As for "milk" concentration of lead in it amounted to 0.005 ± 0.0009 mg/kg; arsenic, 0.005 ± 0.0008 mg/kg; and cadmium, 0.005 ± 0.0007 mg/kg. Mercury contents in "milk" and "sour milk products" were practically the same (0.002 ± ± 0.0001 mg/kg).

To determine non-carcinogenic hazard quotients, we applied values of conditionally tolerable weekly introduction (CTWI) of contaminants which were able to cumulate (MG 2.3.7.2519-09[6]) with calculating weekly exposure. We detected that hazard quotients, both as per their median values and 90-th percentile ones, didn't exceed an acceptable level. Overall hazard index at a median and 90-th percentile level also didn't exceed permissible levels (Table 4).

Risk analysis as per organs and systems revealed that the highest risks were detected for the hormonal system, CNS, kidneys, and immune system; nevertheless, all those risks corresponded to hygienic requirements, both as per median values and 90-th percentile ones (Table 5).

Carcinogenic risks were calculated as per three chemicals, namely, cadmium, arsenic, and lead; arsenic and cadmium are assigned into the first category of carcinogens according to the International Agency for Research on cancer (IARC). Carcinogenic risk assessment revealed that a risk caused by arsenic contents in food products could be estimated as unacceptable for population in general (individual lifelong risk was higher than 1×10^{-4}), both as per a median value and 90-th percentile one. Individual carcinogenic risks caused by exposure to lead and cadmium and calculated as per 90-the percentile were also unacceptable (Table 6).

We calculated population carcinogenic risk for the whole Orenburg region population (553,763 people); the calculation revealed that contamination with arsenic resulted in the maximum probability of oncologic diseases (441.9 new disease cases), and contamination with lead, the minimum one (9.4 new disease cases); contamination with cadmium resulted in 69.2 new cases. Overall, food products consumption could lead to additional 520.5 new oncologic diseases cases for 70 years under the worst possible scenario (90-th percentile).

We analyzed heavy metals introduction with drinking water and revealed that average concentrations of the examined chemicals in it didn't exceed permissible levels, lead concentration being equal to 0.14 MPC; arsenic, 0.2 MPC; cadmium, 0.48 MPC; and mercury, 0.04 MPC.

Daily absorbed doses amounted to 5.58E-05 for lead; 3.96E-05, arsenic; 1.38E-05, cadmium; and–6.14E-07, mercury. We calculated non-carcinogenic risks caused by the examined contaminants contents in drinking water and

Table 4

Hazard quotients related to non-carcinogenic effects occurrence

Chemical		Exposure (mg/kg/ week)	CTWI mg/kg b.w. / week	Hazard quotient (HQ)
Lead	med	0.002	0.025	0.08
	90%	0.003		0.10
Arsenic	med	0.002	0.015	0.13
	90%	0.003		0.25
Cadmium	med	0.002	0.007	0.26
	90%	0.002		0.33
Mercury	med	0.001	0.005	0.12
	90%	0.001		0.27
Overall HI	med			0.59
	90%			0.95

Table 5

Overall hazard index for critical organs and systems in a body

Organs and systems	Hazard index, HI med	Hazard index, HI 90 %
Central nervous system	0.33	0.62
Nervous system	0.21	0.35
Blood	0.08	0.10
Hormonal system	0.59	0.95
Reproductive system	0.20	0.37
Skin	0.21	0.35
Immune system	0.25	0.52
Gastrointestinal tract	0.13	0.25
Cardiovascular system	0.13	0.25
Kidneys	0.39	0.60

Таблица 6

Carcinogenic risks related to food exposure

Chemical	CAS*	IARC**	EPA***	SF_O****	SF_I*****	ICR****** med	ICR 90 %
Cadmium	7440-43-9	1	B1	0	6.3	9.94E-05	1.25E-04
Arsenic	7440-38-2	1	A	1.5	15	4.17E-04	7.98E-04
lead	7439-92-1	2A	B2	0	0.042	1.27E-05	5.4E-04
CRfo Sum	–					5.29E-04	1.5E-03

Notes:
* ChemicalAbstractsService.
** International Agency for Research on Cancer.
*** United States Environmental Protection Agency; EPA.
**** Slope Factor for oral SF_O introduction of a toxicant in a body.
***** Slope Factor for inhalation SF_I introduction of a toxicant in a body.
****** Individual carcinogenic risk.

detected the highest hazard quotient for arsenic (HQ-0.13). Hazard quotients (HQ) calculated for cadmium, mercury, and lead, were lower than 0.01.

Carcinogenic risk assessment revealed that individual carcinogenic risk caused by arsenic contents in drinking water amounted to 5.94E-05; lead contents, 2.62E-06; cadmium contents, 5.25E-06. Probable additional oncologic cases caused by oral introduction of a chemical with drinking water amounted to 32.8 for arsenic; 1.5, for lead; 2.9, for cadmium, for 70 years.

We assessed combined effects produced by heavy metals in food products and drinking water under oral introduction on the basis of hazard index 9HI); the assessment showed that HI for food products amounted to 0.44 (90-the percentile was applied); and to 0.18 for drinking water. Therefore, total hazard index (THI) amounted to 0.62.

We assessed total carcinogenic risk caused by combined oral introduction and revealed that CR for food products amounted to 9.4E-04 (90-th percentile was applied); and to 1.47E-03 for drinking water. Therefore, total carcinogenic risk (TCR) amounted to 1.5E-03.

Basic uncertainties in this work are caused by incomplete data on parameters required to assess exposure as such an assessment depends on more detailed study of a market basket which allows to quantitatively estimate food products consumption by various population groups. The most authentic assessment of influence exerted on health by combined oral introduction of heavy metals can be performed only via targeted and well-planned research with lower variability and uncertainty, based on data specifically obtained for the sake of such research and scenarios which are the most approximate to real life conditions.

Conclusion. We detected that annual food products consumption as per the examined food products groups was by 93 kg higher per capita in Orenburg region than in the country in general.

Calculated median and 90-th percentile values for concentrations of heavy metals (cadmium. lead, mercury, and arsenic) corresponded to the requirements fixed by CU TR 021/2011 "On food products safety"[4] in all the examined food products groups. Average annual concentrations of heavy metals in drinking water didn't exceed hygienic standards.

Milk and dairy products had the first rank place as per their contribution into overall exposure to lead, cadmium, and arsenic; these products were mainly produced in the region. The first rank place as per a contribution into overall exposure to mercury belonged to vegetables and melons. More than 70% of the contribution made into food products contamination with lead, cadmium, mercury, and arsenic are determined by consumption of products which were grown or produced mainly in the region.

Hazard coefficients related to non-carcinogenic effects as well as hazard indexes for

critical organs and systems caused by heavy metals contents both in drinking water and food products didn't exceed acceptable levels.

As we assessed total carcinogenic risk caused by combined oral introduction of heavy metals, we revealed an unacceptable risk level (total carcinogenic risk (TCR) which amounted to 1.5E-03, as per 90-th percentile). Probability of additional oncologic diseases could reach 557 cases under the worst possible scenario.

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DRINKING WATER QUALITY: HEALTH RISK FACTORS AND EFFICIENCY OF CONTROL AND SURVEILLANCE ACTIVITIES BY ROSPOTREBNADZOR

N.V. Zaitseva[1,2,3], A.S. Sboev[4], S.V. Kleyn[1,2], S.A. Vekovshinina[1]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation
[2]Perm State University, 15 Bukireva Str., Perm, 614990, Russian Federation
[3]Perm State Medical University named after Academician E.A. Wagner, 26 Petropavlovskaya Str., Perm, 614000, Russian Federation
[4]Informational methodical center, 19A Varshavskoe shosse, Moscow, 117105, Russian Federation

The paper dwells on issues related to quality of drinking water from centralized water supply systems; spotting out priority health risk factors; and assessing efficiency of control and surveillance activities performed by Rospotrebnadzor in the sphere of drinking water supply to the RF population.

It was detected that in most RF regions control activities performed within social-hygienic monitoring mainly covered contents of substances belonging to the 3rd and 4th hazard categories in drinking water while contents of the 1st hazard category substances were controlled only in several RF regions. The highest shares of samples exceeding hygienic standards as per contents of the 1st hazard category substances were detected for such dangerous chemicals as chloroform, bromodichloromethane, trichloroethylene, 1,2-dichloroethane, tetrachloromethane, tetrachloroethylene, and arsenic.

Chlorine and chlorinated organic compounds, ammonium and chemicals that contain ammonium ion, iron, manganese, arsenic, nickel, and copper compounds, as well as microbiological contamination of water remain priority risk factors with the greatest contribution made into additional incidence associated with poor quality of drinking water taken from communal water supply systems. On average in the RF, in 2018 efficiency of Rospotrebnadzor activities estimated as per prevented GRP (gross regional product) losses caused by drinking water quality amounted to 70.1 ± 10.1 ruble per 1 ruble of costs. The highest values of the criterion were registered for the 3rd cluster (67.7 ruble per 1 ruble of costs); the lowest ones, for the 1st cluster (47.4 rubles per 1 ruble of costs).

Taking into account region peculiarities and different types of territories, it is necessary to develop specific regional programs and action plans aimed at preserving already obtained levels of population provision with qualitative drinking water, improving control and surveillance activities with wide implementation of risk-oriented approach, achieving targets fixed in national project and federal programs.

Key words: *water supply, drinking water quality, risk factors, efficiency, control and surveillance activities.*

High quality and safe drinking water should be available to everyone as it is greatly significant for a person's health, it is his or her basic right; it determines health and life quality of any nation[1]. According to the UN World Water Development Report[2] published in 2019, more than 2 billion people worldwide don't have permanent access to pure drinking water and 844 million people have to spend at least half an hour a day to obtain some water or don't have any access to it at all. Even in Europe and North America about 57 million people don't have centralized water supply in their homes. According to WHO experts' es-

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

Aleksandr S. Sboev – Head, Chief Medical Officer in the Perm Krai (e-mail: urpn@59.rospotrebnadzor.ru; tel.: +7 (342) 239-35-63).

Svetlana V. Kleyn – Doctor of Medical Science, head of the department of methods for sanitary analysis and monitoring (e-mail: kleyn@fcrisk.ru; tel.: +7 (342) 236-32-64; ORCID: https://orcid.org/0000-0002-2534-5713).

Svetlana A. Vekovshinina – Head of Laboratory for conformity assessment techniques and consumer inspections (e-mail: veksa@fcrisk.ru; tel.: +7 (342) 237-18-04; ORCID: https://orcid.org/0000-0002-4833-0792).

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timates[3], each dollar invested into improving drinking water quality, sanitation, hygiene, and water resources management brings 8 dollars as a return.

Despite a certain growth achieved in providing RF population with water corresponding to all safety requirements (by 1.97% in 2018 against 2014), in 2018 12.43% people in the country (5.32% among urban population and 32.72%, among rural one) were not provided with safe drinking water from centralized water supply systems[4].

In 2018 in the RF approximately 16.1 thousand death cases (due to circulatory system diseases, digestive organs diseases, malignant neoplasms, certain infectious and parasitic diseases) and 1,764.49 thousand diseases in the digestive organs, urogenital system, musculoskeletal system and conjunctive tissue, circulatory system, skin and subcutaneous tissue, endocrine system and some other diseases were caused by drinking water that was contaminated with various chemicals (chlorine and chlorinated organic compounds, ammonia, iron, manganese, arsenic, nickel, copper, boron, magnesium, etc.) and microbe agents [1–5].

Rospotrebnadzor's organs and institutions bear the full responsibility for control over drinking water quality and providing sanitary-epidemiologic welfare of the population in the RF; there are also some other state authorities that perform these functions within state sanitary-epidemiologic surveillance[5], social-hygienic monitoring[6,7] , and industrial control[8].

To provide population with high quality drinking water, including that coming from centralized water supply systems, is among priority state tasks that need to be solved in the Russian Federation.

The Federal Project "Pure water"[9], which is a part of "Ecology" National Project[10], stipulates a solution to a problem related to improving drinking water quality via modernization of water supply and water treatment systems with advanced technologies applied in the process.

The Federal Service for Surveillance over Consumer Rights Protection and Human Well-being (Rospotrebnadzor) and other participants in "Ecology" National Project are to solve the following vital tasks [6]: to increase a share of the RF urban population provided with qualitative drinking water from centralized water supply systems up to 99.0% by 2024; to provide 90.8% of the country population with qualitative drinking water from centralized water supply systems by 2024.

These tasks can be solved only if Rospotrebnadzor's activities aimed at improving

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drinking water quality become more efficient and productive; it includes control and surveillance activities based on a risk-oriented model for the Service functioning [7–10]. The latter involves concentrating efforts by Rospotrebnadzor on economic entities that deal with water collection, purification, and distribution as such entities can cause the greatest risks for population health in the RF regions [11, 12]. A possibility to describe ultimate results of the activities performed by Rospotrebnadzor in terms of medical-demographic and economic parameters is truly vital for assessing their productivity and efficiency. All the above-mentioned substantiated the necessity to conduct the given research.

Our research goal was to assess drinking water quality, to highlight priority health risk factors, and to assess efficiency of control and surveillance activities performed by Rospotrebnadzor's bodies in the sphere of providing RF population with drinking water.

Data and methods

Our research focused on drinking water quality parameters, as well as incidence and mortality in 85 RF regions in 2014–2018.

We performed hygienic assessment of drinking water quality as per chemical parameters using data provided by the Federal Information Fund for social and hygienic monitoring (FIF SHM) and a statistical report form No. 18 entitled "Data on sanitary situation in a RF region" collected over 2014-2018.

When assessing drinking water quality, we took into account drinking water quality criteria developed by Rospotrebnadzor in 2019 and outlined in the methodical guidelines MG 2.1.4.0143-19[11].

We assessed drinking water quality taking into account obligatory requirements fixed in the following sanitary regulations:

– HS 2.1.5.1315-03 "Maximum permissible concentrations (MPC) of chemicals in water objects used for drinking, communal and household purposes" (last revised on July 13 2017)[12].

– SER 2.1.4.1074-01 "Drinking water. Hygienic requirements to quality of drinking water taken from centralized water supply systems. Quality control. Hygienic requirements to providing safety of hot water supply systems" (last revised on April 2, 2018)[13].

All the RF regions were classified (a type of each was determined) with a multi-dimensional statistical procedure, namely cluster analysis, as per a set of parameters that described efficiency and productivity of activities performed by Rospotrebnadzor as regards objects under surveillance dealing with water collection and purification and water distribution.

Cluster analysis was performed with a set comprising 8 parameters measured in 2014-2018 in 85 RF regions:

– changes in the parameter "A share of population provided with qualitative drinking water from centralized water supply systems (%)" in 2014-2018;

– changes in the parameter "Incidence among population caused by poor drinking water quality (cases per 1,000 people)" in 2014-2018;

– changes in the parameter "Population mortality caused by poor drinking water quality (cases per 1,000 people)" in 2014-2018;

– changes in the parameter "Frequency of violations concerning Clause 19, The Federal Law No. 52, in the sphere of "Water collection and purification" (%)" in 2014-2018;

– changes in the parameter "Frequency of violations concerning Clause 19, The Federal Law No. 52, in the sphere of "Water distribution" (%)" in 2014-2018;

– changes in the parameter "Incidence among population caused by poor quality

[11] MG 2.1.4.0143-19. Guidelines on assessing increase in quality of drinking water taken from centralized drinking water supply systems / Approved by the RF Chief Sanitary Inspector on March 27, 2019. – M.: Rospotrebnadzor, 2019. – 10 p.

[12] HS 2.1.5.1315-03. Maximum permissible concentrations (MPC) of chemicals in water objects used for drinking, communal and household purposes [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/901862249 (date of visit June 18, 2019).

[13] SER 2.1.4.1074-01 "Drinking water. Hygienic requirements to quality of drinking water taken from centralized water supply systems. Quality control. Hygienic requirements to providing safety of hot water supply systems" (last revised on April 2, 2018) [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/901798042 (date of visit June 18, 2019).

drinking water quality that was prevented due to activities by Rospotrebnadzor (cases per 1,000 people)" in 2014-2018;

– changes in the parameter "Mortality among population caused by poor drinking water quality that was prevented due to activities by Rospotrebnadzor (cases per 1,000 people)" in 2014-2018;

– a ratio of gross regional product per capita in a RF region in 2017 to the same parameter in 2014 (inflation taken into account), times.

When assessing efficiency of control and surveillance activities concerning economic entities that dealt with water collection, purification, and distribution, we applied algorithms and procedures fixed in the Methodical Guidelines MR 5.1.0095–14 and some research works[14] [13–20].

The methodology is based on comparing expenses borne by Rospotrebnadzor to perform control and surveillance to economic damage prevented due to lower incidence and mortality among population in a specific region. Here we determined economic damage caused by population incidence and mortality as production volumes (a part of gross regional product) that were not manufactured due to some employable population falling out of the production process. We determined losses caused by additional mortality that was associated with quality of the environment as per the following calculation: 0.5 year of being economically active per each death case; losses caused by additional incidence were calculated as 1 year of being economically active per each case. We took an average duration of each health disorder that made a person fall out of work being equal to half a calendar year (L = 183) for each death case and to an average duration of temporary disability for each incidence case.

We calculated additional incidence cases and death cases among population caused by environmental factors and cases prevented by Rospotrebnadzor activities; to do that, we modeled relationships between environmental quality parameters, population health, and parameters of activities performed by Rospotrebnadzor in 2010-2017.

To calculate economic efficiency of control and surveillance functions performed by Rospotrebnadzor, we compared expenses on control and surveillance activities aimed at providing sanitary and epidemiologic welfare to prevented economic losses. All our calculations were based on data provided from state statistical reports including those obtained from the Form No. 1 entitled "Data on the results of state control (surveillance) and municipal control collected over 2018" .

Basic results

According to data taken from the Form No. 18 entitled "Data on sanitary situation in a RF region", in 2018 authorities and bodies responsible for the federal state sanitary-epidemiologic surveillance over drinking water quality collected and examined more than 1,965.9 thousand water samples from centralized drinking water supply systems including:

– more than 361.1 thousand samples from centralized drinking water supply sources, more than 44.1 thousand of them taken from surface sources and more than 317.0 thousand samples taken from underground ones;

– more than 192.4 thousand water samples from water supply systems;

– more than 1,412.3 thousand water samples from distribution networks.

Quality control over drinking water from water supply systems (water from them is then directed into water distribution networks) revealed that in the RF obligatory requirements to sanitary and chemical parameters were more frequently violated in 2018 against 2014 (by 0.07%) and their frequency amounted to 16.97%. It could indirectly imply that implemented risk-oriented approaches to control and surveillance activities were more efficient than previously applied ones.

In 2018 water from centralized water supplied systems conformed to hygienic stan-

[14] MR 5.1.0095–14. How to calculate actual economic losses and those prevented due to control and surveillance activities as regards economic losses caused by population mortality, incidence, and disability associated with negative impacts exerted by environmental factors, approved by the RF Chief Sanitary Inspector on October 23, 2014. – M.: Rospotrebnadzor's Federal Center for Hygiene and Epidemiology, 2015 – 43 p.

dards as per all sanitary and chemical parameters in Altai Republic, Mari El Republic, Kamchatka, and Tyva Republic; hygienic standards were violated in 44.92–69.9% samples in the Khanty-Mansi Autonomous Area, Novgorod region, Ivanovo region, Kalmykia, and Dagestan.

A number of drinking water samples from centralized water supply systems that didn't correspond to hygienic standards as per microbiological and parasitological parameters decreased gradually over 2014-2018 by 0.64% and 0.07% correspondingly. Regions where water quality as per microbiological parameters was the highest included Moscow city, Saint Petersburg, Sevastopol, Mari El Republic, Mordovia, Stavropol region, Kamchatka, Tambov region, Magadan region, and Chukotka Autonomous Area. 12.0–13.31% samples taken in Jewish Autonomous Area, Smolensk region, Ingushetia, Karachayevo-Cherkesskaya Republic, and Primorye deviated from hygienic standards as per microbiological parameters.

As a whole, quality of drinking water supplied to population from centralized distribution networks improved in the RF. In 2018 a number of samples that deviated from hygienic standards as per sanitary-chemical parameters decreased by 2.47% against 2014; as per microbiological parameters, by 0.96%. But there was a 0.04% increase in 2018 against 2014 in number of water samples taken from distribution networks that deviated from hygienic standards as per parasitological parameters.

In 2018 quality of drinking water taken from distribution networks was the highest as per sanitary-chemical parameters in Kamchatka, North Ossetia – Alania, Adygei, and Altai Republic (from 0.00 to 0.60% samples not conforming to standards). RF regions with the maximum (%) share of water samples taken from distribution networks that didn't conform to sanitary requirements as per sanitary-chemical parameters included Novgorod region, Tver' region, Chukotka Autonomous Area, Karelia, and Kalmykia (from 34.89 to 58.31% samples not conforming to hygienic standards).

Less than 1% drinking water samples taken from centralized distribution networks didn't conform to hygienic standards as per microbiological parameters in Moscow city, Saint Petersburg, Kamchatka, Chukotka Autonomous Area, Adygei, Orenburg region, Novosibirsk region, Moscow region, Stavropol region, Tomsk region, Murmansk region, Voronezh region, Krasnodar region, and Khanty-Mansi Autonomous Area. All drinking water samples taken from centralized water distribution networks in Sevastopol in 2018 conformed to hygienic standards as per microbiological parameters.

In 2018 RF regions with the maximum (%) share of drinking water samples taken from distribution network that didn't conform to sanitary-epidemiologic requirements as per microbiologic parameters included Chechen Republic, Dagestan, Jewish Autonomous Area, Ingushetia, and Karachayevo-Cherkesskaya Republic (from 9.45% to 23.90% samples deviating from hygienic standards).

As per data provided by the Federal Information Fund of Social Hygienic Monitoring (FIF SHM) in 2015-2016 quality of drinking water was monitored as per its chemical structure in 85 RF regions; in 82 RF regions in 2014 and 2015.

In 2018 and 2017 centers for hygiene and epidemiology located in RF regions and other certified laboratories submitted data to the FIF SHM on 84 chemicals contained in water; in 2016, on 79 chemicals; in 2015, on 105 chemicals; in 2014, on 107 chemicals.

In the RF in 2018 more than 5% water samples deviated from hygienic standards as per contents of the following chemicals: bromine (75.0% non-conforming samples), silicon (21.0%), lithium (17.5%), iron (13.0%), sodium (12.0%), chloroform (11.2%), magnesium (10.2%), boron (8.1%), manganese (6.70%), strontium (5.94%), sulfides and hydrogen sulfide (5.49%).

Over 2014-2018 the highest share of samples deviating from hygienic standards as per the 1st hazard category chemicals contained chloroform (from 7.89 to 11.3% samples). Other hazardous chemicals included (in a descending

order) bromdichloromethane (0.21–1.60% samples with contends higher than MPC), trichloroethylene (0.58–3.21%), 1,2-dichloroethane (0.08–0.80%), dichloromethane (0.72–5.59%), tetrachloromethane (0.12–0.83%), tetrachloroethylene (0.03–0.43%), and arsenic (0.10–0.33%). The highest number of drinking water samples not conforming to sanitary-epidemiologic requirements as per contents of the 2nd hazard category chemicals contained bromine (75.0%). A share of samples with silicon contents deviating from hygienic standards was a bit lower (20.54–24.9% samples with silicon contents higher than MPC), followed by lithium (14.9–38.1%), sodium (11.6–15.0%), boron (8.05–8.69%), strontium (3.50–6.01%), and some other chemicals. Priority chemicals that belonged to the 3rd hazard category included iron (12.1–15.1% samples with its concentration higher than MPC), magnesium (7.35–10.15%), manganese (6.28–7.10%), aluminum (2.44–3.60%), nitrates (1.24–1.64%), and copper (0.003–0.03%); the 4th hazard category chemicals includes sulfides and hydrogen sulfide (1.41–5.49% samples with their concentrations being higher than MPC), ammonia and ammonia-ion (1.42–1.89%), sulfates (1.34–1.74%), chlorides (1.26–2.09%), and phenol (0.00–0.25%).

Comparative analysis revealed that basically contents of chemicals belonging to the 3rd and 4th hazard category in drinking water were under control in the RF regions within social and hygienic monitoring activities. In 2014-2018 more than 620.9 thousand drinking water samples were analyzed as per iron contents (the 3rd hazard category); more than 421.5 thousand samples, as per nitrates contents (the 3rd category); more than 381.1 samples, as per ammonia contents (the 4th category); more than 348.5 thousand samples, as per chlorides contents (the 4th category), etc.

At the same time, overall number of water samples taken to detect chemicals belonging to the1st and 2nd hazard categories in them was significantly lower. Thus, to detect chloroform (the 1st category) in drinking water, experts took more than 56.4 thousand samples; tetrachloromethane (the 1st category), more than 28.2 thousand samples; 1,2-dichloroethane (the 1st category), more than 5.46 thousand samples; bromdichloromethane (the 1st category), more than 16.4 thousand samples; etc.

We established that in 2014-2018 all the RF regions (100%) performed monitoring over iron contents (the 3rd hazard category) in drinking water; 93.9–95.3% RF regions monitored nitrates 9the 3rd hazard category); 88.2–92.7%, chlorides (the 4th hazard category); 88.2–91.5%, ammonia (the 4th hazard category); 87.1–93.9%, manganese (the 3rd hazard category); 84.7–90.2%, sulfates (the 4th hazard category).

But still only some regions in the RF performed control over priority chemicals belonging to the 1st hazard category in drinking water; dichloromethane was under control in 3.5% – 7.3% RF regions; 1,2-dichloroethane, 9.4–11.8%; tetrachloroethylene, 12.9–14.6%; trichloroethylene, 10.6% - 15.9%; bromdichloromethane, 16.5–21.2%; tetrachloromethane, 25.9–28.0%; chloroform, 45.1–52.9%; arsenic, 63.5–70.7% of all the RF regions.

We should note that contents of priority chemical contaminants in drinking water were significantly higher in some RF regions than in the country on average and it caused elevated health risks there. Thus, for example, in 2018 in Rostov region bromdichloromethane contents were higher than hygienic standards in 41.4% drinking water samples. In Primorye in 2017 and 2018 all the 100% water samples didn't conform to sanitary requirements as per trichloroethylene contents. In 2018 chloroform concentrations in drinking water were higher than hygienic standards in 83.3% samples taken in Volgograd region; in 37.9% samples taken in Kirov region; in 36.3% samples taken in Karelia; etc.

Increased chemicals concentrations in drinking water can have adverse effects on the urogenital, musculoskeletal, endocrine, nervous, and cardiovascular system, digestive organs, skin, blood system, and the immune system; they can also exert negative influence on

development processes in a body and the reproductive system[15].

There was a decrease in additional death cases that were probably caused by drinking water contamination in the RF over 2014-2018 (by 8.3% among the overall population). This parameter went down by 1.76% in 2018 against 2016. In 2018 in the RF in general a number of additional death cases caused by any reason associated with poor quality of drinking water from centralized water supply systems probabilistically amounted to 10.93 cases per 100 thousand people or 0.9% of all the death cases.

In 2018 in the RF in general a number of additional diseases caused by drinking water contamination probabilistically amounted to 1,201.3 cases per 100 thousand people (1.5% of the overall incidence among the RF population), and 437.5 cases per 100 thousand children (1.3% of the overall incidence among children). There was a slight decrease in a number of additional disease cases caused by drinking water contamination; the parameter fell by 2.05% against 2014 among the overall RF population.

Chlorine and chlorinated organic compounds, ammonia and ammonia-ion, iron, manganese, arsenic, nickel, and copper in their concentrations in drinking water higher than MPC as well as microbiological contamination of drinking water make the greatest contribution into occurrence of additional incidence cases caused by poor quality of dirking water taken from centralized water supply systems.

In 2018 91.4% of the overall country population was provided with drinking water conforming to safety requirements and it was by 2.0% higher than in 2014[16].

There was a preliminary assessment aimed at determining a share of urban population in the RF who were provided with drinking water from centralized water supply systems with its quality conforming to all the requirements fixed in the methodical guidelines issued in 2019; the assessment revealed that a target figure fixed by in the Federal Project "Pure water" was already reached in 2018 in 6 RF regions including Moscow city (99.63%), Saint Petersburg (99.99%), Kabardino-Balkaria (100%), Stavropol region (99.99%), Kamchatka (99.95%), and Astrakhan region (99.01%)[17].

According to preliminary assessments, in 2018 another target figure for 2024 fixed in the Federal Project "Pure water" (90.8% of the overall country population provided with qualitative drinking water from centralized water supply systems) was achieved in 7 RF regions including Saint Petersburg (100%), Moscow city (99.6%), Kamchatka (98.7%), Sevastopol (97.4%), Stavropol region (95,5%), North Ossetia - Alania (94.1%), and Lipetsk region (90.8%).

We applied clusterization as per a set of 8 parameters including "changes in a share (%) of population provided with qualitative drinking water from centralized water supply systems" as a result, all 85 RF regions were divided into 3 clusters (3 types of territory) (Figure and Table).

The first cluster included 44 regions in the RF (Figure). All these regions had similar trends such as an increase (by 3.08% on average) in a share of population provided with qualitative drinking water from centralized water supply systems, a considerable decrease in a number of additional incidence cases (by 4.7 cases per 1,000 people) and death cases (by 0.04 cases per 1,000 people) among population caused by poor quality of drinking water, and less frequently detected violations of Clause 19, 52-FZ, by economic entities dealing with water collection and purification, and

[15] On sanitary-epidemiologic welfare of the population in the Russian Federation in 2018: The state report. – M.: The Federal Service for Surveillance over Consumer Rights Protection and Human Well-being, 2019. – 254 p.

[16] On sanitary-epidemiologic welfare of the population in the Russian Federation in 2018: The state report. – M.: The Federal Service for Surveillance over Consumer Rights Protection and Human Well-being, 2019. – 254 p.

[17] MG 2.1.4.0143-19. Guidelines on how to assess improvements in quality of drinking water taken from centralized water supply systems / approved by the RF Chief Sanitary Inspector on March 27, 2019. – M.: The Federal Service for Surveillance over Consumer Rights Protection and Human Well-being, 2019. – 10 p.

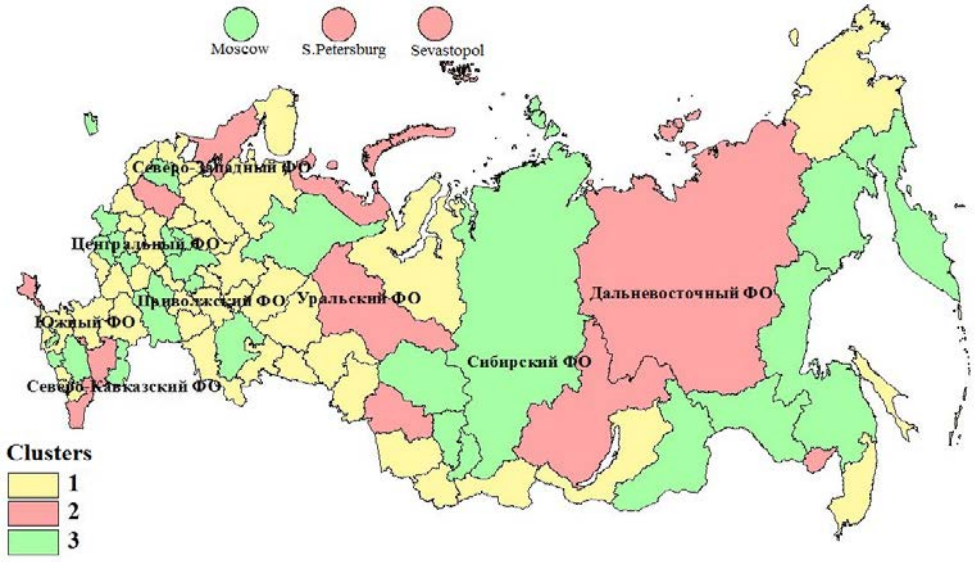

Figure. Results of cluster analysis: RF regions divided into 3 clusters as per a set of parameters

Table

Average parameter values in clusters

Parameters	Average parameter value in a cluster		
	1	2	3
Changes (Δ) in a share of population provided with qualitative drinking water from centralized water supply systems (%) in 2014-2018	3.08%	-1.76%	7.75%
Changes (Δ) in incidence among population in a region caused by poor quality of drinking water (cases per 1,000 people) in 2014-2018	-4.70	11.37	-0.19
Changes (Δ) in population mortality in a region caused by poor quality of drinking water (cases per 1,000 people) in 2014-2018	-0.04	0.08	-0.01
Changes (Δ) in frequency of violations concerning Clause 19, 52-FZ, in a region in the sphere of water collection and purification (%) in 2014-2018	-0.44%	-0.48%	-0.20%
Changes (Δ)in frequency of violations concerning Clause 19, 52-FZ, in a region in the sphere of water distribution (%) in 2014-2018	-0.20%	-3.44%	0.03%
Changes (Δ) in incidence among population in a region caused by poor quality of drinking water and prevented by Rospotrebnadzor activities (cases per 1,000 people) in 2014-2018	-1.48	-1.37	9.32
Changes (Δ) in mortality among population in a region caused by poor quality of drinking water and prevented by Rospotrebnadzor activities (cases per 1,000 people) in 2014-2018	-0.02	-0.05	0.09
A ratio of gross regional product per capita in a RF region in 2017 to the same parameter in 2014 (inflation taken into account), times	0.92	1.07	0.96

water distribution (by 0.44 % and 0.20% respectively, Table). Activities performed by Rospotrebnadzor in the regions from **the first cluster** and aimed at preventing and reducing population incidence and mortality caused by poor quality of drinking water resulted in a decrease in these parameters, by 1.48% and 0.02% accordingly. Gross regional product per capita decreased in these regions over the analyzed period (by 0.92 times, Table).

Overall, these regions have quite favorable trends as regards a growing share of population provided with qualitative drinking water and declining incidence and mortality among population caused by poor quality of drinking water; however, activities performed

by Rospotrebnadzor in these regions and aimed at preventing drinking water contamination and reducing health disorders caused by such contamination are becoming less efficient.

Besides, average cluster efficiency of Rospotrebnadzor activities (as per prevented GRP losses caused by drinking water quality) was the lowest among all three clusters in 2018 and amounted to 40.71 rubles per 1 spent ruble (average value for the country in general is 58.47 rubles per 1 spent ruble). The highest efficiency in this cluster was achieved in the following regions (in the descending order): Tyumen region, Perm region, and Tula region (127.93–115.29 rubles per 1 spent ruble).

The situation in these regions requires systematic work on increasing efficiency of Rospotrebnadzor activities aimed at improving drinking water quality including those based on the risk-oriented approach.

The second cluster included 13 regions: Saint Petersburg, Jewish Autonomous Area, Irkutsk region, Nenets Autonomous Area, Novosibirsk region, Dagestan, Kalmykia, Karelia, Yakutia, Tver' region, Khanty-Mansi Autonomous Area, Crimea, and Sevastopol (Figure).

There was the greatest spread of average parameter values in this cluster (Table): a decrease in a share of population provided with qualitative drinking water from centralized water supply systems (–1.76%); a growth in mortality (+0.08 cases per 1,000 people) and incidence (+11.37 cases per 1,000 people) among population caused by poor quality of drinking water; less frequently detected violations of Clause 19, 52-FL, by economic entities dealing with water collection and purification (–0.48%) and water distribution (–3.44%); a decrease in number of deaths and incidence cases caused by poor quality of drinking water and prevented by Rospotrebnadzor activities (by 1.37 cases per 1,000 people and by 0.05 cases per 1,000 people, accordingly); an insignificant growth in gross regional product per capita in 2017 against 2014 (by 1.07 times).

Average cluster efficiency of activities performed by Rospotrebnadzor in 2018 was higher in the 2nd cluster than in the 1st one, 48.09 rubles per 1 spent ruble, but still it was lower than in the country on average. The highest efficiency in this cluster was achieved in 2018 in the following regions (in the descending order): Saint Petersburg, Khanty-Mansi Autonomous Area, and Sevastopol (116.02–77.75 rubles per 1 spent ruble).

So, **the second cluster** regions require developing and implementing certain measures aimed at reducing health disorders caused by poor quality of drinking water, wider implementation of the risk-oriented approach and an increase in efficiency of Rospotrebnadzor activities.

The third cluster included 28 regions where a share of population provided with qualitative drinking water from centralized water supply systems grew the most rapidly.

Besides, all the regions **in the third cluster** had a certain decrease in population mortality and incidence caused by poor quality of drinking water (–0.01 cases per 1,000 people and –0.19 cases per 1,000 people, respectively).

Also in these regions there was a slight decrease in frequency of violations by economic entities dealing with water collection and purification (–0.20%) and a slight growth in the parameter in the sphere of water distribution (+0.03%) (Table).

RF regions from the 3rd cluster had a slight decrease in their gross regional product per capita in 2017 against 2014 (by 0.96 times).

Activities performed by Rospotrebnadzor aimed at preventing mortality and incidence caused by poor quality of drinking water were the most efficient in these regions (+0.09 cases per 1,000 people and +9.32 cases per 1,000 people, respectively).

Average cluster efficiency of activities performed by Rospotrebnadzor in 2018 was the highest in this cluster among all three, 60.62 rubles per 1 spent ruble, and it was close to its average value in the country (58.47 rubles per 1 spent ruble). The highest efficiency in this cluster was achieved in 2018 in the following regions (in the descending order): Moscow city, Kemerovo region, Amur region, and Orel region (194.47–100.20 rubles per 1 spent ruble).

Overall, regions **in the third cluster** tend to have the highest share of population pro-

vided with qualitative drinking water; steadily low incidence and mortality among population caused by poor quality of drinking water; high efficiency and productivity of activities by Rospotrebnadzor aimed at preventing such health disorders among population (60.62 rubles per 1 spent ruble in the cluster on average); and a slight decrease in the gross regional product in 2017 against 2014.

Conclusions and recommendations:

1. The research has revealed that certain chemicals can be considered priority ones in the RF as their contents in drinking water were higher than hygienic standards in more than 5% samples; they are bromine, silicon, lithium, iron, sodium, magnesium, boron, manganese, strontium, sulfides and hydrogen sulfide, and some other compounds.

2. Within social and hygienic monitoring, it is necessary to performed stricter control over contents of chemicals that belong to the 1st hazard category including chlorinated organic compounds (chloroform, bromdichloromethane, trichloroethylene, 1,2-dichloroethane, dichloromethane, tetrachloromethane, tetrachloroethylene etc.), and arsenic.

3. Chlorine and chlorinated organic compounds, ammonia and ammonia-ion, iron, manganese, arsenic, nickel, and copper in their concentrations in drinking water higher than MPC as well as microbiological contamination of drinking water make the greatest contribution into occurrence of additional incidence cases caused by poor quality of dirking water taken from centralized water supply systems.

4. Over the last 5 years (2014–2018) there has been a steady growth in a share of RF population provided with safe drinking water from centralized drinking water supply systems. In 2018 91.4% of the total country population was provided with drinking water that conformed to safety requirements and it was by 2.0% higher than in 2014.

5. All the RF regions were divided into three clusters (three types of territories) as per results of cluster analysis performed with a set of parameters that characterized efficiency of Rospotrebnadzor activities concerning objects under surveillance dealing with water collection, purification, and distribution:

– regions in **the first cluster** had quite favorable trends such as a growing share of population provided with qualitative drinking water, falling incidence and mortality among population caused by poor quality of drinking water; however, efficiency of Rospotrebnadzor activities performed in them decreased over 2014-2018. It is necessary to systematically increase efficiency of activities performed by Rospotrebnadzor and aimed at improving drinking water quality;

– regions in **the second cluster** had an increase in mortality and incidence among population caused by poor quality of drinking water, Rospotrebnadzor activities aimed at reducing them were not efficient, but there was a rapid growth in gross regional product per capita in those regions. For these regions, it is necessary to develop and implement urgent measures aimed at preventing health disorders caused by poor quality of drinking water, wider implementation of the risk-oriented approach, and increasing efficiency of Rospotrebnadzor activities;

– activities performed by Rospotrebnadzor in regions in **the third cluster** were the most productive and efficient; a share of population provided with qualitative drinking water was also the highest there; population incidence and mortality caused by poor quality of drinking water were steadily low in those regions; activities performed by Rospotrebnadzor and aimed at preventing health disorders caused by poor quality of drinking water were the most productive and cost-effective (60.62 rubles per 1 spent ruble in the cluster on average); there was a slight decrease in the gross regional product in those regions in 2017 against 2014.

6. In 2018 efficiency of Rospotrebnadzor activities as per prevented GRP losses caused by drinking water quality amounted to 58.47 rubles per 1 spent ruble in the country on average. The highest cost efficiency was detected in the 3rd cluster; and the lowest one, in the 1st cluster (40.71 rubles per 1 spent ruble).

7. It is necessary to develop regional action plans aimed at maintaining provision of population with qualitative drinking water that has already been reached; improving control and surveillance activities with the risk-oriented approach being widely implemented; setting goals to achieve target figures fixed in national projects and federal programs (in particular, The Federal Project “Pure water” and the National Project “Ecology”). All this should be done taking into account regional peculiarities and cluster analysis results that allowed dividing all the RF regions into three different clusters.

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ASSESSING RISKS OF RESPIRATORY ORGANS DISEASES AND CO-MORBID PATHOLOGY IN CHILDREN CAUSED BY AMBIENT AIR CONTAMINATION WITH TECHNOGENIC CHEMICALS (COHORT STUDY)

O.A. Maklakova[1,2]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation
[2]Perm State University, Россия, 15 Bukireva Str., Perm, 614990, Russian Federation

We conducted a cohort study which included 144 children who were divided into 3 groups. The test group A was made up of 47 children who lived on a territory where ambient air was contaminated with benzene, phenol, formaldehyde, and particulate matter. The test group B included 45 children exposed to aerogenic introduction of metals (vanadium and manganese). The reference group was made up of 22 children who lived on a territory which was safe in terms of its sanitary-hygienic state.

It was detected that 87.2 % children aged 4–6 who were exposed to aerogenic impacts by benzene, phenol, formaldehyde, and particulate matter had allergic rhinitis, and two thirds of children with respiratory organs diseases had secondary immune failure. By the first school year, probability of allergic rhinitis, bronchial asthma, recurrent bronchitis and functional pathologies in the gastrointestinal tract grew by 4.6–7.9 times. When children reached 11–14 years, frequency of chronic diseases in the gastrointestinal tract among them grew by 14.5 times, and probability of secondary immune failure and disorders in the vegetative nervous system was 6.0–6.6 times higher.

It was noted that ambient air contamination with metals resulted in chronic diseases in the lymphoid tissue of the nasopharynx diagnosed in 65.8 % children; children who were exposed to metals in ambient air ran 1.9 times higher risks of combined pathologies in their respiratory organs and the nervous system. When children reached 7–10 years, probability of allergic rhinitis, pathologies in the lymphoid tissue of the nasopharynx, and functional disorders in the digestive system was 3.9–5.3 times higher. Senior schoolchildren who suffered from chronic respiratory organs diseases ran 2.7–3.0 times higher risks of vegetative dystonia and secondary immune failure.

Key words: *children, respiratory organs diseases, co-morbid pathology, particulate matter, benzene, phenol, formaldehyde, metals, cohort study.*

Over the last decades diseases in the respiratory organs have permanently taken the leading place in the structure of overall incidence among children younger than 14. This pathology accounts for 45.8–59.4% among children of various ages [1–3]. In 2017 in the Russian Federation prevalence of the respiratory pathology diagnosed in a child for the first time amounted to 117,449.94 cases per 100 thousand children. Prevalence of the pathology in 51 RF regions, Perm region included, was higher than in the country on average[1]. Epidemiologic research revealed that technogenic environmental factors exerted adverse impacts on population health; according to the WHO data, each third disease in a child is caused by adverse effects produced by environmental factors [4–7]. Growing emissions from motor transport and industrial enterprises result in greater ambient air contamination in large industrial centers with a mixture of chemicals that contains particulate matter, metals, aromatic hydrocarbons, formaldehyde, and other compounds [8, 9]. Despite some improvements in the ambient air quality detected in recent years, in 2017 in the RF high air contamination levels were detected in 15 regions; the air was predomi-

Ol'ga A. Maklakova – Head of the Advisory and Polyclinic Department; Associate Professor at Human Ecology and Life Activities Safety Department (e-mail: olga_mcl@fcrisk.ru; tel.: +7 (342) 236-80-984; ORCID: http://orcid.org/0000-0001-9574-9353).

[1] Incidence among children (aged 0–14) in Russia in 2017: statistical data // the Department for monitoring, analysis, and strategic development of the public healthcare, The RF Public Healthcare Ministry. – M., 2018. – Part V. – p. 143.

nantly contaminated with heavy metals, phenol, and particulate matter[2].

According to numerous research works, an adverse ecological situation causes risks of health disorders among children, first of all, in the respiratory organs [4–7, 11–16]. Incidence with respiratory pathologies tends to be 2.0-4.6 times higher in industrial cities; it is especially true for allergic diseases and secondary immune failures with their prevalence being by 1.5 times higher than in the country on average [4, 6, 7, 14–17]. Diseases of the respiratory organs are known to be mostly recurrent under exposure to adverse technogenic chemical factors and they are often combined with various syndromes and diseases [13–16]. When a concomitant pathology occurs, it makes drastic changes in a clinical picture of the main pathologic process and determines nature and gravity of possible complications; all the above mentioned makes it difficult to diagnose and treat a disease and deteriorates a patient's life quality [4, 16–18].

At present there are a lot of vital tasks the preventive medicine is to solve; one of them is to assess risks of co-morbid pathology occurrence in children with chronic diseases in the respiratory organs under aerogenic exposure to technogenic chemicals.

Our research goal was to perform a cohort study in order to assess structure and dynamics of diseases in the respiratory organs and co-morbid pathology occurrence in children who permanently lived on territories where ambient air was contaminated with various technogenic chemicals.

Data and methods

We performed a two-direction cohort study on children who lived under various combined aerogenic exposure to chemical environmental factors; the basic goal was to reveal peculiarities related to development of diseases in the respiratory organs and co-morbid pathology in them. Children were included into our study with random sampling technique; all of them underwent in-patient treatment and regular medical check-ups in 2000–2017 at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. We divided the children into three cohorts.

The focus group A was made up of 47 children (55.3% boys and 44.7% girls, their average age was 5.33±0.35 at the moment when the study was performed), who were born and permanently lived in an industrial city in Perm region. According to data obtained within social and hygienic monitoring activities performed by the Perm Regional Center for Hygiene and Epidemiology, children from the focus group A lived on a territory where in 2000–2016 maximum single concentrations of particulate matter in the air amounted to 1.6-2.8 MPC; phenol, 1.5-3.5 MPC: formaldehyde, up to 2.2 MPC; benzene, up to 14.7 MPC. Average annual concentrations of phenol amounted to 1.3-2.3 MPC a.a.; particulate matter, 1.1–1.3 MPC a.a.; formaldehyde, 1.4–3.0 MPC a.a. (in 2011–2013).

The focus group B included 45 children (51.1% boys and 48.9% girls, their average age being 5.66±0.29) who permanently lived in a city in Perm region where a ferrous metallurgy plant was located. Experts from the Perm Regional Center for Hygiene and Epidemiology and the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies assessed ambient air quality within social and hygienic monitoring activities in 2010–2013 and 2015 on a territory where children from the focus group B lived. As a result, they detected concentrations of manganese and vanadium that were higher than MPC (up to 2.19 MPC a.a. and 1.2 MPC a.a. accordingly); particulate matter concentrations were up to 0.6 MPC a.a.

The reference group included 22 children (54.5% boys and 45.5% girls, their average age being 5.93±0.30) who lived on a territory that was sanitary and hygienically safe. All three groups were comparable in terms of sex, age, and social status.

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Cohort observation period lasted from 4 to 10 years. The second medical examination was accomplished at age 7–10 on 37 children from the focus group A (their average age was 8.27±0.32 at that moment), 38 children from the focus group B (their average age was 8.43±0.42), and 16 children from the reference group (their average age was 8.67±0.46). 46 children had the third medical examination when they were 11-14 (16 children from the focus group A, 18 children from the focus group B, and 12 children from the reference group).

Medical and biological research was performed in full conformity with the ethical principles stated in Helsinki Declaration (1975) and the RF State Standard GOST-R 52379-2005[3]. The examination included medical and social questioning; analysis of data from outpatient case histories (the Form No. 122/u); inspections by medical experts (pediatrician, neurologist, otorhinolaryngologist, allergologist-immunologist, and gastroenterologist); laboratory diagnostics (complete blood count, biochemical blood test, and immunoassay); functional and instrumental research techniques (spirography, electrocardiography, heart rate assessment, ultrasound examination of the abdominal cavity organs). All the examinations were conducted according to conventional procedures.

Statistical analysis included conventional techniques of descriptive statistics; we calculated odds ratio (OR), relative risk, and 95% confidence interval (CI) and evaluated validity of discrepancies as per Pearson χ^2 test and Fischer's test. Probability calculated with Student's t-test was taken as a statistical significance level when it was $p \leq 0.05$ [20].

Research results

Results of the first medical examination revealed that pre-school children from the focus groups A and B predominantly suffered from allergic diseases in the upper respiratory tracts (J30.0, J30.3, J31.0), which were 1.3 times more frequently detected in them than in children from the reference group (p=0.05) (Table 1). We revealed that bronchial asthma (J45.0) and recurrent bronchitis (J39.8, J44.8) were 1.7 times more frequently detected in the focus group A, and chronic lymph-proliferative diseases in the nasopharynx (J35.0, J35.1, J35.2, J35.3) were 2.1 times more frequently detected in the focus group B than in the reference group (p=0.05–0.007).

It was detected that when ambient air was contaminated with benzene, phenol, formaldehyde, and particulate matter, it caused 14.6 times higher risk of allergic rhinitis for pre-school children (OR=14.64; CI=4.24–50.62); exposure to adverse aerogenic impacts by metals caused 4.3 higher risks of tonsils and adenoids hypertrophy (OR=4.29; CI=1.44–12.75), against children living on a territory where an ecological situation corresponded to sanitary-hygienic standards.

Secondary immune failure (D83.9) was diagnosed in 70.2% children aged 4–6 in the focus group A and it was 1.3 times more frequently than in the focus group B, and 1.4 times more frequently than in the reference group (p=0.06–0.04) (Table 2). Disorders in the autonomic nervous system (G90.8) were registered 1.4 times more frequently in children from the focus group B than in those from the reference group (p=0.05). Functional pathologies in the gastrointestinal tract (K30, K83.8, K86.8) were detected with the same frequency in pre-school children from the focus groups A and B and it was 1.3–1.4 times more frequent than in the reference group (p=0.04–0.014). Overall, functional disorders in the gastrointestinal tract were 3.3–4.6 times more probable for 4–6-year-old children exposed to the environment contaminated with technogenic chemicals (the focus group A had OR=4.57; CI=1.28–16.32; the focus group B had OR=3.27; CI=1.00–10.66).

Our research revealed that two thirds of 4-6-year-old children from the focus group A suffering from respiratory organs diseases also had secondary immune failure and it was 1.5 times higher than in the reference group (p=0.04). 71.1% children who were exposed to

[3] GOST R 52379-2005. The RF State Standard. Good Clinical Practice (ICH E6 GCP) [Web-source]. – M.: Standartinform, 2005. – URL: http://docs.cntd.ru/document/1200041147 (date of visit January 26, 2019).

Table 1

Incidence among children aged 4–6 (the first examination), %

Nosology	Focus group A	Focus group B	Reference group	Validity of discrepancies		
				p_1	p_2	p_3
Chronic tonsillitis, hypertrophy of tonsils and adenoids (J35.0, J35.1, J35.2, J35.3)	42.6	65.8	31.8	0.39	0.007	0.026
Allergic rhinitis, pollinosis (J30.0, J30.1, J30.3)	87.2	86.8	68.2	0.05	0.05	0.96
Recurrent bronchitis, tracheitis (J39.8, J44.8)	6.4	7.9	0	0.31	0.30	0.78
Bronchial asthma (J45.0)	57.4	47.4	36.4	0.06	0.78	0.34
Common variable immunodeficiency (secondary) (D83.9)	70.2	52.6	50.0	0.06	0.80	0.04
Disorders of the autonomic nervous system (G90.8)	61.7	71.1	54.5	0.57	0.05	0.34
Functional dyspepsia, biliary dyskinesia, reactive pancreatitis (K30, K83.8, K86.8)	88.9	85.1	63.6	0.014	0.04	0.59
Chronic gastritis, chronic gastroduodenitis (K29.5, K29.9)	4.3	11.1	9.1	0.29	0.32	0.15

Note:
p_1 is validity of discrepancies between the focus group A and the reference group;
p_2 is validity of discrepancies between the focus group B and the reference group;
p_3 is validity of discrepancies between the focus group A and the focus group B.

Table 2

Incidence among children aged 7–10 (the second examination), %

Nosology	Focus group A	Focus group B	Reference group	Validity of discrepancies		
				p_1	p_2	p_3
Chronic tonsillitis, hypertrophy of tonsils and adenoids (J35.0, J35.1, J35.2, J35.3)	48.6	65.8	31.2	0.24	0.019	0.06
Allergic rhinitis, pollinosis (J30.0, J30.1, J30.3)	91.9	86.8	62.5	0.01	0.043	0.23
Recurrent bronchitis, tracheitis (J39.8, J44.8)	10.8	7.9	0	0.23	0.34	0.28
Bronchial asthma (J45.0)	59.5	47.4	31.2	0.04	0.27	0.29
Common variable immunodeficiency (secondary) (D83.9)	72.9	52.6	50.0	0.07	0.86	0.04
Disorders of the autonomic nervous system (G90.8)	67.6	71.1	56.2	0.43	0.29	0.74
Functional dyspepsia, biliary dyskinesia, reactive pancreatitis (K30, K83.8, K86.8)	94.6	92.1	68.7	0.02	0.04	0.33
Chronic gastritis, chronic gastroduodenitis (K29.5, K29.9)	24.3	31.6	18.7	0.26	0.18	0.48

Note:
p_1 – is validity of discrepancies between the focus group A and the reference group;
p_2 – is validity of discrepancies between the focus group B and the reference group;
p_3 – is validity of discrepancies between the focus group A and the focus group B.

the air contaminated with metals suffered from respiratory organs diseases combined with vegetative-vascular dystonia and it was 1.4 times higher than in the reference group (p=0.05). Relative risk of pathologies in the nervous system amounted to 1.86 for children from the focus group B suffering from chronic diseases of the respiratory organs (RR=1.86; 95% CI: 1.16–2.99).

The second control medical check-up revealed a greater number of respiratory pathologies among children under aerogenic exposure to technogenic chemicals (Table 3). There was still high prevalence of allergic rhinitis on the examined territories, which was 1.4–1.5 times higher among children from the focus groups than among those from the reference group (p=0.043–0.01). We detected 6.8 times higher probability of allergic rhinitis among children from the focus group A (OR=6.80; CI=1.44–32.20), and 3.9 times higher probability of the disease among children from the focus group B (OR=3.96; CI=0.99–15.77). Diseases of the lymphoid tissue in the nasopharynx were detected in ⅔ junior school children who were exposed to adverse aerogenic impacts exerted by metals, and the value was 2.1 times statistically significantly higher than in the reference group (p=0.019), and 1.4 times higher than in the focus group A. We detected that chronic lymphproliferative diseases of the nasopharynx were 4.2 times more probable among junior schoolchildren from the focus group B (OR=4.23; CI=1.21–14.79).

We noted that prevalence of chronic allergic pathologies in the lower respiratory tracts (J45.0, J39.8, J44.8) was 2.25 times higher on the territory where ambient air was contaminated with benzene, phenol, formaldehyde, and particulate matter (the focus group A), than on the reference territory (p=0.012), and 1.3 times higher than on the territory exposed to contamination with metals (the focus group B, p=0.08) (Table 2). We detected that junior schoolchildren from the focus group A ran 4.6 times higher risks of bronchial asthma and recurrent bronchitis

Table 3

Incidence among children aged 11–14 (the third examination), %

Nosology	Focus group A	Focus group B	Reference group	Validity of discrepancies		
				p_1	p_2	p_3
Chronic tonsillitis, hypertrophy of tonsils and adenoids (J35.0, J35.1, J35.2, J35.3)	31.3	66.7	33.3	0.31	0.06	0.03
Allergic rhinitis, pollinosis (J30.0, J30.1, J30.3)	93.8	83.3	66.7	0.06	0.20	0.28
Recurrent bronchitis, tracheitis (J39.8, J44.8)	0	8.3	0	–	0.60	–
Bronchial asthma (J45.0)	62.5	58.3	50.0	0.51	0.65	0.80
Common variable immunodeficiency (secondary) (D83.9)	68.7	75.0	25.0	0.02	0.007	0.68
Disorders of the autonomic nervous system (G90.8)	75.0	91.7	33.3	0.03	0.001	0.12
Functional dyspepsia, biliary dyskinesia, reactive pancreatitis (K30, K83.8, K86.8)	100.0	83.3	66.7	0.02	0.29	0.09
Chronic gastritis, chronic gastroduodenitis (K29.5, K29.9)	62.5	27.8	25.0	0.05	0.86	0.04

Note:
p_1 – is validity of discrepancies between the focus group A and the reference group;
p_2 – is validity of discrepancies between the focus group B and the reference group;
p_3 – is validity of discrepancies between the focus group A and the focus group B.

(OR=4.58; CI=1.30–16.18) than children from the reference group who lived on a hygienically safe territory. Besides, air contamination with benzene, phenol, formaldehyde, and particulate matter caused 1.4–1.5 more frequent secondary immune failure among children from the focus group A than among those from the focus group B and the reference group (p=0.07–0.04). We revealed functional pathologies of the gastrointestinal tract in 92.1–94.6% children aged 7–10 living on a territory exposed to adverse effects produced by technogenic chemicals and it was 1.3–1.4 times statistically significantly higher than in the reference group (p=0.04–0.02). Junior schoolchildren who were exposed to adverse impacts by technogenic chemicals ran 5.3–7.9 times higher risks of functional disorders in the gastrointestinal tract (the focus group A: OR=7.95; CI=1.35–46.90; the focus group B: OR=5.30; CI=1.09–25.84) than children from the reference group who lived on a hygienically safe territory.

The third check up that was performed on senior schoolchildren revealed that there still was high prevalence of allergic rhinitis under aerogenic exposure to adverse impacts by technogenic chemicals (93.8–83.3%) and it was 1.2 times higher than in the reference group (66.7%, p=0.06–0.20) (Table 4). ⅔ of children aged 11–14 who lived under aerogenic exposure to metals suffered from chronic diseases of the lymphoid tissue in the nasopharynx and it was 2.0–2.1 times higher than in the focus group A and the reference group (p=0.06–0.03). We noted that secondary immune failure among children from the focus group A aged 11–14 remained practically the same as it had been revealed at the second examination; but there was a 1.4-time growth in its prevalence over 3 years among children from the focus group B. There was a 2.0-time decrease in prevalence of this pathology among children from the reference group, and discrepancies between this group and focus groups A and B became statistically significant (p=0.02–0.007). We detected that 11–14-year-old children who lived on a territory where the air was contaminated with technogenic chemicals or heavy metals ran 6.6-9.0 times higher risks of secondary immune failure (the focus group A: OR=6.60; CI=1.23–35.44; the focus group B: OR=9.0; CI=1.42–57.12) than children from the reference group.

Over a 6-year-period a number of children with disorders in the autonomic nervous systems grew by 1.2–1.4 times (Table 4), and it was 2.3–2.7 times statistically significantly higher than in the reference group (p=0.03–0.001). We detected that children aged 11–14 who were exposed to ambient air contamination with technogenic chemicals ran 6.0-22.0 times higher risks of disorders in the autonomic nervous system (G90.8) than children from the reference group (the focus group A: OR=6.00; CI=1.15–31.23; the focus group B: OR=22.0; CI=2.05–236.05). Besides all the children who lived on a territory where ambient air was contaminated with benzene, phenol, formaldehyde, and particulate matter, turned out to have functional pathologies in the gastrointestinal tract, and ⅔ of them suffered from chronic diseases of the upper gastrointestinal tract section (K29.5, K29.9) and it was 1.2–2.5 times statistically significantly higher than in the reference group and the focus group B (p=0.05–0.02). Prevalence of chronic pathologies in the gastrointestinal tract grew by 14.5 times in the focus group A over the total observation period, but it went up only by 2.5–2.7 times in two other groups.

We also detected that senior schoolchildren who suffered from chronic respiratory organs diseases also had secondary immune failure 2.7 times more frequently in the focus group A and 3.0 times more frequently in the focus group B than in the reference group (p=0.02–0.007). Relative risk of combined chronic respiratory diseases and secondary immune deficiency amounted to 3.0 among children exposed to adverse aerogenic effects produced by metals (RR=3.00; 95% CI: 1.07–8.43). Number of children who suffered from chronic respiratory pathologies and disorders in the autonomic nervous system and who were ex-

posed to negative impacts by technogenic chemicals was 2.2 times higher in the focus group A and 2.7 times higher in the focus group B (p=0.03–0.001) than in the reference group. We detected that children who were under aerogenic exposure to metals ran the maximum risk of combined chronic respiratory diseases and vegetative-vascular dystonia which was equal to 2.75 (RR=2.75; 95% CI: 1.21–6.23).

Conclusions:

1. Most pre-school children (87.2%) who were exposed to ambient air contamination with benzene, phenol, formaldehyde, and particulate matter, suffered from allergic rhinitis, and each third child had bronchial asthma and recurrent bronchitis; respiratory organs diseases were combined with immune failure in ⅔ cases. We detected that probability of allergic rhinitis, bronchial asthma, and recurrent bronchitis grew by 4.6–6.8 times over 3–5 years of cohort observations over children by the time they were 7–10 years old. Probability of functional pathologies in the gastrointestinal tract grew by 7.9 times over the same period. By the time children were 11–14 years old, prevalence of chronic diseases in the gastrointestinal tract grew by 14.5 times, and secondary immune failure and disorders in the autonomic nervous system were diagnosed 6.0–6.6 times more frequently.

2. Each third child aged 4–6 who lived on a territory where ambient air was contaminated with metals (vanadium and manganese) suffered from a chronic disease of the lymphoid tissue in the nasopharynx, and ¾ of such children had disorders in the autonomic nervous system and ran 1.9 times higher risk of combined respiratory diseases and nervous system pathologies. Probability of allergic rhinitis and pathologies of the lymphoid tissues in the nasopharynx was 3.9–4.2 times higher among junior schoolchildren; probability of functional disorders in the digestive organs, 5.3 times higher. Senior schoolchildren who suffered from chronic respiratory organs diseases ran 2.7–3.0 times higher risk of disorders in the autonomic nervous system and secondary immune failure.

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CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF MALIGNANT NEOPLASMS IN THE THYROID GLAND IN CHELYABINSK REGION POPULATION OVER 1998–2016

S.A. Shalaginov[1], L.Yu.Krestinina[1], A.S. Domozhirova[2], S.B. Sergiyko[3,4]

[1]Urals Research Center for Radiation Medicine, 68 A Vorovsky Str., Chelyabinsk, 454076, Russian Federation
[2]Chelyabinsk Regional Clinical Center for Oncology and Nuclear Medicine, 42 Blyuhera Str., Chelyabinsk, 454076, Russian Federation
[3]South-Ural State Medical University of the Ministry of Healthcare of the Russian Federation, 64 Vorovsky Str., Chelyabinsk, 454092, Russian Federation
[4]City Clinical Hospital № 1, 16 Vorovsky Str., Chelyabinsk, 454048, Russian Federation

The paper dwells on assessment of dynamics in incidence with malignant neoplasms in the thyroid gland among Chelyabinsk region population over 1998-2016. Dynamics is taken in different age groups; incidence with different malignant neoplasms is characterized among men and women. The authors took data from major medical and diagnostic institutions in Chelyabinsk region where information about patients suffering from malignant neoplasms in the thyroid gland could be accumulated. We detected 4,467 people with the diagnose confirmed with histological (93.2%) and cytological (6.8%) research.

We detected that primary incidence with malignant neoplasms in the thyroid gland tended to grow in Chelyabinsk region and it completely corresponded to the dynamics in the Russian Federation as a whole. This growth was mostly due to an increase in primary incidence among people aged 60 and older. Papillary carcinoma is the most widely spread malignant neoplasm with 68.1% cases in the overall structure of thyroid gland carcinoma in Chelyabinsk region. This malignant neoplasm tended to grow steadily from 64.2% in the beginning of the observation period to 73.0% at the end of it. But at the same time follicular carcinoma tended to decrease from 25.5% to 18.2% cases; just as an aggregated share of other carcinoma types which were relatively rare. Average age of men at the moment malignant neoplasms in the thyroid gland were detected in them was lower than that of women; they were 49.6±0.22 and 50.9±0.9, $p<0.001$, respectively. The highest average age was detected for people with non-differentiated malignant neoplasms (66.9±0.7); the lowest one, for people with follicular carcinoma (49.7±0.1), $p<0.001$.

Key words: *thyroid gland, malignant neoplasm, Chelyabinsk region, population, age, incidence, structure.*

A large number of studies performed at different times in different countries, as well as in different regions of Russia were devoted to examining thyroid cancer epidemiology. One would think that all the aspects of thyroid cancer have been covered in these papers. Possible range of incidence rate was estimated as being equal to 0.8–11.2 per 100,000 persons over the period 1998–2016. The range among males was 0.6–5.0; and among females, 1.2–16.0. The incidence rate among people aged 0–19 ranged from 0.5–-4.2, and it ranged from 2.1 to 16.8 per 100,000 persons among people older than 60. However, there are good reasons still attracting epidemiologists' attention to the problem of thyroid cancer. Perhaps, the main one is the increase in the thyroid cancer incidence over the recent years. In Russia, for example, the growth was from 5.8 to 8.3 cases per 100,000 persons [1–6]. Nowadays, it is not clear to what extent this growth is due to improved quality of diagnos-

Sergey A. Shalaginov – Candidate of Medical Sciences, Senior researcher at the epidemiological laboratory (e-mail: Shalaginov@urcrm.ru; tel.: +7 (351) 232-79-14; ORCID: https: //orcid.org/0000-0003-0497-5879).

Lyudmila Yu. Krestinina – Candidate of Medical Sciences, Head of the epidemiological laboratory (e-mail: ludmila@urcrm.ru; tel.: +7 (351) 232-79-18; ORCID: https: //orcid.org/0000-0003-0497-5879).

Alla S. Domozhirova – Doctor of Medical Sciences, Professor, Deputy to Chief Physician responsible for organizational and methodical work (e-mail: 2356563@mail.ru; tel.: +7 (351) 235-65-63; ORCID: https: //orcid.org/0000-0003-0806-3164).

Sergey V. Sergiyko – Doctor of Medical Sciences, Professor, Head of the General and Pediatric Surgery Department (e-mail: ssv_1964@mail.ru; tel.: +7 (351) 728-48-47; ORCID: https: //orcid.org/0000-0001-6694-9030).

tics, and to what extent due to environmental, medical, and biological or some other factors.

The number of published papers on the problem of thyroid cancer sharply increased after the Chernobyl accident and the catchment area went further beyond the borders of the territory that was actually contaminated with radiation. The problem of thyroid cancer acquired a different meaning for other radioactively-contaminated territories (except the territory around Chernobyl nuclear power station) including the resettlement territory for persons exposed as a result of the Mayak PA activities.

Long time after the onset of radiation contamination in the Urals, the assessment of thyroid cancer risk becomes relevant among the offspring of the exposed population. Up to now, a large number of persons exposed as a result of the Mayak PA activities and their offspring have settled in Chelyabinsk region [7–9]. Taking into account high variability of epidemiological data on thyroid cancer incidence [1–6, 10–19], obtained in different regions, solid substantiation for radiogenic risk assessment requires evaluation of background incidence rate among the population living in Chelyabinsk region.

Iodine deficiency, hypothyroidism associated with it, and a high level of TSH stand out among the main factors contributing to the onset of tumor progression in thyroid gland [20, 21]. The possibility of tumor development in thyroid gland depends on its functional and morphological status which is gender and age dependent [22]. Malignization risk increases against the background of nodular euthyroid goiter, adenomas and thyroiditis [21, 23].

The thyroid gland is highly radiosensitive and it is especially true for children. A lot of papers have been published so far concerning high risks of malignant neoplasms in the thyroid gland among various groups of irradiated population[1] [23–25]. When a solid variant of papillary carcinoma develops, it can have a particular importance. Until recently, this variant of papillary carcinoma has been treated as rather rare [27]. However, as it has been shown by recent studies, it amounted to 37 % of papillary carcinomas associated with thyroid gland exposure as a result of Chernobyl accident, and it can now be regarded as radiation-induced thyroid cancer [28].

Thus, **our research goal** was to study primary incidence and structure of thyroid cancer among the population living in Chelyabinsk region over the period 1998-2016 and it seems quite justified.

Materials and methods. We used information on all cases of thyroid cancer contained in the Chelyabinsk Regional Population Oncology Register, the medical database of the Urals Research Center for Radiation Medicine, in the archive of the histological laboratory at the anatomic pathology department of the City Clinical Hospital №1 of Chelyabinsk (in 1985, on the basis of City Clinical Hospital No. 1, a regional center of endocrine surgery was established, where all persons (including children) from Chelyabinsk region who have endocrine pathology are sent to for treatment). As a result of combining information from all available sources and primary analysis, a sampling was made up of 4,467 individuals who lived in Chelyabinsk region from 1998 to 2016, inclusive, with the newly diagnosed thyroid cancer. Repeated references to the same patient were removed from the general list due to duplication of information in different institutions or due to return visit regarding treatment, clarification of the diagnosis, recurrence of the disease or detection of metastases. All relevant consistent information taken from any of the sources mentioned above is taken into account for each patient and thyroid cancer case. These retrospective data were in some way incomplete. For example, the age at which a diagnosis was put was not known for 39 persons (0.9 %), no sex was indicated for 123 persons (2.8 %), there was no histological

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confirmation of the diagnosis for 302 persons (6.8%). Data on sex were incomplete due to the following: first, direct indication of sex could be absent; second, there could be surnames that are the same for feminine and masculine gender; third, there could be incomplete information on names and patronymics (only initials are given). When there is no histological examination data, the diagnosis of thyroid cancer was confirmed by cytological examination.

Only in 69 cases out of 4,467 (1.5%) thyroid cancer was first diagnosed post mortem (neglected cases of thyroid cancer, which led to the death of a patient, or it was accidentally detected during an autopsy in the event of death from other causes). In all these cases the diagnosis was confirmed histologically. For 98.5% of patients thyroid cancer was diagnosed in their lifetime by an endocrinologist. Thus, calculated standardized parameters of newly diagnosed thyroid cancers can be considered as indicators of primary incidence. The incidence rate was calculated as a number of newly diagnosed thyroid cancers over a period x 100,000/number of person-years over the period under consideration.The data on the population living in Chelyabinsk region over the study period are taken from statistical books of the Chelyabinsk Department of Statics. Alignment of time-series data on thyroid cancer incidence were performed with the standard method of least squares [29]. Statistical processing was carried out with χ^2 test and Student's test [30].

Results and discussion.

Analysis of thyroid cancer incidence and structure dynamics over the period 1998–2016

Distribution of males and females from different age groups with thyroid cancer reveals features of obvious similarities (Figure 1). Predominance of males aged from 20 to 24 y.o. can be an exception (6.4% and 2.8% respectively, $p < 0.001$). The maximum percentage of males with thyroid cancer (14.1%) is detected among people aged 55–59; the highest percentage of females (12.9%), among people aged 50–54. Males and females with thyroid cancer from the 50–59 age group account for 26.7% and 25.3% respectively, which is consistent with the published data (15.2%–31.1%) [1–3, 6, 18, 19].

Taking into account a similar type of distribution of males and females by age, as well as taking into account a relatively low number of males with thyroid cancer (629 persons, 14.1%) the analysis of incidence was carried out for males and females together. As it can be seen from Figure 2, the incidence in Chelyabinsk region as well as in the Russian Federation as a whole is uneven, while in both cases there is a tendency to an increase in the incidence of thyroid cancer.

We applied the least squares method to calculate parameters of incidence with thyroid cancer taking into account preliminary alignment of time-series data on thyroid cancer incidence over 19 years for Chelyabinsk region

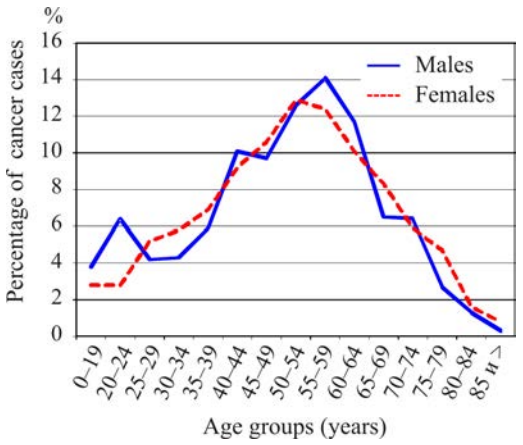

Figure 1. Thyroid cancer distribution (C73 – ICD-10) by age and sex, %

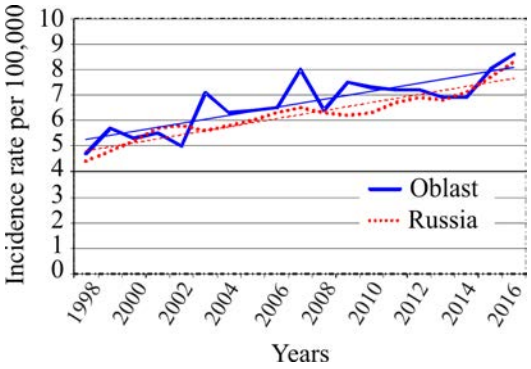

Figure 2. Dynamics of newly diagnosed thyroid cancers over the period 1998-2016 (newly diagnosed cancers per 100,000 persons per a particular year)

and the RF as a whole; the results turned out to be almost identical [29]. An absolute growth in incidence rates amounted to 0.170 and 0.174, respectively; an average growth rate was 1.3% and 1.4%; average value of 1% of growth – 0.026 and 0.028 cases per 100,000 persons.

Thus, the obtained data indicate that this increase in incidence with thyroid cancer in Chelyabinsk region is a reflection of the general Russian trend.

It can be seen that the trend lines of incidence growth go almost parallel (Figure 2). The increase in incidence over the period 1998–2016 in Chelyabinsk region was 3.91; in the Russian Federation, 3.90 cases per 100,000 person-years during the period under review. The rate of changes in incidence, especially in Chelyabinsk region, is uneven. In Chelyabinsk region, the maximum growth was registered in 2007 (by 1.74 cases compared with the previous year), in the Russian Federation the maximum growth was only 0.63 cases and it was achieved in 2015.

Table 1 presents the incidence rates of thyroid cancer for the period 1998–2016 inclusive. Over the whole reviewed period, the primary incidence rate was 6.6 cases per 100,000 person-years and it is consistent with the published epidemiological data for the most countries and the Russian Federation (according to published data, the incidence rate varied within the range 5–10 per 100,000 persons) [1–6, 10–19]. A steady growth of thyroid cancer incidence is registered in Chelyabinsk region as well as in most regions in the RF [31]. As it can be seen in Table 1, there is a steady increase in the dynamics of incidence rates per 100,000 person-years, starting from 5.2 in 1998–2002 to 7.6 in 2013–2016, while an increase in the second five-year period is statistically significant ($p < 0.01$).

The incidence rate among persons aged 0–19 decreased from 0.9 to 0.3 per 100,000 person-years (Table 2). There were no significant differences in incidence rate between the calendar periods. The average value was 0.6 cases per 100,000 person-years, which corresponds to the published data [32, 33].

Table 1

Dynamics of thyroid cancer incidence

Period, years	Amount of person-years for the period	Number of cases over the period	Incidence rate per 100,000 person-years
1998–2002	18,267.677	956	5.2
2003–2007	17,722.374	1,213	6.8*
2008–2012	17,411.500	1,239	7.1
2013–2016	13,973.315	1,059	7.6
Total	67,374.866	4,467	6.6

Note: * – $p < 0.001$ when compared with the previous period.

Table 2

Dynamics of thyroid cancer incidence among people aged 0–19

Period, years	Amount of person-years for the period	Number of cancer cases	Incidence rate per 100,000 person-years
1998–2002	4,874.823	43	0.9
2003–2007	4,277.526	25	0.6
2008–2012	3,767.369	20	0.5
2013–2016	3,051.365	10	0.3
Total	15,971.083	98	0.6

The maximum incidence rate among people aged 20–59 (Table 3) was detected in the period 2003–2007 and amounted to 8.2 cases per 100,000 person-years, the minimum was registered for the initial period – 6.5 cases per 100,000 person-years, the differences are statistically significant when the given periods are compared, $p < 0.001$.

Table 3

Dynamics of thyroid cancer incidence among people aged 20–59

Period, years	Amount of person-years	Number of cancer cases	Incidence rate per 100,000 person-years
1998–2002	10,007.895	652	6.5
2003–2007	10,325.207	844	8.2*
2008–2012	10,475.736	797	7.6
2013–2016	8,116.241	654	8.1
Total	38,925.079	2947	7.6

Note: * – $p<0.001$ when compared with the previous group.

Table 4

Dynamics of thyroid cancer incidence among people aged 60 and older

Period, Years	Amount of person-years for the period	Number of cancer cases	Incidence rate per 100,000 person-years
1998–2002	3384,959	261	7.7*
2003–2007	3119,641	344	11.0
2008–2012	3168,395	422	13.3**
2013–2016	2805,709	395	14.1
Whole period	12478,704	1422	11.4

Note:* – $p < 0.001$ when compared to other groups; ** – $p < 0.025$ when compared to the previous group.

Thyroid cancer incidence statistically significantly increases among people aged ≥ 60 (Table 4) from 7.7 cases per 100,000 person-years during the initial period to 14.1 cases per 100,000 person-years in the final period, $p<0.001$.

Over the entire examined period from 1998 to 2016 (Table 2–4), the highest primary incidence with thyroid cancer was observed among people older than 60, 11.4 cases per 100,000 person-years; the minimum value was detected among people aged 0–19, 0.6 cases per 100,000 person-years. The intermediate values of thyroid cancer incidence were detected among people aged 20–59 and amounted to 7.6 cases per 100,000 person-years. The differences in incidence rates between the age groups are statistically significant, $p<0.001$.

For the majority of thyroid cancer types (Table 5) their percentage corresponded to that in the world and in Russia [11, 13, 14, 18].

Papillary and follicular thyroid cancers predominated in Chelyabinsk region (68.1% and 22.6%, respectively) and it corresponded to the published data. Percentage of different thyroid cancers was comparable for males and females. However, the percentage of the most frequent cancers (papillary and follicular) tended to be higher in females as compared to males, whereas the percentage of rare types of cancer (medullary, undifferentiated cancers) was higher in males.

The dynamics detected in the structure of the main thyroid cancer types was characterized by a gradual increase in the percentage of the papillary cancer from 62.9% in 2003–2007 to 73.0% in 2010–2016, with the respective decrease in the percentage of the follicular cancer from 25.5% to 18.2% (Table 6). Upward tendency in the percentage of medullary cancer, downward tendency in the percentage of other, relatively rare, types of thyroid cancer was also noted.

The age of the patients with newly diagnosed thyroid cancer varied in the range equal to 9–99 years. Mean age at which the disease became apparent, which was assessed mainly based on the date when histological sampling was performed, was 50.7±0.07 years (Table 7). It was slightly higher as compared to the data given in the majority of published papers [2, 11, 12, 14, 16, 21, 22]. Mean age at which thyroid cancer was diagnosed was 50.9±0.09 for females, whereas it was 49.6±0.22 for males. The differences are statistically significant ($p < 0.001$).

The oldest mean age at which thyroid cancer was diagnosed (66.9±0.7 years) (Table 8) was observed for people with undifferentiated

Table 5

Percentage of different thyroid cancers among the population in Chelyabinsk region for the period of 1998 through 2016 depending on sex

Sex	Types of thyroid cancer					
	Papillary, %	Follicular, %	Medullary, %	Undifferentiated, %	Lymphoma, %	Other types, %*
Male	66.8	21.3	4.9	2.7	1.5	2.7
Female	68.5	22.6	3.6	2.2	1.8	1.3
Total	68.1	22.6	3.7	2.3	1.8	1.6

Note: * – other types include solid cancers, squamous cancers, embryonal tumors, and metastases of other tumors into the thyroid gland.

Table 6

Distribution of different thyroid cancers among the population in Chelyabinsk region taken in dynamics

Calendar period, years	Types of thyroid cancer		
	Papillary	Follicular	Other
1998–2002	64.2%	25.5%	10.3%
2003–2007	62.9%	27.8%	9.3%
2008–2012	71.4%	19.5%	9.1%
2013–2016	73.0%	18.2%	8.8%
Total	68.1%	22.6%	9.4%

Table 7

Distribution by sex and mean age at the time of thyroid cancer development among the population in Chelyabinsk region in 1998–2016

Sex	Number of cases	Percentage, %	Mean age (years)
Male	629	14.1	49.6±0.22*
Female	3715	83.2	50.9±0.09
Sex is unknown	123	2.7	49.6±0.94
Total	4467	100.0	50.7±0.07

Note: * – $p<0.001$ when compared to females.

Table 8

Mean age of the patients with different types of thyroid cancer

Types of thyroid cancer	Mean age		
	Male	Female	Total, including persons with unascertained sex
Papillary	48.6±0.4	50.7±0.2***	50.4±0.1*
Follicular	48.7±0.8	49.8±0.2	49.7±0.2**
Medullary	49.8±1.1	54.0±0.6***	53.0±0.6
Undifferentiated (anaplastic)	66.6±1.1	66.5±0.96	66.9±0.7
Other types	57.7±1.1	53.0±1.0****	54.1±0.8

Note: * – $p<0.002$ when compared with the follicular cancer, $p<0.001$ when compared with all the other types of cancer;

** – $p<0.001$ when compared with all the other types of cancer, except the papillary cancer;

*** – $p<0.001$ when compared with males;

**** – $p<0.002$ when compared with males.

cancers, the youngest one (49.7±1.2 years), for people with follicular cancers ($p<0.001$). For the majority of thyroid cancer types the oldest age of the disease manifestation was registered for females as compared to that for males. No differences were revealed in case of undifferentiated cancers. In the group of people with "other cancers" males were even older than females (57.7±1.1 years and 53.0±1.0, respectively, $p<0.002$).

Among 4,344 patients with thyroid cancer with ascertained sex, the females to males ratio was 5.9:1.0. It is slightly higher than the same ratio given in the majority of published papers dealing with thyroid cancer. According to them the sex ratio among people with thyroid cancer is mainly in the range 3:1–5:1 [1–6, 13, 14, 34].

Sex ratio is notably shifted in favor of females with the most frequent types of thyroid cancer: papillary and follicular, and malignant lymphomas. Sex ratio with respect to medullary cancer for the studied group of people from Chelyabinsk region was 4.3, which is lower than that for papillary and follicular cancers. At the same time it was noted in a number of published papers that the sex ratio for medullary cancers is 1:1 [14].

Conclusion. The increase in the number of newly diagnosed thyroid cancers in Chelyabinsk region over the period from 1998 through 2016 could be determined by a number of reasons. It could be associated with improved quality of diagnostics; first and foremost, with large-scale screening of persons suspected of thyroid gland diseases introduced into routine practice, and targeted histological examination of these people. One of the reasons for the increase in the number of cancers, including thyroid cancers, could also be eco-

logical factor which takes on greater and greater importance with the economic revival and industrial production growth in the whole Russian Federation, and in Chelyabinsk region in particular. It has been stated that the dynamics of the growth in the incidence rate in Chelyabinsk region is generally consistent with that in Russia as a whole. It implies common reasons for the increase in thyroid cancer incidence.

A significant increase in thyroid cancer incidence in elderly people (>60 years) that was noted in the paper could be due to the increased life expectancy in Chelyabinsk region, higher percentage of elderly people in the population structure, and shift in the critical physiological age of thyroid cancer development to higher values.

A descending trend in thyroid cancer incidence among children and teenagers could not be explained straightforwardly. However, relatively low statistics of thyroid cancers among people aged 0–19 years attracts certain attention (only 98 cases were registered over the whole examined period in Chelyabinsk region).

Some parameters characterizing the structure of thyroid cancers are in line with the published data for the majority of the world and the Russian Federation. The older mean age of females in comparison to that of males at thyroid cancer manifestation (50.9±0.09 and 49.6±0.22, respectively, $p<0.001$), is probably associated with longer life expectancy for females, or with physiological aging of males as compared to females. The revealed age-dependent differences are mainly due to papillary and, to a lesser extent, follicular cancer, i.e. those types of cancer for which main etiological factors are iodine deficit and pre-existing thyroid gland diseases which are more frequent in females.

Some increase in the percentage of follicular thyroid cancers is probably associated with regional ecological peculiarities, such as low content of iodine in the atmosphere and soil in the Urals region. The percentage of certain cancer types in males and females turned out to be comparable. It suggests similar pathogenetic mechanisms of thyroid cancer development in representatives of both sexes despite great differences in cancer detectability.

It is difficult to explain a higher rate and older mean age of the medullary thyroid cancer occurrence in females as compared to males. It is known that medullary cancers could be one of the manifestations of familial or sporadic form of hereditary complex disorder MEN-2, caused by mutations of the c-RET proto-oncogene on chromosome 10q11.2. It means that it could occur in either male or female with equal probability. Thus, it could be assumed that in Chelyabinsk region a greater part of medullary thyroid cancers were initiated by the effect produced by exogenous factors, and the revealed differences between two sexes are associated with higher predisposition of females to these factors.

It could be stated that in 1998–2016 the rate and dynamics of thyroid cancer incidence among the population in Chelyabinsk region were mainly consistent with the nation-wide rate (Figure 2). The revealed low incidence rate in childhood and young age requires further follow up and control of the data completeness.

Radioactive contamination of the territory in certain districts in the region and exposure of the population living there in 1950–1960 did not influence the average regional incidence rate of thyroid cancer in the long-term period. Nevertheless the task to determine a radiation-induced risk of thyroid cancer development in representatives from particular groups of exposed population and their offspring continues to be relevant.

From a practical perspective, the significance of this work is in the fact that the obtained results provide reasonable grounds to refuse from large-scale (within the Chelyabinsk region) activities aimed at targeted health check-ups of the exposed persons and their offspring, paying special attention to areas of their compact settlement.

The information that people of older ages (as compared to 1980–1990s) are prone to additional risk of thyroid cancer development is important for Chelyabinsk region. And it

should be taken into account by endocrinologists and oncologists who are involved in thyroid cancer detection.

The obtained regularities and tendencies will be taken into account in future risk analysis of thyroid cancer development associated with the study of the radiation effects on residents of Chelyabinsk region who were grouped into the cohort of exposed population.

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ASSESSING DIETARY RISKS CAUSED BY FOOD ADDITIVES: A CASE STUDY OF TOTAL DIET IN VIETNAM

Nguyen Hung Long[1], Le Thi Hong Hao[2], Vu Thi Trang[2], Tran Cao Son[2], Lam Quoc Hung[1]

[1]Vietnam Food Administration, 138A Giảng Võ Ba Đình, Hanoi, Vietnam
[2]National Institute for Food Control, 65 Phạm Thận Duật, Hanoi, Vietnam

A risk assessment study of 6 types of food additives (including benzoates, sorbates, cyclamate, saccharin, tartrazine, and sunset yellow) in Vietnamese diets was conducted based on the food consumption research and food additives concentration followed by the guideline of World Health Organization. Surveys on food consumption and food sampling were conducted in 6 provinces including Ha Noi, Ho Chi Minh, Thua Thien Hue, Nam Dinh, Tay Ninh and Quang Tri. The survey results have determined the amount of food consumed for each different food product groups on different age groups. Test results of 6 food additives including benzoates, sorbates, saccharins, cyclamates, tartrazine and sunset yellow FCF using HPLC method show that benzoates and sorbates are the two most discovered groups of substances in the tested samples. The highest concentrations of these compounds were on jelly, soft drinks, ground meat, chilli sauce ... Types of food additives used as sweeteners are common in dried fruits, jam; the cyclamate content was very high in these two product groups. The colouring agents content were at a much lower level, mainly found in chili sauce. Risk assessment results show that total intake of sorbate and benzoate in the group of children under 5 years old were the highest value, which was 38 % of ADI. For all other age groups, the risk ranged from 10.6 to 34.0 % ADI for benzoates and from 0.56 to 1.8 % ADI for sorbates. For the remaining 4 food additives, total consumption was much lower than their ADIs. With the assumption that people used all types of food, 0.8 % of the population had the intake of benzoate exceed its ADI.

Food additives are commonly used worldwide. Many types of food additives have been accepted by Codex Alimentarius to be used in foods such as preservatives, sweeteners, coloring products, flavorings... [1]. In Vietnam, some commonly used food additives include benzoates, sorbates, cyclamate, saccharin, tartrazine, and sunset yellow. Although there are regulations on the maximum limits of these substances in many food categories, the total intake of these compounds maybe higher because there are many types of foods that contain the same compounds. According to previous investigations, there were a number of food additives in different food matrices, including sodium benzoate, potassium sorbate, sodium oxalate, sodium citrate, artificial sweeteners and coloring agents [2, 3]. The level of each food additive on each commodity were found within the regulatory limit, but people consume more than one types of food, then the total intake of food additives may exceed the safety level.

Risk assessment is a component of the process of risk analysis besides risk management and risk communication. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have published the principles of chemical risk assessment in food. JECFA, which is the FAO/WHO joint committee, is responsible for risk assessment for food additives. The risk assessment process is based on the general principles guided by the "Princi-

Nguyen Hung Long – Deputy director (e-mail: nguyenhunglong1002@gmail.com; tel.: +8 (491) 225-05-27; ORCID: https: //orcid.org/0000-0002-6589-2439).

Le Thi Hong Hao – Director general (e-mail: lethihonghao@yahoo.com; tel.: +8 (490) 424-81-67; ORCID: https: //orcid.org/0000-0003-3570-8570).

Vu Thi Trang – Head of Laboratory of Food Quality and Food Additives Testing (e-mail: trang2103@gmail.com; tel.: +8 (491) 614-91-06; ORCID: https: //orcid.org/0000-0003-0924-6376).

Tran Cao Son – Head of Laboratory of Food Toxicology and Allergens Testing (e-mail: caoson32@gmail.com; tel.: +8 (498) 8683282; ORCID: https: //orcid.org/0000-0002-9779-2715).

Lam Quoc Hung – Head of Department of Food poisoning monitoring (e-mail: lamquochungmoh@gmail.com; tel.: +8 (491) 331-99-36; ORCID: https: //orcid.org/0000-0002-0158-8905).

ples and methods of chemical risk assessment in food" and consists of four main stages including hazard identification, hazard characterization and exposure assessment and risk characterization [4].

The first two steps ussually performed before approval of any food additive base on invitro and invivo studies in laboratory animal. The extent to which a food additive can pose a health risk depends upon its toxicity and the dietary exposure. JECFA establishes acceptable daily intake (ADI) values for food additives. ADI values are calculating using a safety factor which ensures that if the additive is consumed daily at that level for the rest of one's life, there would be no «appreciable health risk» [5]. According to the Codex Alimentarius reports and EFSA studies [6–11], the definition of food additives, their INS number and the acceptable daily intake (ADI) for each compound were shown in Table 1. Exposure assessment is the next step which requires the information of food additives consumption. Usually the 24-hour dietary recall survey or the food frequency questionnaire (FFQ) is the tool of choice for estimating the intake of foods likely to contain additives. Concentration of the additive in different foods is chemically estimated to ultimately calculate the dietary exposure to the additive. And finally, the probability of occurrence of adverse toxic effects in humans as a result of exposure to food additive is assessed. This is usually done by comparing ADI values of the additive with exposure levels among humans.

Many risk assessment studies have been published worldwide. According to Cressey and Jones study in New Zealand, mean population level estimates of dietary exposure were well below the respective acceptable daily intakes (ADIs) for all age-gender groups for all preservatives at 7–27%, 1–4% and 1–8% of the ADI for sulfites, sorbates and benzoates, respectively [12]. Another research of Bemrah et al. about the assessment of dietary exposure to 13 selected food colours, preservatives, antioxidants, stabilizers, emulsifiers and sweeteners in French population showed that the intake estimates are reassuring for the majority of additives studied since the risk of exceeding the ADI was low, except for nitrites, sulfites and annatto, whose ADIs were exceeded by either children or adult consumers or by both populations under the modelling assumptions [13]. Another study of Chung et al. in Korea for saccharin, stevioside, D-sorbitol and aspartame stated that the EDIs of artificial sweeteners

Table 1

The studied food additives with their definition and ADI values [6–11]

Food additives	Definition	INS number	ADI (mg/kg b.w.)
Benzoates	Acid benzoic Natri benzoate Kali benzoate Calci benzoate	210 211 212 213	0–5
Sorbates	Acid sorbic Kali sorbate Calci sorbate	200 202 203	0–25
Saccharin	Saccharin Calci saccharin Kali saccharin Natri saccharin	954(I) 954(II) 954(III) 954(IV)	0–5
Cyclamate	Acid cyclamic Calci cyclamate Natri cyclamate	952(I) 952(II) 952(III)	0–7
Tartrazine	Tartrazine	102	0–7,5
Sunset yellow FCF	Sunset yellow FCF	110	0–1

such as saccharin and aspartame in Korea are significantly lower than ADI set by the JECFA [14]. Then, Ha et al. assessed the consumer exposure to sodium saccharin, aspartame and stevioside and confirmed that for most Korean consumers, the EDIs were no greater than 20% of their corresponding ADI; however, the EDI of sodium saccharin for conservative consumers aged 1–2 years reached 60% of their ADI [15]. These authors also assessed the synthetic colours in Korea [16]. Rao et al. assessed intakes of synthetic food colours: tartrazine, sunset yellow and erythrosine in India to be 7.5, 2.5 and 0.1 mg/kg body weight, respectively [17]. Another research in India showed that Though sunset yellow FCF and tartrazine were the two most popular colours, many samples used a blend of two or more colours [18].

In Vietnam, risk assessment has been recently applied for the risk based control of chemicals and microorganism. Some risk assessments have been done for mycotoxins or heavy metals [19–21]. However, there has not been any risk assessment study of food additives. Therefore, assessing the total amount of chemical exposure based on the Vietnamese diet is an important parameter to study the impact of these chemicals on the health of Vietnamese people.

This study conducted a risk assessment of the 6 additives namely benzoates, sorbates, cyclamate, saccharin, tartrazine, and sunset yellow for Vietnamese health. The assessment includes steps according to FAO/WHO guidelines and is compared with acceptable daily intake (ADI) recommended by the Codex Alimentarius.

Materials and Methods

The study evaluated 6 groups of food additives (benzoates, sorbates, cyclamate, saccharin, tartrazine and sunset yellow FCF) in the food samples of the diet at risk of containing of food additives, including confectionery, soft drinks, processed meat products, jam, spices, canned food, instant cereals, instant coffee, dairy products, and supplements.

The food consumption study has been conducted in urban and rural areas in 6 provinces in the North, Central and South of Vietnam including Hanoi, Nam Dinh, Thua Thien Hue, Quang Tri, Ho Chi Minh and Tay Ninh. The respondents were divided into different age groups including young children (≤5 years), elementary students (6–10 years), high school students (11-18 years), adults (19–40 age) and middle/elderly people (> 40 years). The total number of households surveyed was 2700, which was calculated according to the sample size calculating formula. All participants were interviewed about their diet recall for 24 hours and for 1 week that related to the studied food group. They weight were also assessed by using a health scales (for young children) or by interviewing (for other age group).

The total number of food samples collected in the provinces was 2970 samples. Samples were analyzed at the ISO/IEC 17025:2017 accredited laboratory of National Institute for Food Control (NIFC) using HPLC methods.

The survey data on food consumption are collected by Epidata software 3.1. SPSS 16.0 software is used to evaluate food consumption statistics and test results of food additives.

Results and Discussion

General characteristics of the research object

Of the 2700 households interviewed, total 10499 people were assessed. The age group distribution and the average weight of the study subjects are presented in Table 2.

The age distribution of the research group and their average weight shows that the majority of the study population was adulthood

Table 2

Average weight by different age groups

Age groups	Frequency	Percentage	Weight	
			Average	SD
≤ 5	847	8.1	14.3	5.18
6–10	720	6.9	26.1	19.82
11–18	1051	10.0	43.7	11.53
19–40	3452	32.9	56.3	17.81
> 40	4429	42.2	56.7	13.91
TỔNG	10499	100.0	49.5	–

(over 18 years) accounts for more than 75%. The average weight of this group was about 56.5 kg, while the average weight of all subjects was 49.5 kg. These results are also consistent with the current convention that the average weight of Vietnamese people is 55 kg. In this study, the actual average weight of adults was taken from the actual data of 56.5 kg.

Determination of food additive content

Detection limit values of food additives according to ISO 17025 accredited method for benzoates, sorbates, saccharin, cyclamate, tartrazine, sunset yellow FCF are 10 mg/kg, 2 mg/kg, 40 mg/kg, 0.5 mg/kg and 0.5 mg/kg, respectively. In this study, as food additives are often added to foods with a fairly high content, for samples with not detected results, they were assessed to be zero.

Benzoates and sorbates are the two most common groups of food additives in tested samples. In particular, most notably on jelly, soft drinks, grind meat rolls, chili sauces. Types of sweeteners are common in dried fruits and jams. Especially, the cyclamate content is very high in these two product groups. The coloring agents were at much lower content, mainly in chili sauces.

Some groups of food products with low additive content such as instant noodles, instant cereals, instant coffees, instant teas, ice cream, yogurt, sausage, dried meats, roasted meats, canned meats, canned vegetables and fruits.

Table 3

The amount of food additives in different food groups (gram)

Food category	Sorbate	Benzoate	Saccharin	Cyclamate	Tartrazine	Sunset yellow FCF
Cake/pie	38.01	6.84	4.35	0	0.17	0.42
Candy	6.17	44.83	19.89	6.27	4.77	3.25
Jelly	256.2	124.1	7.43	21.67	2.22	2.45
Snack	149.2	74.04	14.38	71.33	2.38	1.44
Soft drinks	19.36	76.6	28.07	2.3	1.84	4.37
Fruit juices	7.62	45.74	13.88	4.86	0.29	1.92
Jam	26.78	64.57	30.39	170.4	0.83	2.91
Dried fruits	127.3	172.5	88.46	149.9	0.35	0.74
Dried grind meats	82.6	108.7	0	0	0	0
Grind meat rolls	163.8	287.6	0	0	0	0
Sausages	30.9	17.74	0	0	0	0
Roasted meats	23.25	16.21	0	0	0.07	0
Canned meats	0	4.66	0	0	0	0.08
Dried meats	2.3	25.03	3.41	0	0.83	4.26
Chili sauces	106.8	297.1	10.32	7.05	1.34	19.85
Soy sauces	71.2	201.8	15.84	0	0	1.07
Fish sauces	75.81	181.2	49.74	0	0	0
Instant noodles	0	9.2	0	0	0.76	0.42
Instant cereals	0	0.78	0.69	0.74	0.08	0.07
Instant coffees	0	0	24.54	0	0	0
Instant teas	0	2.13	2.54	0	0.08	0.09
Canned vegetables	12.46	31.1	7.33	6.34	0.04	0.01
Ice cream	0	0	0	0	0.13	0.44
Yogurt	0	2.37	2.91	0	0.46	0.53
Food supplements	10.83	26.17	5.79	0	5.09	8.21

Because many products were not detected with additives, the standard deviations of some cases were quite high. However, in the framework of this study, average results are used as data in risk assessment.

Evaluation of food consumption and the intake of food additives by food groups

Based on statistical survey data on food consumption, consumption characteristics of each food product by age group were evaluated. Among the food assessed, the beverage group is the food group that has individuals exceeding ADI for benzoate. However, for each food group and each type of additives, average consumption dose of these additives were lower than ADI. The data of soft drink group is shown in table 4 and table 5 as an example.

It can be seen that the rate of people using soft drink was quite high, the largest among the 11–18 year old teenagers (71.4%), but the average one-time use was highest in the 6–10 years old. Calculating for all age groups, average usage was about 400 g. This consumption was used to evaluate the intake dose by age group and is summarized in table 5.

The highest average intake was found for the benzoate group and in the group under 5 years old, which was 1.81 mg/kg b.w. For other food additives, the intake dose on soft drink is also higher than that of other food product groups.

Evaluation of the total intake of food additives in the total diet

With the hypothesis that is consumers use at least 1 food product to all types of food products on the same day, the total consumption of each food additive was evaluated and presented in table 6.

The results in table 5 show that the total intake of sorbate and benzoate is highest and in the group of children under 5 years old, which were 1.5 and 1.9 mg/kg b.w., respectively. These doses were still within the limits of ADI for both groups of these substances. For benzoates, the estimated daily intake (EDI) was from 10.6 to 38% of it corresponding ADI. This figure for sorbate was much lower, which were just 0.56% to 1.8% of ADI. For the remaining 4 food additives, total consumption is much lower than their ADI. The coloring group (tartrazine and sunset yellow) has the lowest consumption. In the sweetener group, the total consumption of saccharin is higher than that of cyclamate, but still 15–40 times lower than ADI depending on the age group.

The number of total consumption of food additives for each individual which is higher than the ADI is also evaluated, and presented in Table 7.

With the above hypothesis, the number of people with total intake of higher than benzoate's ADI accounts for the highest percentage, especially in low age groups. In the group of less than 5 years of age and the group of 6–10 years, there were 4.6% and 2.6% exceeding ADI, respectively. On average, about 0.8% of people assessed have total consumption exceeding ADI for benzoates.

In addition, there are a few other individuals whose total intake of sorbate, saccharin and sunset yellow exceeds ADI, and they are also concentrated in the lower age group. The risk for these compounds is negligible.

Table 4

Soft drink consumption by age group

Age group	Total	Number of people used		Intake (g/day)			
		n	%	Average	SD	Min	Max
≤ 5 years old	695	248	35.7	337.2	229.84	25	1500
6–10 years old	626	347	55.4	429.5	488.99	250	6000
11–18 years old	788	563	71.4	381.7	260.37	250	4500
19–40 years old	1965	1161	59.1	407.2	316.61	250	6000
> 40 years old	2259	989	43.8	411.6	297.44	250	3000
Total	6333	3308	52.2	401.3	320.28	25	6000

Table 5

Intake of food additive by age group (mg/kg b.w.) when consuming soft drink

Age group	Food additives	N	Average	SD	Min	Max
≤ 5 years old	Sorbate	248	0,456568	0,311178	0,033850	2,030770
	Benzoate	248	**1,806463**	1,231212	0,133920	8,034970
	Saccharin	248	0,661977	0,451176	0,049070	2,944410
	Cyclamate	248	0,054241	0,036968	0,004020	0,241260
	Tartrazine	248	0,043393	0,029575	0,003220	0,193010
	Sunset yellow FCF	248	0,103058	0,070240	0,007640	0,458390
6–10 years old	Sorbate	347	0,318595	0,362717	0,185440	4,450570
	Benzoate	347	**1,260555**	1,435130	0,733720	17,609200
	Saccharin	347	0,461929	0,525902	0,268870	6,452870
	Cyclamate	347	0,037850	0,043091	0,022030	0,528740
	Tartrazine	347	0,030280	0,034473	0,017620	0,422990
	Sunset yellow FCF	347	0,071914	0,081874	0,041860	1,004600
11–18 years old	Sorbate	563	0,169111	0,115353	0,110760	1,993590
	Benzoate	563	**0,669107**	0,456406	0,438220	7,887870
	Saccharin	563	0,245194	0,167249	0,160580	2,890500
	Cyclamate	563	0,020091	0,013704	0,013160	0,236840
	Tartrazine	563	0,016073	0,010963	0,010530	0,189470
	Sunset yellow FCF	563	0,038172	0,026038	0,025000	0,450000
19–40 years old	Sorbate	1161	0,140013	0,108876	0,085970	2,063230
	Benzoate	1161	**0,553978**	0,430780	0,340140	8,163410
	Saccharin	1161	0,203005	0,157859	0,124640	2,991470
	Cyclamate	1161	0,016634	0,012935	0,010210	0,245120
	Tartrazine	1161	0,013307	0,010348	0,008170	0,196090
	Sunset yellow FCF	1161	0,031604	0,024576	0,019400	0,465720
> 40 years old	Sorbate	989	0,140532	0,101561	0,085360	1,024340
	Benzoate	989	**0,556029**	0,401839	0,337740	4,052910
	Saccharin	989	0,203756	0,147253	0,123770	1,485190
	Cyclamate	989	0,016695	0,012066	0,010140	0,121690
	Tartrazine	989	0,013356	0,009653	0,008110	0,097350
	Sunset yellow FCF	989	0,031721	0,022925	0,019270	0,231220

Table 6

Total intake of food additives of age groups (mg/kg b.w.)

Age group	Food additives	N	Average	SD	Min	Max
≤ 5 years old	Sorbate	764	**1,548362**	2,160869	0,000000	36,947490
	Benzoate	764	**1,886320**	1,947033	0,000000	21,280590
	Saccharin	764	0,330762	0,483127	0,000000	3,420870
	Cyclamate	764	0,177728	0,241519	0,000000	3,265000
	Tartrazine	764	0,029699	0,038514	0,000000	0,392030
	Sunset yellow FCF	764	0,055880	0,079646	0,000000	0,568370
6–10 years old	Sorbate	645	**1,246741**	1,326715	0,002490	20,205960
	Benzoate	645	**1,711453**	1,702170	0,005500	24,146250
	Saccharin	645	0,348452	0,501063	0,000000	7,567360

Age group	Food additives	N	Average	SD	Min	Max
	Cyclamate	645	0,150885	0,164616	0,000000	1,788870
	Tartrazine	645	0,028883	0,035141	0,000000	0,549210
	Sunset yellow FCF	645	0,057721	0,079524	0,000000	1,220920
11–18 years old	Sorbate	813	0,763875	0,640765	0,000000	5,181750
	Benzoate	813	**1,222627**	0,822839	0,000000	7,933700
	Saccharin	813	0,234662	0,202015	0,000000	2,908430
	Cyclamate	813	0,085542	0,081287	0,000000	0,647320
	Tartrazine	813	0,017807	0,013804	0,000000	0,194340
	Sunset yellow FCF	813	0,038611	0,032411	0,000000	0,453840
19–40 years old	Sorbate	2551	0,411440	0,373940	0,000000	4,182480
	Benzoate	2551	0,771790	0,640250	0,003870	10,731790
	Saccharin	2551	0,131729	0,176981	0,000000	3,751350
	Cyclamate	2551	0,037838	0,057337	0,000000	0,504090
	Tartrazine	2551	0,008916	0,011403	0,000000	0,214140
	Sunset yellow FCF	2551	0,021114	0,028083	0,000000	0,576460
> 40 years old	Sorbate	2228	0,272799	0,366641	0,000000	6,053790
	Benzoate	2228	0,538597	0,579170	0,000000	6,313950
	Saccharin	2228	0,127510	0,162306	0,000000	1,512330
	Cyclamate	2228	0,040153	0,057071	0,000000	0,683800
	Tartrazine	2228	0,008904	0,010481	0,000000	0,100320
	Sunset yellow FCF	2228	0,020258	0,025695	0,000000	0,234780

Table 7

The number and proportion of consumers with total intake of food additives exceeding ADI with the above hypothesis

Age group	Sorbates		Benzoates		Saccharin		Cyclamate		Tartrazine		Sunset yellow FCF	
	n	%	n	%	n	%	n	%	n	%	n	%
≤ 5 years old	1	0.1	35	4.6	–	–	–	–	–	–	–	–
6–10 years old	–	–	17	2.6	2	0.3	–	–	–	–	2	0.3
11–18 years old	–	–	3	0.4	–	–	–	–	–	–	–	–
19–40 years old	–	–	2	0.1	–	–	–	–	–	–	–	–
> 40 years old	–	–	2	0.1	–	–	–	–	–	–	–	–
Total	1	0.01	59	0.8	2	0.03	–	–	–	–	2	0.03

No individual had a total intake of cyclamate and tartrazine exceeding ADI, indicating a very low risk for both groups of these food additives.

Conclusion

The first total diet study was conducted in the study of dietary risk assessment of 6 food additives. The data showed that the average food additive intakes of consumer in Vietnam were within the recommendation of Codex Alimentarius. This study is a recommendation to a better food additives management and communication.

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COMPARATIVE ASSESSMENT OF ARTIFICIAL SWEETENERS TOXICITY VIA EXPRESS BIOTEST

A.V. Samoilov[1], N.M. Suraeva[1], M.V. Zaitseva[1,2], M.N. Kurbanova[1], V.V. Stolbova[2]

[1]V.M. Gorbatov's Federal Scientific Center for Nutrition Systems, RAS, 78 Shkolnaya Str., Vidnoe, 142703, Russian Federation
[2]Moscow State University, 12 Bldg., 1 Leninskie Gory, Moscow, 119991, Russian Federation

Various food additives are being produced and consumed by population in greater and greater quantities and risks of probable toxic effects exerted by them are growing as well. These additives frequently occur in various combinations in food products and the environment, they can be consumed for a long period of time and produce hazardous mutagenic and carcinogenic effects. Therefore, it is extremely vital to assess combined impacts exerted by food additives so that their safety would be proven. There are certain advantages related to vegetative test-systems and cytogenetic analysis procedures for biological tests data when it comes to screening for toxic and mutagenic effects produced by chemicals. Allium-test which applies Allium cera bulb onion roots as a test-object is quite distinctive. When compared with other tests that employ animals and various cell cultures, this test turns out to be less complicated and costly and more sensitive as well.

Our research goal was to examine influences exerted by such artificial sweeteners as aspartame and sucralose on living weight gain and mitotic anomalies frequency in apical meristem cells in Allium cera bulb onion roots. We also assessed a synergy effect caused by combined exposure to both these chemicals. We detected that aspartame caused a significant decrease in root living weight against the control while there were no toxic effects caused by sucralose. Maximum toxicity was detected when a test-system was exposed to both artificial sweeteners together and it was considered to result from the above mentioned synergy effect. Chromosome aberrations frequency in test samples differed insignificantly from the control but we also detected authentic changes in chromosome anomalies spectrum in root meristem cells. Disorders in chromosome disjunction and anomalies in the mitotic apparatus were the most frequently registered ones.

Key words: *sucralose, aspartame, Allium cepa, biological testing, cytogenetic analysis, toxicity, chromosome aberrations, anomalies in the mitotic apparatus.*

Aspartame and sucralose are popular artificial sweeteners; they are applied in manufacturing a wide range of food products such as soft drinks, confectionary, chewing gum, dairy products, jams, various sauces and flavors as well as low-calorie products. Aspartame is an artificial synthesized chemical, namely L-aspartil-L-phenylalanine. It dissolves in water quite well, is 200 times sweeter than saccharose, and can't be used in food products that are to be thermally treated as it is usually destroyed under heating and loses its sweet taste. Sucralose (trichlorogalactosaccharose), another widely spread artificial sweetener, is obtained from saccharose via chemical transformation. It is thermally stable and is quite like usual sugar in its taste but still it is 600 times sweeter than saccharose. These sweeteners were officially approved by Food and Drug Administration in 1981 and 1998 respectively. Aspartame is applied as a sweetener in more than 90 countries and in manufacture of 6,000 various food products [1]. Sucralose is detected to be applied in more than 70 countries worldwide. Besides, sucralose, due to high chemical stability of its molecule and good

Artem V. Samoilov – candidate of biological sciences, head of laboratory of quality and safety of food products (e-mail: molgen@vniitek.ru; tel.: +7 (495) 541-87-00; ORCID: https://orcid.org/0000-0001-5655-174X).

Natal'ya M. Suraeva – doctor of biology, chief researcher inlaboratory of quality and safety of food products (e-mail: nsuraeva@yandex.ru; tel.: +7 (495) 541-87-00; ORCID: https://orcid.org/0000-0002-0144-8128).

Mariya V. Zaitseva – junior researcher in laboratory of quality and safety of food products (e-mail: mascha.zajtseva2014@yandex.ru; tel.: +7 (495) 541-87-00; ORCID: https://orcid.org/0000-0002-6624-883X).

Madinat N. Kurbanova – candidate of biological sciences, senior researcher inlaboratory of quality and safety of food products (e-mail: sk5969@yandex.ru; tel.: +7 (495) 541-87-00; ORCID: https://orcid.org/0000-0001-5798-554X).

Valeriya V. Stolbova – candidate of biological sciences, senior lecturer, department of radioecology and ecotoxicology, faculty of soil science (e-mail: vstol@bk.ru; tel.: +7 (495) 939-22-11; ORCID: https://orcid.org/0000-0001-5433-5533).

water solubility, is considered a potential environmental pollutant. Sucralose has already been found in water basins in some countries and even in the Gulf Stream in the Atlantic in concentrations within 0.1-1.0 g/l; therefore, there is an issue related to probable risks of technogenic environmental pollution with the substance [2].

Food products manufacturing is permanently growing and population tend to consume more and more variable food additives; given that, health risks related to such additives and risks of probable environmental pollution with them are also increasing substantially. Data on artificial sweeteners safety have been analyzed in foreign literature and the results are rather controversial. Tests were performed on models of human cells cultures, animal cells cultures, bacteria, and on laboratory animals. Most publications in early periods confirmed aspartame and sucralose to be safe provided that existing consumption standards were met; conclusions were made due to no genotoxic and acute toxic effects being detected via standard tests; although there were some works mentioning that the substances could induce chromosome aberrations [1, 3–7]. However, over recent years new facts about toxic effects produced by these sweeteners have been discovered, and, consequently, it was recommended to strictly control their consumption. Thus, sucralose was considered to be hazardous for human health due to its chlorinated structure as it is well-known that chlorinated sugar and products of its hydrolysis that are quite similar to sucralose in their structure destroy nerve cells. *In vitro* research performed on human liver cells allowed to detect that sucralose reacted with reduced B12 vitamin and such reactions were suggested to influence metabolism of the vitamin [2]. Negative influence exerted by artificial sweeteners, sucralose included, on intestinal microbiota was also confirmed; this influence resulted in dysbacteriosis and inflammatory reactions in mice's livers [8]. According to literature data, even more serious consequences for health can be caused by aspartame contents in food products. It was shown that when aspartame was added into drinking water during 2 months, it led to damages to rats' livers due to induced hyperglycemia and accumulation of various lipids under oxidative stress [9]. Histological and immune-histochemical research on generative tissues of rats that had aspartame in their ration revealed significant disorders in placenta and fetal tissues [10]. It was also revealed that chronic consumption of aspartame, especially at earlier stages in development and during long-term periods, could cause changes in the nervous system, astrogliosis, and lower vital capacity of prefrontal cortex neurons, tonsil, hippocampus, and hypothalamus in rats [11].

It should be noted that ideal test-systems applied to imitate human or animal detoxification systems and metabolism of chemical compounds haven't been created yet. And unreasonably little attention has been paid to mechanisms of systemic (combined) effects produced by negative factors on a test-organism; therefore, new trends in strategic planning of test procedures are aimed at making changes into toxicity assessment algorithms as regards mixtures and food matrices [12]. The examined sweeteners can often be consumed together as they occur in various food products; therefore, they can produce both additive and synergic negative effects on a human body. So, to assess safety of impacts exerted by synthetic compounds, nowadays it is truly vital to apply a complex approach that can be implemented with express-biotests being added into a set of test techniques.

Vegetative test-systems have certain advantages as regards complex screening of toxic, cytotoxic, and mutagenic effects; Allium-test is a specific one among such tests. The test uses *Allium cepa* bulb onion roots as a test-object and its test-function is changes in length/living weight, as well as some cytological and cytogenetic parameters of cells in the apical meristem. At present this test is recommended by WHO experts as a standard one to be applied in cytogenetic environmental monitoring. Moreover, the method was also successfully tested to examine a wide range of factors, such as ionizing and non-ionizing radiation [13–16], food additives [17], herbi-

cides [18], medications [19], and even antimutagenic potential [20]. When compared with other tests that employed animal and vegetative cell cultures, Allium-test turned out to be more sensitive [21], and reliability of data on mutagenicity obtained with it is usually beyond question as there are very few false-negative results [22]. We assume that tests on aspartame and sucralose in *in vivo* experiments with Allium-test will allow to obtain new data on biological effects produced by these widely used food additives.

Our research goal was to examine toxicity and genotoxicity of aspartame and sucralose and their combination with two Allium-test modifications.

Data and methods. We applied the following sweeteners in our experiments: sucralose (Alfa Aesarby Thermo Fisher Scientific) and aspartame (Alfa Aesar by Thermo Fisher Scientific). Our test-organisms were *Allium cepa L* bulb onions (Schtutgarten sort) of the same size (diameter 2.5–3 cm) and weight (5–7 grams). Onions didn't have green leaves. Prior to incubation, we removed dried peels from onions. Then, onions from the control groups were put in to 10-ml vials with bottled drinking water; onions from test groups were put into sweeteners solutions in the same water in concentrations equal to 1 g/l and 2 g/l each, and their combinations in concentrations equal to 0.5 g/l and 1 g/l. Concentrations of sweeteners in solutions didn't exceed domestic standards for their contents in food products. Both test and control groups were incubated in darkness under 25ºC for 5 days. Solutions in vials were refreshed daily. After incubation was over, roots were cut off from each onion, fluid was removed off them with filter paper; onions were them weighed and used to perform cytogenetic analysis of cells from the apical meristem. Preparations were then dried with acetoorcein (1 gram of orcein was dissolved in 50 ml of 45-% CH_3COOH). In order to preserve roots for a long time, we stored than in 70% ethanol. Then we prepared instant squash preparations and analyzed them with Axioskop 40 (Zeiss) light microscope. About 17,000 cells were viewed with a microscope during cytogenetic research. Obtained results were statistically processed with Microsoft Excel and Statistica, mean values were compared with Student's T-test and Fischer's transformation; we also compared two samplings with non-parametric Chi-square criterion.

Results and discussion. To assess toxic, mitosis-modifying and genotoxic effects produced by aspartame and sucralose with Allium-test, we examined the following parameters: a growth in root living weight under various concentrations and combinations of sweeteners, mitotic index, frequency and range of chromosome aberrations. Besides, we applied two Allium-test modifications, with preliminary 2-day sprouting and further selection of onions with their root length being not shorter than 1 cm (the first and the second modification accordingly) and without preliminary sprouting. We assume that this set of parameters allows to achieve the most valid detection of different physiological and cytogenetic disorders reflecting overall toxic effects and cyto-(geno) toxic ones respectively. We'd like to dwell a bit more on toxicity parameters as a conventional Allium-test techniques involved measuring root length to assess them, but we chose living weight instead. It has a number of advantages, including higher sensitivity [23]. We also tested combinations of these two substances in order to reveal hidden potential of their toxicity as a synergic effect occurrence.

As we can see from Tables 1 and 2, we detected authentic discrepancies between test and control groups related to a smaller growth in root living weight in onions treated with aspartame solution; these discrepancies were detected in both test modifications. Sucralose didn't have any toxic effects.

When assessing effects produced by mixtures of these two substances, we obtained significant results in the second test modification. Aspartame in concentration equal to 1 g/l led to an authentic almost 2-time decrease in root living weight against the control group, but if sucralose was added in the same concentration, then mixture toxicity authentically grew by more than 4 times. Moreover, the

Table 1

Effects produced by sweeteners on a growth in root living weight, mitotic activity and frequency of chromosome aberrations in apical meristem cells of onion roots in the first test modification (n = 6)

A test variant	Average root weight per an onion, grams	Mitotic index, %	Chromosome aberrations recalculated per, %	
			Overall number of cells	Dividing cells
Control	0.217±0.061	5.91	0.29	4.83
Sucralose, 1 g/l	0.228±0.049	6.23	0.34	5.45
Sucralose, 2 g/l	0.281±0.081	6.26	0.96*	15.36*
Aspartame, 1 g/l	0.161±0.047	4.28*	0.55*	12.77*
Aspartame, 2 g/l	0.108±0.041**	4.65*	0.29	6.31*
Sucralose + Aspartame, 0.5 g/l	0.101±0.039**	6.95*	0.33	4.72
Sucralose + Aspartame, 1 g/l	0.115±0.036**	4.76*	0.23	4.79

Note: * means p<0.05; **means p<0.2.

Table 2

Effects produced by sweeteners on a growth in root living weight, mitotic activity and frequency of chromosome aberrations in apical meristem cells of onion roots in the second test modification (n = 5)

A test variant	Average root weight per an onion, grams	Mitotic index, %	Chromosome aberrations recalculated per, %	
			Overall number of cells	Dividing cells
Control	0.469±0.070	12.05	1.24	10.26
Sucralose, 1 g/l	0.429±0.100	12.44	1.13	9.09*
Sucralose, 2 g/l	0.478±0.064	14.37*	1.82	12.65*
Aspartame, 1 g/l	0.206±0.045*	14.62*	1.34	9.19
Aspartame, 2 g/l	0.224±0.043*	15.49*	1.73*	11.18
Sucralose + Aspartame, 0.5 g/l	0.245±0.141*	16.55*	1.31	7.93*
Sucralose + Aspartame, 1 g/l	0.140±0.027*	9.72*	0.82*	8.48*

Note: * means p <0.05.

effect was dose-dependent. So, these data proved there was a systemic synergic effect that made a mixture of two chemical substances more toxic than each of them separately. It is especially alarming as these two chemicals can be introduced together into a body or occur as environmental pollutants in different combinations.

It is known that deviations in mitotic index of test-organisms, both towards growing or falling, are signs that a test medium is cytotoxic [24]. We analyzed this parameter in test groups exposed to aspartame in both test modifications and detected statistically significant discrepancies in its values against control samples (Tables 1 and 2); it was lower in the first test modification and higher in the second one. As for samples exposed to sucralose, an authentic discrepancy was detected only in the second test modification and high concentration of this sweetener. Therefore, we detected a mitotoxic effect in samples exposed both to aspartame and sucralose but it was insignificant as a share of mitotic cells in all test groups had insignificant statistical discrepancies form control groups (less than 40%), while food preservatives and flavor enhancers can reduce this parameter by several times [25].

A share of chromosome aberrations after exposure to aspartame and sucralose, both

separately and in a combination, remained low in both test modifications. We also didn't detect any dose-dependent effects (Tables 1 and 2). These data are quite consistent with other research results proving that these two substances didn't have any genotoxic effects [26]. We analyzed a range of chromosome aberrations as a probable outcome of adaptation reorganizations caused by impacts exerted by these substances or as a sign that their impacts were quite specific (Figure 1).

We distributed all the detected aberrations into the following groups: group A included disorders in chromosome disjunction (forwarding or lagging); group B, mitotic apparatus anomalies (adhesion, multi-polar mitosis); group C, aberrations reflecting clastogene effects by a factor (bridges, fragments); group D, other aberrations (chromosome fragmentation, agglutination, or pulverization) respectively. It turned out that there were no authentic discrepancies between test and control groups detected in the first test modification, while in the second one almost all test samples had authentic discrepancies from control ones, and disorders were predominantly in groups A and B (Figure). And the most significant discrepancies were again detected in samples exposed to a combination of aspartame and sucralose in 1 g/l concentration just as it was with root living weight measuring.

According to these data, aspartame and a combination of aspartame and sucralose within fixed standards for their contents in food products produced an authentic dose-dependent toxic effect. The results were obtained mostly via examining microscopic parameters, namely a growth in root living weight, as this parameter reflected a sum of all distorting effects and, therefore, was more sensitive than other microscopic parameters [17]. So, the above-mentioned literature data on these sweeteners determined in animal experiments to be health risk factors have also been confirmed by the present work by a vegetative biotest. In this context, arguments that call for reducing recommended permissible safe doses of aspartame in food products seem even more convincing [27].

Conclusion. We exposed onions to aspartame in two Allium-test modifications and analyzed a growth in root living weight; it allowed us to come to a valid conclusion that aspartame was toxic in a dose equal to 1 g/l (50% delay in a living weight growth against the control). We didn't detect any similar effects when samples were exposed to sucralose. All the examined doses didn't exceed hygienic standards for contents of these substances in

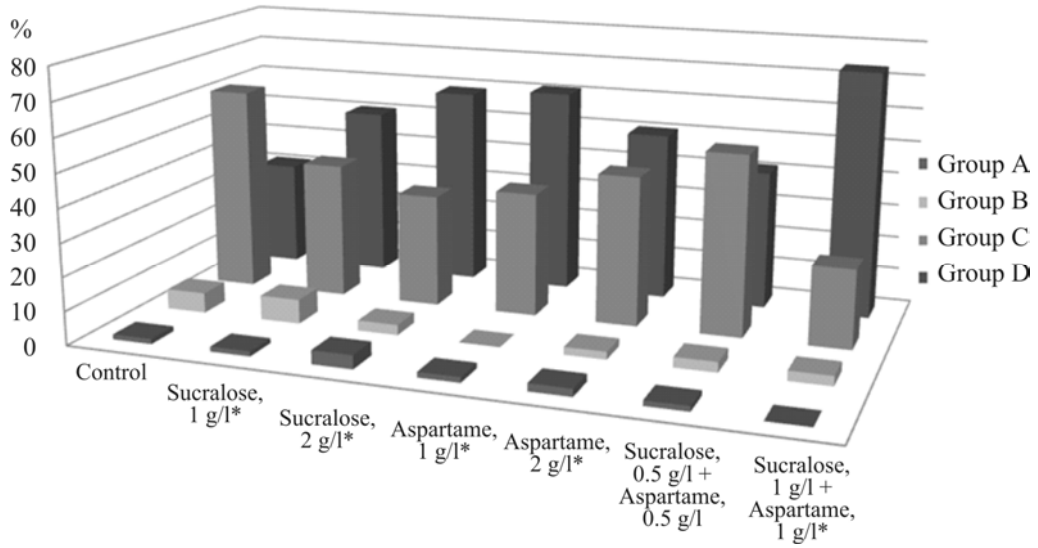

Figure 1. Distribution of chromosome aberrations as per groups in the second test modification (%), * means $p<0.05$

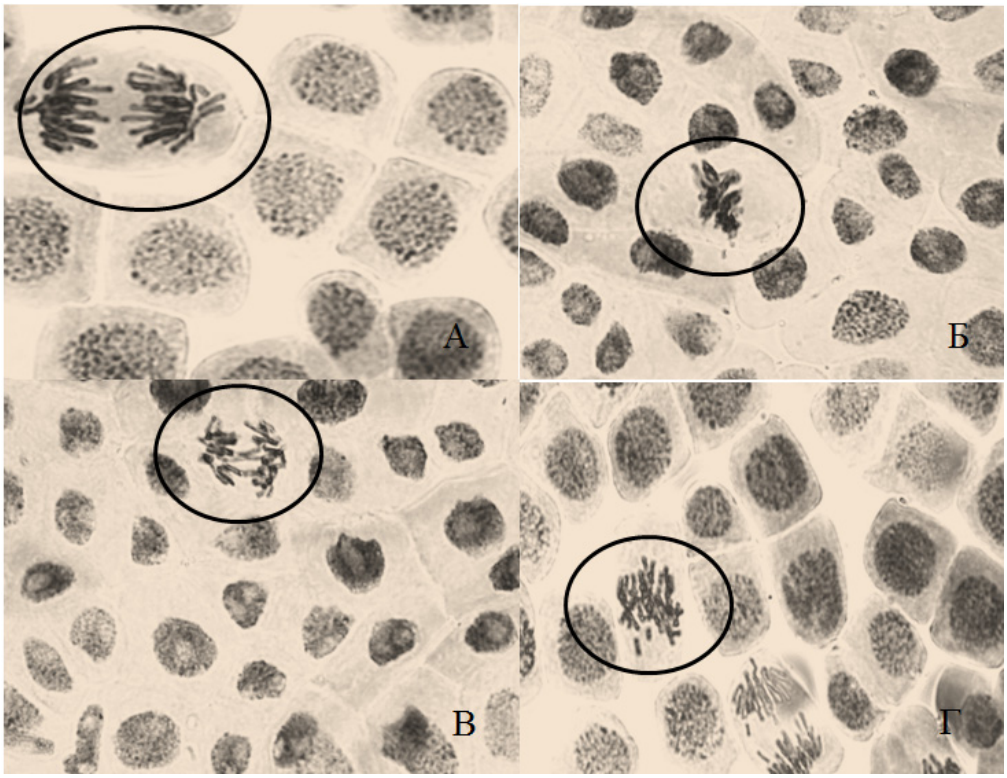

Figure 2. Dried preparations of onion meristem cells. A is forwarding in anaphase, B is adhesion in metaphase, C is a bridge in anaphase, D is fragmentation in metaphase

food products. We were the first to obtain some data on combined effects produced by these sweeteners and to register a synergic effect. It was revealed that these chemicals exerted an authentic impact on a range of chromosome aberrations in meristem cells against the control. It turned out that the most informative results as regards assessing the above mentioned toxicity and genotoxicity parameters were obtained via Allium-test with preliminary sprouting (the second modification).

The obtained results are consistent with an above-mentioned theoretical assumption that a mixture of aspartame and sucralose would be more toxic than each of them separately, and it should be taken into account when hygienic standards for contents of these substances in food products are developed.

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ASSESSMENT OF CORRELATION BETWEEN HETEROGENEOUS RISK FACTORS AND MORBIDITY AMONG WORKING POPULATION IN RUSSIAN REGIONS WITH DIFFERENT BACKGROUND OF HEALTH FORMATION

N.A. Lebedeva-Nesevrya[1], A.O. Barg[1], M.Yu. Tsinker[2], V.G. Kostarev[3]

[1]Perm State University, 15 Bukireva Str., Perm, 614990, Russian Federation
[2]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation
[3]Federal Service for Surveillance over Consumer Rights protection and Human Well-being, Perm regional office, 50 Kuibyisheva Str., Perm, 614016, Russian Federation

The research focuses on classifying Russian regions as per their sanitary-hygienic and social-economic welfare, as well as on assessing (for certain nosologies) correlations between heterogeneous risk factors and morbidity with temporary disability among working population. The RF regions were classified (with k-average cluster analysis) as per their sanitary-hygienic and social-economic welfare in order to spot out territories with similar "background" for formation of working population health.We used data provided by the Federal Statistic Service (as per the RF regions) collected in 2016 as our empiric base. As per cluster analysis results, we assigned the RF regions into four specific categories, namely "ill-being", "moderately ill-being", "moderately well-being", and "well-being" (the obtained data are visualized on the map of the country).The performed correlation-regression analysis allowed us to obtain more than twenty authentic models that described correlations between various factors and morbidity with temporary disability among working population.We calculated determination coefficient R^2 for each model that characterized a share of explained variation in a health parameter caused by a factor that was considered in a model.We paid special attention to the 1st cluster that had the least favorable background for health formation (here we detected most apparent influence exerted by social and economic factors on analyzed health parameters of working population). The 2nd clyster was also examined thoroughly as it was characterized with the highest morbidity among working population (we revealed that social-hygienic welfare on territories belonging to this cluster had greater influence on health parameters than social and economic one).Our data can be appliedto create federal and regional programs aimed at preserving and improving working population health.

Key words: *working population, morbidity with temporary mortality, heterogeneous health risk factors.*

National targets to be reached and strategic development tasks to be solved in the RF over a period of time up to 2024 are fixed in the RF President Order dated May 07, 2018. They are all aimed at streamlining and speeding up technologic development of the country, as well as at providing economic growth rates higher than average ones in the world. All that should result in Russia taking its place among the top five economies in the world[1]. These plans are quite ambitious and they require high-quality labor resources that can provide relevant labor productivity. Russian workers employed at enterprises and companies are assumed to be highly-qualified professionals and to have intellectual and innovative potential as well as good health. The latter is a significant issue given decreasing number of population and a growth in average age of employable population in the country which oc-

Natalya A. Lebedeva-Nesevrya – Doctor of Sociological Sciences, Associate Professor, Head of Laboratory for Social Risks Analysis Techniques, Professor at Sociology Department (e-mail: natnes@fcrisk.ru; tel.: +7 (342) 237-25-34; ORCID: https: //orcid.org/0000-0003-3036-3542).

Anastasiya O. Barg – Candidate of Sociological Sciences, Associate Professor at the Department of Sociology (e-mail: an-bg@yandex.ru; tel.: +7 (342) 239-63-29; ORCID: https: //orcid.org/0000-0003-2901-3932).

Mikhail Y. Tsinker – junior research associate of Department of Mathematical Modeling of Systems and Processes (e-mail: cinker@fcrisk.ru; tel.: +7 (342) 237-18-04; ORCID: https: //orcid.org/0000-0002-2639-5368).

Vitalii G. Kostarev – Candidate of Medical Sciences, Chief State Sanitary inspector in Perm region, Head of Rospotrebnadzor office in Perm region (e-mail: urpn@59.rospotrebnadzor.ru; tel.: +7 (342) 239-35-63).

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curs, among other things, due to a planned increase of the retirement age.

Preservation and improvement of workers' health in Russia is most often considered to be dependent on minimizing effects produced by occupational risk factors. Impacts exerted by occupational factors and working conditions on mortality and morbidity among employable population have been proven by research performed in the North America [1–3], Europe [4–6], and Russia [7–10]. A considerable number of studies on occupational morbidity and industrial injuries have been accomplished in Russian regions [11–15]. Their results allow concluding that all regions in Russia differ significantly as per health parameters of employable population; it is determined not only by peculiarities related to employment and working conditions, but also by anthropogenic contamination of the environment [16], and in some Russian regions also by climatic factors (low temperatures [17, 18], significant discrepancies in average temperatures in warm and cold seasons [19], a regional climate being too windy [20]). An extent to which people pursue health-preserving life style also influences their health greatly [21–23]; it is also true for social and economic context of workers' life [24, 25]. Impacts exerted by social and economic ill-being of a territory on mortality and morbidity among employable population are determined by a limited access to medical aid [26], poor living conditions [27], and prevalence of hazardous behavioral attitudes as regards health [28–30]. Besides, poor social and economic development of the country, frequent financial crises, and decreasing population incomes are able to create intensive (chronic) "social stress" [31].

Our research goal was to classify Russian regions as per sanitary-hygienic and social-economic welfare as well as to assess correlations between heterogeneous factors and parameters of morbidity with temporary disability among working population (for each specific category).

Data and methods. RF regions were classified via k-cluster analysis as per their sanitary-hygienic and social-economic welfare; it was done in order to spot out territories with similar "background" for formation of working population's health. Our empiric data were taken from the Federal State Statistics Service (as per RF regions); we used data for 2016. To determine sanitary-hygienic welfare in a region, we applied three parameters that characterized anthropogenic burden on the environment:

a) a share of ambient air samples not conforming to hygienic standards (%);

b) a share of drinking water samples deviating from hygienic standards as per sanitary-chemical parameters (%);

c) a share of soils samples not conforming to hygienic standards as per sanitary-chemical parameters (%).

We analyzed social-economic welfare in a region as per three macro-parameters that usually determined more particular socioeconomic ones:

a) adjusted gross regional product per capita (gross added value) (rubles);

b) specific weight of dilapidated housing in the overall housing stock (%);

c) population's purchasing power which is calculated as a ratio of average population incomes per capita to a living wage fixed in a specific RF region.

To assess working population's health in regions assigned into different clusters, we applied the following parameters of morbidity with temporary disability: a) a number of temporary disability cases (as per 100 workers), b) a number of days during which a worker was temporarily disabled (as per 100 workers in general, and as per sex as well), c) average duration of 1 temporary disability case.

We applied correlation-regression analysis for regions in each cluster to determine correlations between an extended list of various risk factors and morbidity with temporary disability among working population. Parameters related to sanitary-hygienic factors included a number of examined drinking water samples with hazardous chemicals contents higher than MPC; a number of examined samples taken from centralized water supply systems that didn't conform to hygienic standards (%); a number of examined soil samples taken in settlements that didn't conform to hygienic standards (%); a number of examined ambient air samples taken in cities and rural settle-

ments that contained hazardous chemicals in concentrations higher than MPC (%); a share of examined objects that didn't conform to hygienic standards as per noise (%); a share of examined objects that didn't conform to sanitary standards as per vibration (%); a share of examined objects that didn't conform to hygienic standards as per electromagnetic radiation (%). We applied the following social-economic factors in our analysis: value of the national wealth (value of fixed assets in various branches) per capita (rubles); investments into fixed capital per capita (rubles); unemployment rate (as per WLO methodology, %); expenses on education (rubles/person); expenses on public healthcare (rubles/person); number of physicians (all specialties; per 1 thousand people); number of nurses (per 1 thousand people); a living wage fixed in a region (rubles): average population incomes per capita (rubles); a ratio of average incomes per capita to a living wage (%); average monthly wage paid to an employed (rubles); a share of people with incomes lower than a living wage (%); a number of families who were provided with housing or improved their housing conditions on their own, a ratio to overall population; a share of housing without centralized water supply (%); a share of housing without sewerage (%); specific weight of housing equipped with centralized heating.

Results and discussion. Cluster analysis results allowed assigning RF regions into four different clusters. The first cluster included regions where the situation was the worst in terms of both sanitary-hygienic and social-economic situation there; there were 19 RF regions assigned into it. They were predominantly located in the Far East Federal District (Jewish Autonomous Area, Primorye region, Yakutia, and Khabarovsk region) and Siberia Federal District (Transbaikal region, Irkutsk region, Kemerovo region, Krasnoyarsk region, Novosibirsk region, Buryatia Republic, Tyva Republic) (Figure). Kurgan region and Chelyabinsk region from the Urals Federal District were also included into this cluster as well as Kirov region and Saratov region from the Privolzhskiy Federal District. This cluster also included Kursk region, Murmansk region, Ingushetia Republic, and Crimea Republic. The cluster had the highest share of ambient air samples that didn't conform to hygienic standards (average cluster value was 1.89%); soils samples not conforming to hygienic standards (10.38%); and the highest specific weight of dilapidated housing in the overall housing stock; purchasing power was the lowest in this cluster (2.36). Morbidity with temporary disability was not critical in this cluster as most examined parameters were close to average country values excluding "average duration of 1 temporary disability case" that amounted to 14.4 days in 2016.

The second cluster that included regions where a situation was "moderately adverse" had the highest share of drinking water samples that didn't conform to hygienic standards (39%). A share of dilapidated housing in the overall housing stock was also high in this cluster (4.4%). Other sanitary-hygienic and social-economic parameters were close to average country values but morbidity with temporary disability could be considered too high as actually four out of five parameters related to it were the highest in RF regions included into this cluster. A number of temporary disability cases amounted to 47.17 per 100 employed, and a number of days during which a person was off sick amounted to 643 (overall) per 100 employed, 737.9 (women), 598.2 (men).

Almost half of the regions located in the Central Federal District were assigned into this cluster (Vladimir region, Ivanovo region, Kostroma region, Smolensk region, Tver' region, Tula region, and Yaroslavl region), together with neighboring Novgorod region. Besides, the cluster included three more regions from the North-West Federal District (Arkhangelsk region, Leningrad region, and Karelia). Also some southern regions were ranked as being "moderately adverse": they were Rostov region, Dagestan, and Kalmykia; it included several "Siberian" regions, such as Komi Republic, Tomsk region, and Khanty-Mansi Autonomous Area and two regions from the Privolzhskiy Federal District, namely Perm region and Mordovia, and two regions from the Far East Federal District (Amur region and Magadan region). Overall, there were 21 RF regions in the second cluster.

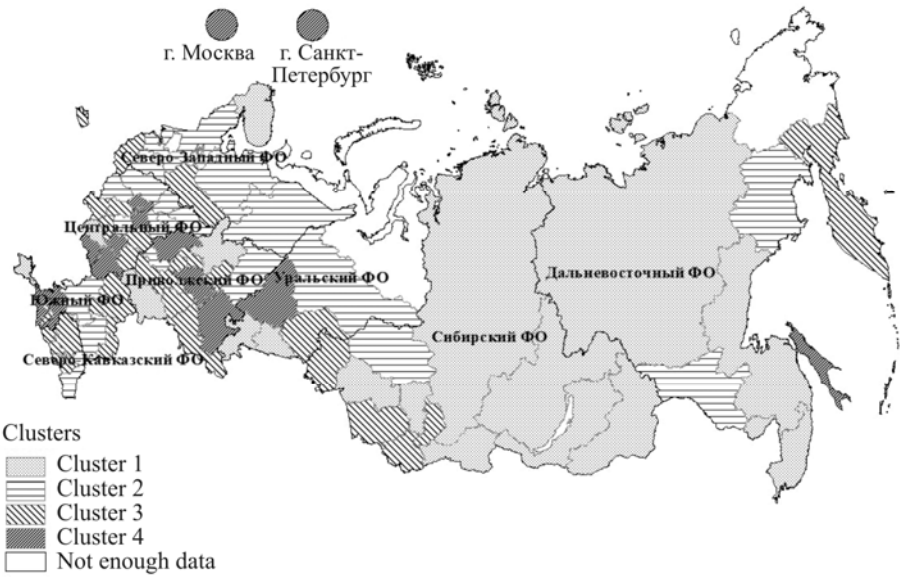

Figure. RF regions assigned into different clusters as per sanitary-hygienic and social-economic welfare

Table

Average values of social-economic and sanitary-hygienic parameters and temporary morbidity among employed population in clusters and in the country as a whole

Parameters	Average value in a cluster				Average in RF
	1	2	3	4	
Examined ambient air samples with chemicals in concentrations higher than MPC, %	**1.89**	0.17	0.34	0.43	0.67
Examined samples of water taken from centralized water supply systems not conforming to hygienic standards as per sanitary-chemical parameters, %	15.64	**39.01**	8.59	14.32	18.93
Examined soils samples taken in settlements not conforming to hygienic standards as per sanitary-chemical parameters, %	**10.38**	3.05	2.44	6.23	5.04
Purchasing power	2.36	2.65	2.55	**3.69**	2.71
Share of dilapidated housing, %	**5.14**	4.40	2.32	2.46	3.53
Gross regional product per capita (billion rubles)	768.81	772.28	517.49	**3 522.84**	1 117.43
A number of days during which a person is off sick per 100 employed	606.97	**643.09**	614.83	566.54	612.59
A number of temporary disability cases per 100 employed	42.23	**47.17**	45.23	42.07	44.53
Average duration of 1 temporary disability case	**14.44**	13.67	13.61	13.44	13.79
A number of days during which a person is off sick per 100 employed (men)	555.48	**598.25**	567.26	567.54	572.51
A number of days during which a person is off sick per 100 employed (women)	675.19	**737.94**	674.61	655.22	687.89

The third cluster was made up of "conditionally well" regions; only one parameter here ("adjusted gross regional product") was the lowest among all the clusters (517.4 billion rubles). All other parameters were close to average values, and "share of dilapidated housing in the overall housing stock" and "share of soils samples not conforming to hygienic standards" were the lowest in this cluster, 2.32% and 2.44% respectively (Table).

The third cluster included 29 RF regions, among them 10 Republics (Kabardino-Balkaria, Karachai-Cherkess, North Ossetia, Chechnya, and Chuvashia from the North-Caucasian Federal District, as well as Adygei, Altai Republic, Mari El, Khalassia, and Udmurtia. There were also five regions from the Central Federal District (Bryansk region, Kaluga region, Orel region, Ryazan region, and Tanbov region); four regions form the Privolzhskiy Federal District (Orenburg region, Penza region, Samara region, and Ulyanovsk region); three regions from the North-West Federal District (Vologda region, Kaliningrad region, and Pskov region); two regions from the Southern Federal District (Astrakhan region and Volgograd region) and the Siberian Federal District (Altai region and Omsk region) in this cluster; it also included Stavropol region, Kamchatka, and Tyumen region.

The fourth cluster where the situation was the safest and most favorable was the smallest one and included only 13 RF regions. First of all, they were Moscow city and Saint-Petersburg (federal cities); secondly, there were so called "federal regions" such as Krasnodar region, Moscow region, Bashkortostan, Tatarstan, and Sverdlovsk region; thirdly, the cluster included regions that were rich with mineral resources, namely Sakhalin and Yamal-Nenets Autonomous Area. This cluster also included Belgorod region, Voronezh region, Lipetsk region, and Nizhniy Novgorod region.

Regions in the fourth cluster had the highest gross regional product (average cluster value was 3,522 billion rubles) and purchasing power (average cluster value was 3.69); share of dilapidated housing in the overall housing stock was relatively low and amounted to 2.46%. Sanitary-hygienic well-being was a bit lower than social-economic one as regions from the cluster held the second rank place as per air and soils samples not conforming to hygienic standards just after the most unfavorable first cluster. Morbidity parameters were among the lowest; it was true for a number of temporary disability cases (42.07 per 100 employed), average duration of one temporary disability case (13.44 days), and a number of days during which a worker was off sick (566.5 per 100 employed overall; 655.22 per 100 employed women).

Our next step was to assess correlations between specific social-economic and sanitary-hygienic factors and morbidity with temporary disability among employed population; to do that, we took the above-mentioned cluster analysis results into account. Correlation-regression analysis allowed us to obtain several dozens of authentic pair models that described those correlations. We also calculated determination coefficient R^2 for each model; it described a share of explained variations in a health parameter caused by a factor considered in a model. We paid special attention to the first cluster as a background situation for health formation was the least favorable there, and to the second one as morbidity among employed population was the highest there.

We detected "factor – effect" relationships between certain social and economic factors and morbidity with temporary disability among employed people in the first cluster:

between investments into fixed capital per capita and a number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (-0.37), at p=0.003, a moderate correlation, R^2=0.14); number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (-0.38), at p=0.003, a moderate correlation, R^2=0.14); a number of temporary disability cases per 100 employed (correlation coefficient was equal to (-0.29), at p=0.025, a weak correlation, R^2=0.08);

between living wage and number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (-0.25), at p=0.047, a weak correlation, R^2=0.06); number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (-0.25), at p=0.049, a weak correlation, R^2=0.06); a number of temporary disability cases per 100 employed (correlation coefficient was equal to (-0.36), at p=0.004, a moderate correlation, R^2=0.13);

between average income per capita and a number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (-0.27), at p=0.035, a weak correlation, R^2=0.07), and a similar negative correlation was also detected in the fourth

("the most favorable") cluster (the correlation coefficient was equal to (-0.49), at p=0.002, a moderate correlation, R^2=0.24); a number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (-0.34), at p=0.006, a moderate correlation, R^2=0.12); a number of temporary disability cases per 100 employed (correlation coefficient was equal to (-0.31), at p=0.015, a moderate correlation, R^2=0.10);

There was a correlation between average wages paid to employed people and a number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (-0.29), at p=0.021, a weak correlation, R^2=0.08), and a similar negative correlation was also detected in the fourth ("the most favorable") cluster (correlation coefficient was equal to (-0.48), at p=0.002, a moderate correlation, R^2=0.23); a number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (-0.34), at p=0.007, a moderate correlation, R^2=0.11); a number of temporary disability cases per 100 employed (correlation coefficient was equal to (-0.29), at p=0.024, a weak correlation, R^2=0.08);

There was a correlation between unemployment (calculated as per WLO methodology) and a number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (-0.37), at p=0.003, a moderate correlation, R^2=0.14); a number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (-0.28), at p=0.027, a weak correlation, R^2=0.08).

As we can see, there tends to be an apparent correlation between health parameters and welfare of working population in the first cluster; in other words, working population health tends to depend on how economically developed these territories are. As all the above mentioned correlations are reverse, we can state that if social-economic welfare grows, morbidity with temporary disability among working population decreases.

We also detected significant influences exerted by specific sanitary-hygienic factors of working population health in the first cluster; there were several "factor – effect" relationships reveled in those regions.

A number of days during which a worker was off sick per 100 employed men depended on an increase in a share of examined samples containing the following chemicals in concentrations higher than MPC: ammonia and ammonia ion (correlation coefficient was equal to (0.34), at p=0,007, a moderate correlation, R^2=0.12); boron (correlation coefficient was equal to (0.50), at p=0,004, a significant correlation, R^2=0.25), and there was also a correlation between this parameter and a number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (0.47), at p=0.000, a moderate correlation, R^2=0.22) and a number of temporary disability cases per 100 employed (correlation coefficient was equal to (0.34), at p=0.008, a moderate correlation, R^2=0.11); manganese (correlation coefficient was equal to (0.40), at p=0.002, a moderate correlation, R^2=0.16), and there was also a correlation between this parameter and a number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (0.32), at p=0.001, a moderate correlation, R^2=0.10). There was a correlation between a number of days during which a worker was off sick per 100 employed men and a greater share of examined ambient air samples taken in urban and rural settlements that contained the following substances in concentrations higher than MPC: fluorine and its compounds (recalculated as per fluorine) (correlation coefficient was equal to (0.42), at p=0.005, a moderate correlation, R^2=0.17); hydrogen fluoride (correlation coefficient was equal to (0.40), at p=0.008, a moderate correlation, R^2=0.16); this parameter also depended on a increase in a share of objects examined with laboratory tools and not conforming to sanitary standards as per electromagnetic radiation (correlation coefficient was equal to (0.35), at p=0.006, a moderate correlation, R^2=0.13); on an increase in a share of objects that were examined with laboratory tolls and didn't conform to sanitary standards as per vibration (correlation coefficient was equal to (0.34), at p=0.009, a moderate correlation, R^2=0.11).

An increase in a share of examined drinking water samples that contained boron in concentrations higher than MPC determined an increase in

number of days during which a worker was off sick per 100 hundred employed women (correlation coefficient was equal to (0.54), at p=0.002, a significant correlation, R^2=0.29).

We determined the following correlations for the second cluster. Just as it was in the first one, when unemployment grew, it resulted in a decrease in a number of days during which a worker was off sick per 100 hundred employed men (correlation coefficient was equal to (-0.57), at p=0.000, a significant correlation, R^2=0.32), a decrease in a number of days during which a worker was off sick per 100 hundred employed people (overall) (a correlation coefficient was equal to (-0.45), at p=0.000, a weak correlation, R^2=0.20), and a decrease in a number of temporary disability cases per 100 employed (correlation coefficient was equal to (-0.36), at p=0.003, a moderate correlation, R^2=0.13). This can be due to people being afraid to lose a job as they are aware it will be rather difficult to find a new one.

When a ratio of average population incomes per capita to a living wage grew, it led to a decrease in a number of days during which a worker was off sick per 100 hundred employed women (a correlation coefficient was equal to (-0.61), at p=0.000, a significant correlation, R^2=0.38), and there was also a decrease in this parameter per 100 employed people (overall) (a correlation coefficient was equal to (-0.27), at p=0.032, a weak correlation, R^2=0.07).

It is interesting to note that an increase in an average duration of one temporary disability case correlated with a growth in various social and economic parameters at micro and macro-levels in the second cluster only. For example, growing expenses on public health care determined an increase in an average duration of one temporary disability case (correlation coefficient was equal to (0.44), at p=0.000, a moderate correlation, R^2=0.20); the same effect was produced by growing average monthly wages paid to employed population (correlation coefficient was equal to (0.54), at p=0.000, a significant correlation, R^2=0.30), an increase in a living wage (correlation coefficient was equal to (0.63), at p=0.000, a significant correlation, R^2=0.40). Growing investments into fixed capital per capita determined duration of temporary disability (correlation coefficient was equal to (0.61), at p=0.000, a significant correlation, R^2=0.37), and the same effect was produced by an increase in number of families that were provided with housing or improved their housing conditions on their own taken as a ratio to overall population number (correlation coefficient was equal to (0.33), at p=0.008, a moderate correlation, R^2=0.11). Better housing conditions also led to a longer duration of one temporary disability case in the fourth cluster (correlation coefficient was equal to (0.62), at p=0.000, a significant correlation, R^2=0.38). Number of nurses (per 1,000 people) in the 2nd and 4th clusters had a positive correlation with an average duration of one temporary disability case (correlation coefficient was equal to (0.59) and (0.55), at p=0.000, a significant correlation, R^2=0.37 and R^2=0.31 respectively). Duration of a disease case in other clusters correlated with only one or two socioeconomic factors but in the second cluster there were six such parameters; and it is only in the second cluster that this correlation was solely positive while, for example, in the third cluster (which was "conditionally well") growing investments into fixed capital per capita resulted in a shorter duration of one temporary disability case (correlation coefficient was (-0.28), at p=0.010, a weak correlation, R^2=0.08).

As for sanitary-hygienic factors, there were several most significant ones that determined parameters of working population health in the second cluster; they were:

– a share of examined drinking water samples that contained iron (including iron chloride) in concentrations higher than MPC; it resulted in greater number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (0.60), at p=0.000, a significant correlation, R^2=0.36) and women (correlation coefficient was equal to (0.38), at p=0.003, a moderate correlation, R^2=0.14), as well as per 100 employed population (overall) (correlation coefficient was equal to (0.44), at p=0.000, a moderate correlation, R^2=0.20); this sanitary-hygienic parameter also caused a growth in a number of temporary disability cases (correlation coefficient was equal to (0.41), at p=0.000, a moderate correlation, R^2=0.17);

– a share of drinking water samples that contained manganese in concentrations higher than MPC resulted in a greater number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (0.34), at p=0.009, a moderate correlation, R^2=0.11); the same was detected for excessive concentrations of copper in drinking water (correlation coefficient was equal to (0.38), at p=0.006, a moderate correlation, R^2=0.15)

– a share of examined soil samples taken in settlements that did not conform to hygienic standards as per cadmium resulted in a growing number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (0.30), at p=0.006, a weak correlation, R^2=0.09); the same effect was produced by a share of soil samples not conforming hygienic standards as per microbiological parameters (correlation coefficient was equal to (0.31), at p=0.012, a moderate correlation, R^2=0.10), and this parameter also correlated with a greater number of days during which a worker was off sick per 100 employed people (overall) (correlation coefficient was equal to (0.47), at p=0.012, a moderate correlation, R^2=0.22) and a greater number of temporary disability cases per 100 employed people (correlation coefficient was equal to (0.53), at p=0.000, a significant correlation, R^2=0.28). A greater share of soil samples not conforming to hygienic standards as per radioactive substances led to a greater number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (0.34), at p=0.006, a moderate correlation, R^2=0.12); the same effect was produced by soils samples not conforming to standards as per sanitary-chemical parameters (correlation coefficient was equal to (0.37), at p=0.003, a moderate correlation, R^2=0.13). A higher share of soils samples not conforming to hygienic standards as per lead contents resulted in a greater number of days during which a worker was off sick per 100 employed people (overall) (correlation coefficient was equal to (0.35), at p=0.000, a moderate correlation, R^2=0.12) and in a greater number of temporary disability cases per 100 employed people (overall) (correlation coefficient was equal to (0.30), at p=0.000, a weak correlation, R^2=0.09); a greater share of soils samples deviating from standards as per heavy metals contents led to a greater number of days during which a worker was off sick per 100 employed men (correlation coefficient was equal to (0.32), at p=0.011, a moderate correlation, R^2=0.10), a greater number of days during which a worker was off sick per 100 employed (overall) (correlation coefficient was equal to (0.31), at p=0.000, a moderate correlation, R^2=0.10), and a greater number of temporary disability cases per 100 employed (overall) (correlation coefficient was equal to (0.33), at p=0.000, a moderate correlation, R^2=0.11).

Conclusions. RF regions were assigned into four various clusters as per a set of sanitary-hygienic and social-economic parameters; regions in each cluster have similar "background" for formation of working population's health. The first cluster, with the most adverse conditions, included 19 RF regions; there were high shares of examined ambient air samples and soils samples taken in settlements that contained adverse chemicals in concentrations higher than MPC and didn't conform to hygienic standards as per sanitary-chemical parameters, a significant share of dilapidated housing in the overall housing stock, and high average duration of one disease case. The second cluster, "moderately adverse" one, included 21 RF regions; there were high shares of examined water samples taken from centralized water supply systems that didn't conform to hygienic standards as per sanitary-chemical parameters, the highest number of days and cases of temporary disability per 100 employed (overall, men, and women). The third cluster, or 'conditionally well" one, included 29 RF regions; there was the lowest shares of dilapidated housing, and sanitary-hygienic parameters that didn't conform to hygienic standards were lower than on average in the country. The fourth cluster, or the most favorable one, included 13 RF regions; there was the highest purchasing power and gross regional products per capita, and sanitary-hygienic parameters that deviated from standards were lower than on average in the country.

We detected negative correlations between health parameters and certain social and economic parameters in the first ("the most

adverse") cluster; these parameters were investments into fixed capital per capita, living wage, average population income per capita, average monthly wages paid to employed people, and unemployment rate (calculated as per WLO methodology). It substantiates the necessity to increase welfare of population living in RF regions from this cluster as it will lead to improvement in their health.

As for the second cluster ("moderately adverse" one), we detected the strongest correlations between health parameters and sanitary-hygienic parameters, especially those related to drinking water and soil samples.

Cluster analysis results revealed that an average share of water samples taken from water supply systems that didn't conform to hygienic standards as per sanitary-hygienic parameters amounted to 39% in RF regions from the second cluster (and it was more than 2 times higher than on average in the country and in other clusters); given that, it is quite reasonable to pay greater attention exactly to sanitary-hygienic parameters in the second cluster.

Such a social-economic parameters as unemployment rate turned out to be significant for health formation in all four clusters.

Therefore, when developing federal and regional programs aimed at preserving and improving health of overall population and employable population in particular, it is necessary to take detected risk factors into account depending on a cluster a territory belongs to and treat them as priority ones.

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OCCUPATIONAL PATHOLOGY IN WORKERS EMPLOYED AT DEEP AND SURFACE MINING OF APATITE ORES IN THE KOLA ZAPOLYARYE

S.A. Syurin, S.A. Gorbanev

North-west Public Health Research Center, 4, 2nd Sovetskaya Str., Saint Petersburg, 191036, Russian Federation

Ore mining technologies are constantly developed and improved; nonetheless, most miners employed at apatite mines in Zapolyarny regions of the Kola Peninsula run higher risks of occupational diseases (OD). The research goal was to explore peculiarities related to OD occurrence in miners employed at deep and surface apatite mines. We examined data collected within social-hygienic monitoring activities in 2007–2017, the section "Working conditions and occupational morbidity"; the data were collected for the overall Murmansk region population, there were 470 patients with OD and 749 OD cases revealed in the region over the selected time period. We detected that in case of deep mining OD (first of all, in the musculoskeletal system) occurred at a younger age and after a shorter working period due to increased labor hardness (59.6 %). As regards surface mining, labor hardness and overall vibration were the basic etiologic factors there, and OD structure was characterized with considerable fractions of diseases in the musculoskeletal system (32.7 %) and vibration disease (hand-arm vibration syndrome, 31.8 %). OD number in one worker employed at deep mines was higher than in one worker employed at surface mining (1.68 ± 0.07 and 1.49 ± 0.5, $p<0.02$). Workers employed at deep mines run higher risks of deforming arthrosis (RR=6.88; 95 % CI 3.21–14.74; χ^2=35.7; p<0.001) and forearms myofibrosis (RR=8.11; 95 % CI 1.92–34.1; χ^2=11.8; p=0.0005); workers employed at surface mining run higher risks of vibration disease (RR=1.40; 95 % CI 1.08–1.80; χ^2=6.69; p=0.009) and radiculopathy (RR=1.47; 95 % CI 1.12–1.93; χ^2=7.61; p=0.006). Both occupational groups have to face unfavorable working conditions mostly due to technological processes and workplaces being out-of-date. We came to a conclusion that it was necessary to create programs aimed at prevention of occupational diseases in workers employed at apatite mines in the Arctic taking into account peculiarities related to hazardous occupational impacts and accompanying adverse factors associated with the chilling environment.

Key words: *apatite ores, deep and surface mining, miners, occupational pathology, the Arctic, occupational diseases, musculoskeletal system diseases, vibration disease.*

Khibiny apatite-nepheline ore mines that are located on the Cola Peninsula are among the richest in the world with their explored ore reserves exceeding 4 billion tons [1]. Ores have been mined there both in deep mines and on the surface since 30ties last century and nowadays 26–28 million tons are extracted annually. On average, overall number of workers who directly deal with ore extraction under severe climatic conditions in this polar region amounts to 4-5 thousand people [2]. Technologies that are applied at mining and chemical enterprises are constantly developing but still most miners employed at apatite-nepheline ores in Polar Regions of the Cola Peninsula have to work under hazardous working conditions[1] [3]. Overall and local vibration, labor hardness, noise, chilling microclimate at workplaces, dust and gas mixtures, work in forced and uncomfortable postures, as well as some other occupational factors lead to elevated risks of common and occupational diseases (OD) [4–7]. Natural and climatic conditions in the Far North are known to potentiate influences exerted by hazardous occupational factors on workers' health [8–10]. All the above mentioned indicates that it is relevant

Sergey A. Syurin – Doctor of Medical Sciences, Chief researcher (e-mail: kola.reslab@mail.ru; tel.: +7(812)-7179783; ORCID: http://orcid.org/0000-0003-0275-0553).

Sergey A. Gorbanyov – Doctor of Medical Sciences, Director (e-mail: s-znc@mail.ru; tel.: +7(812)-7179783; ORCID: http://orcid.org/0000-00025840-4185).

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to reveal peculiarities related to health disorders and their prevention among miners who are exposed to hazardous occupational factors varying in their nature and intensity.

Our research goal was to examine peculiarities related to OD development in miners employed at apatite-nepheline mines and dealing with underground and surface mining in Polar Regions of the Cola Peninsula.

Data and methods. We examined social and hygienic monitoring data contained in «Working conditions and occupational incidence» section; the data were collected in Murmansk region over 2007–2017 and provided by the Rospotrebnadzor's Federal Center for Hygiene and Epidemiology.

We processed our research results with Microsoft Excel 2010 and IBM SPSS Statistics v.22 software. We determined Student's t-test for independent samplings, fitting criterion χ^2, relative risk (RR), and 95% confidence interval (95% CI). Numeric data were given as simple mean and standard error ($M\pm m$). Critical significance of a zero hypothesis was considered to be 0.05.

Results. We examined data on 470 miners employed at deep (n=256), and surface (n=214) apatite-nepheline mines who had 749 occupational pathology cases diagnosed in them over 2007–2017.

Almost all miners (98.9%) were males. Their average age at which occupational pathology was first detected in them amounted to 52.6±0.3, and their working experience in mining industry was equal to 26.0±0.3 лет. Age and working experience were higher among workers employed at surface mines (p<0,001) than among those employed at deep ones (Table 1).

Given significant differences between deep and surface mining technologies [2, 11], occupations that examined workers had were beyond compare. As regards deep miners, OD were diagnosed in 48 (18.8%) hole men, 39 (15.2%) shaft men, 29 (11.5%) drilling machine operators, 28 (10.9%) load-haul-damper operators, 17 (6.6%) underground miners, and 15 (5.95) timbermen. Deep miners who had other occupations suffered from OD considerably less frequently.

Table 1

Overall and occupational characteristics of miners employed at deep and surface apatite mines and suffering from occupational diseases

Parameters	Deep mines	Surface mines	Total
Sex: males females	254 (99.2%) 2 (0.8%)	211 (98.6%) 3 (1.4%)	465 (98.9%) 5 (1.1%)
Age, years	51.6±0.5	53.8±0.4*	52.6±0.3
Work experience, years	24.9±0.5	27.4±0.5*	26.0±0.3

Note: * means there are statistically significant discrepancies (p<0.05) between miners employed at deep and surface mines.

As regards surface miners, OD were detected in 104 (48.6%) mining track drivers, 43 (20.1%) earthmover drivers, 26 (12.15) drilling machine operators, and 21 (9.8%) bulldozer drivers. Workers with other occupations almost never suffered from OD.

Annually there were considerable changes in number of miners with first detected occupational pathologies over the examined periods, with both increases and decreases in it. In some cases, we even detected opposite dynamics in two workers' groups being compared. Workers employed at deep mines ran higher OD risks than those employed at surface mines in 2007 (RR = 2.68; 95% CI 1.08–6.66; $\chi^2 = 4.13$; p = 0.042); 2008 (RR =2.31; 95% CI 1.04–5.15; $\chi^2 = 4.44$; p = 0.035); and 2012 (OP = 2.41; 95% CI 1.18–4.91; $\chi^2 = 5.17$; p = 0.023). Only in 2015 workers employed at surface mines ran higher OD risks (RR = 1.64; 95% CI 1.00–2.69; $\chi^2 = 5.18$; p = 0.023). Over 11-year examined period, occupational incidence was the highest in 2011, 2014, and 2015. Overall, there was an ascending trend detected for first detected OD among miners over 2007–2015; but their number tended to decrease in 2016–2017 (Figure).

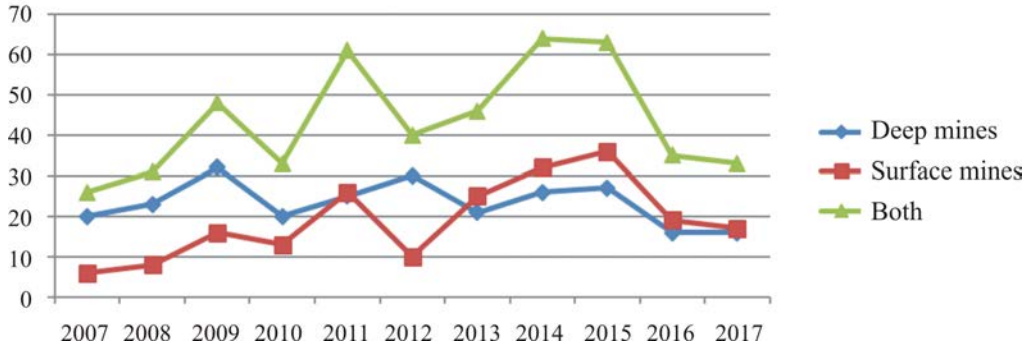

Figure. Annual number of miners with first detected occupational pathology

Number of OD cases was higher among deep miners than among those employed at surface mines ($p<0.02$). Structure of occupational pathology was quite similar in both groups. The first three rank places belonged to 1) musculoskeletal system diseases; 2) injuries, intoxications, and some other outcomes caused by external impacts; 3) nervous system diseases. Musculoskeletal system pathologies had bigger share in OD structure among deep miners than among those employed at surface mines ($p<0.001$). Three most widely spread OD nosologies detected in deep miners included vibration disease, radiculopathy, and deforming arthrosis; as regards surface miners, they suffered from vibration disease, radiculopathy, and mono-polyneuropathy.

Workers employed at deep mines ran elevated risks of deforming arthrosis (RR = = 6.88; 95% CI 3.21–14.7; $\chi^2 = 35.7$; $p<0.001$), and forearm myofibrosis (RR =8.11; 95% CI 1.92–34.1; $\chi^2 = 11.8$; $p = 0.0005$); those employed at surface mines ran elevated risks of vibration disease (RR = 1.40; 95% CI 1.08–1.80; $\chi^2 = 6.69$; $p=0.009$), and radiculopathy (RR = 1.47; 95% CI 1.12–1.93; $\chi^2 = 7.61$; $p = 0.006$).

There were six acute intoxications caused by carbon oxide and dioxide as well as by some other toxicants at deep mines. There were no such cases at surface mines. OD were more frequently detected as per regular medical check-ups results among deep miners ($p<0.001$) than among those employed at surface mines (Table 2).

There were significant discrepancies in hazard categories of working conditions that existed at deep and surface apatite mines. Working conditions at surface mines often belonged to 3.1 and 3.2 hazard category ($p<0.001$); 3.3 hazard category was rarer ($p<0.001$); and we did not detect any working conditions that could be ranked as having 3.4 or 4 hazard category. At deep mines, there were several factors that caused occupational pathology occurrence such as toxic substances belonging to I–IV hazard category ($p<0.01$), local vibration ($p<0.001$), labor hardness with 3.1 hazard category or higher ($p<0.001$); overall vibration was the most significant factor at surface mines ($p<0.001$).

Technological conditions that caused OD occurrence were similar in both groups of miners. The most significant technological factors were poorly developed technological processes, out-of-date organization of workplaces, machines, mechanisms, tools, accessories and equipment having constructive drawbacks (Table 3).

Discussion. Our research revealed that though technological processes were being developed at apatite mines in Polar Regions of the Cola Peninsula, it didn't lead to any substantial improvement of working conditions for miners; two thirds of them still had to work in a hazardous environment, with working conditions at their workplaces belonging to 3.2 hazard category. As it has always been, miners are among those workers

Table 2

Occupational pathology among miners employed at apatite mines in the Arctic

Parameters	Deep mines	Surface mines	Total
Disease cases	431	318	749
Diseases per 1 worker	1.68±0.07	1.49±0.05*	1.59±0.03
Occupational nosologies:			
Musculoskeletal system diseases	207 (48.0)	104 (32.7)*	311 (41.5)
Injuries, intoxications, and some other outcomes caused by external impacts	97 (27.0)	101 (31.8)	198 (26.4)
Nervous system diseases	66 (15.3)	58 (18.2)	124 (16.6)
Ear and mastoid diseases	54 (12.5)	48 (15.1)	102 (13.6)
Respiratory organs diseases	7 (1.6)	5 (1.6)	12 (1.6)
Most frequent diseases:			
Vibration disease	90 (20.9)	101 (31.8)*	191 (25.5)
Radiculopathy	77 (17.9)	91 (28.6)*	168 (22.4)
Mono-polyneuropathy	64 (14.8)	56 (17.6)	120 (16.0)
Sensorineural hearing loss (noise effects in inner ear)	54 (12.5)	48 (15.1)	102 (13.6)
Deforming arthrosis	75 (17.4)	7 (2.2)*	82 (10.9)
Myofibrosis	23 (5.3)	2 (0.6)	25 (3.3)
Chronic bronchitis	6 (1.4)	3 (0.9)	9 (1.2)
Nature of a disease:			
Acute	6 (1.4)	–	6 (0.8)
Chronic	425 (98.6)	318 (100.0)	743 (99.2)
Occupational diseases detection:			
Medical check-up	301 (69.8)	179 (56.3)*	922 (63.0)
A patient applying for medical aid	130 (30.2)	139 (43.7)*	541 (37.0)

Note: * means there are statistically significant discrepancies ($p<0.05$) between miners employed at deep and surface mines.

Table 3

Factors that caused occupational pathology occurrence among workers employed at apatite mines in the Arctic

Parameters	Deep mines	Surface mines	Total
Working conditions categories:			
Hazard category 2	3 (1.2)	2 (0.9)	5 (1.1)
Hazard category 3.1	30 (11.7)	52 (24.3)*	82 (17.4)
Hazard category 3.2	150 (58.6)	157 (73.4)*	307 (65.3)
Hazard category 3.3	40 (15.6)	3 (1.4)*	43 (9.1)
Hazard category 3.4	28 (10.9)	0*	28 (6.0)
Hazard category 4	5 (1.9)	0	5 (1.2%)
Factors causing occupational pathologies:			
Labor hardness (3.1 or higher hazard category)	257 (59.6)	148 (46.5)*	394 (52.6)
Overall vibration	57 (13.2)	109 (34.3)*	166 (22.2)
Noise	54 (12.5)	48 (15.1)	102 (13.6)
Local vibration	48 (11.1)	8 (2.5)*	56 (7.5)
I–IV hazard category toxicants	14 (3.2)	2 (0.6)*	16 (2.1)
Fibrogenic aerosols	1 (0.2)	3 (0.9)	4 (0.5)

Technological conditions causing occupational pathologies:			
Technological processes being poorly developed	255 (59.2)	193 (60.7)	448 (59.8)
Out-of-date organization of workplaces	117 (27.1)	86 (27.0)	203 (27.1)
Machines, mechanisms, tools, accessories and equipment having constructive drawbacks	52 (12.1)	38 (11.9)	90 (12.0)
Technical regulations being violated	5 (1.2)	–	5 (0.7)
Out-of-date sanitary-technical equipment	2 (0.5)	1 (0.3)	3 (0.2)
Safety rules being violated	2 (0.5)	–	2 (0.3)

Note: * means there are statistically significant discrepancies ($p<0.05$) between miners employed at deep and surface mines.

who run elevated risks of occupational pathologies [11–13]. We detected that there were several significant factors causing occupational pathologies such as labor hardness, overall and local vibration, and noise. Poorly developed technological processes, out-of-date organization of workplaces and constructive drawbacks of machines, mechanisms, tools, accessories and equipment intensified negative influences exerted by risk factors on workers' health.

Musculoskeletal system diseases, just as before, took the first rank place in occupational pathology structure among miners employed at apatite mines [7, 14, 15]. The most widely spread nosologies in the category were radiculopathy, deforming arthrosis, and forearm myofibrosis. There were also such most widely spread pathologies as vibration disease and sensorineural hearing loss and it was quite consistent with the existing hazardous occupational factors.

Working conditions existing at deep mines were less favorable than those at surface ones. It can explain why OD occur among deep miners at younger age (51.6±0.5 against 53.8±0.5, $p<0.001$) and after a shorter working experience period (24.9±0.5 against 27.4±0.5, $p<0.001$). Labor hardness and local vibration caused high prevalence of musculoskeletal system diseases among workers employed at deep mines while workers employed at surface mines tended to suffer from vibration disease more frequently. We should also note that acute OD were detected only among workers employed at deep mines.

Since we performed our research in the Arctic region, we can well assume that adverse (chilling) microclimate at workplaces had its etiological significance [9, 16]. Chilling, both overall and local, is well known to result in lower physical and mental working capacity, coordination disorders, elevated risks of health disorders and work injuries [17, 18]. However, we didn't detect any OD caused by this factor. Probably, impacts exerted by exposure to cold on miners' bodies were not fully explored due to meteorological defects in control and surveillance activities. Another issue is serious differences (both decreases and increases) in number of OD cases annually detected in workers employed at deep and surface apatite mines. Inconsistence existing in cause-and-effect relations between working conditions and workers' health make us assume there are influences exerted by other factors such as low-quality medical check-ups, OD number being regulated by administrative personnel, etc. [19, 20].

Conclusion. Programs aimed at preventing occupational pathology among workers employed at apatite mines in the Arctic should be developed taking into account peculiarities of impacts exerted by adverse occupational factors. It is vital to make labor processes easier both for deep and surface miners and to decrease overall vibration at surface mines. Technical measures aimed at preventing health disorders should primarily involve technological processes improvement and better workplace organization, elimination of constructive defects in machinery and other equip-

ment, as these factors cause more than 85% occupational pathology cases detected among miners employed at apatite mines in Cola Polar Regions. It is also necessary to improve techniques for assessing influences exerted on workers' health by chilling microclimate at workplaces as it can enhance and modify effects produced by adverse occupational factors.

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ON QUANTITATIVE ASSESSMENT OF MICROBE RISK CAUSED BY EXPOSURE TO ENTERIC VIRUSES IN DRINKING WATER

E.V. Baydakova[1,2], T.N. Unguryanu[1,2], R.I. Mikhailova[3]

[1]Arkhangelsk Region Department of the Federal Service on Customers' Rights Protection and Human Well-Being Surveillance, 24 Gaidar St., Arkhangelsk, 163000, Russian Federation
[2]Northern State Medical University, 51 Troitsky Av., Arkhangelsk, 163000, Russian Federation
[3]Centre for Strategic Planning and Management of Biomedical Health Risks, Russian Ministry of Health, Bldg. 1, 10 Pogodinskaya Sr., Moscow, 119121, Russian Federation

The authors assessed microbiological risks of acute intestinal infections (AII) with viral etiology caused by drinking water taken from centralized water supply systems among overall urban population in Arkhangelsk region over 2006-2017. The research was performed with Quantitative Microbial Risk Assessment (QMRA) procedure. It was revealed that acute intestinal viral infections prevailed among intestinal infections; the most widely spread ones were rotavirus infection (86.9%), norovirus infection (7.7%), and enterovirus infection (3.7%). The authors also performed comparative analysis of spatial distribution and long-term dynamics of incidence with AII which were possibly caused by infectious agents entering a body with water. The analysis revealed that rotavirus and norovirus infections frequently occurred in Arkhangelsk, Novodvinsk, Koryazhma, and Kotlas. Incidence with rotavirus infection among population in Koryazhma and Arkhangelsk grew 1.5-1.6 times faster than epidemiological processes on the reference territory. Coliphages contents were equal to P_{95}in drinking water taken from centralized water supply systems in Arkhangelsk and Koryazhma, and it was 1.4 and 2.2 times higher respectively than the hygienic standard. Rotavirus, norovirus, and enterovirus infections were highly likely to occur in Arkhangelsk (R=0.97-0.99), and rotavirus infection, in Koryazhma (R=0.95). Average probability of norovirus infection (R=0.58) and enterovirus infection (R=0.43) was detected in Koryazhma. The research results indicate that Quantitative Microbial Risk Assessment (QMRA) procedure is feasible and significant within the system of sanitary-epidemiologic surveillance over water treatment; it substantiates the necessity to create and implement virology monitoring over centralized drinking water supply.

Key words: *drinking water, water supply, acute intestinal infections, viral infections, coliphages contents, risk assessment, microbiological risk, QMRA.*

To ensure safety of drinking water supply is an efficient way to prevent diseases caused by drinking water consumption. In Russia, centralized water supply systems which are used to supply drinking water to consumers require urgent improvement [1]. Even if water purification is qualitative, there is still a possibility that infectious agents can penetrate water supply networks, for example, when water distribution systems are worn out, or in case of a damage done to water pipelines [2, 3]. Cases of acute enteric infections caused by infectious agents caught from water haven't been examined sufficiently due to epidemiologic analysis insensitivity and absence of research that allows to establish a direct correlation between drinking water contamination and a growth in sporadic incidence [4, 5].

Domestic techniques applied to assess microbe risks are based on factors which are directly related to contagion from water, for example, communal water supply and communal improvement of settlements, as well as quality of water taken from recreation water

Elena V. Baydakova – chief expert at the Epidemiologic Surveillance Department; junior lecturer at the Hygiene and Medicine Ecology Department (e-mail: elenabaydakova@yandex.ru; tel.: +7 (8182) 20-06-56; ORCID: https://orcid.org/0000-0002-1570-6589).

Tatiana N. Unguryanu – Doctor of Medical Sciences, chief expert at the Department for Activities Organization and Provision; Professor at the Hygiene and Medicine Ecology Department (e-mail: unguryanu_tn@mail.ru; tel.: +7 (8182) 20-06-56); ORCID: https://orcid.org/0000-0001-8936-7324).

Rufina I. Mikhaylova – Doctor of Medical Sciences, Head of the Laboratory for Drinking Water Supply Hygiene and Water Biophysics (e-mail: awme@mail.ru; tel.: +7 (499) 246-76-74; ORCID: https://orcid.org/0000-0001-7194-9131).

reservoirs and water sources[1]. Microbe risk assessment as a complex scientific approach based on quantitative assessment of influences exerted by microbe-related factors is widely used in foreign studies [6, 7]. Foreign techniques have some differences from domestic ones. In particular, Quantitative Microbial Risk Assessment, or QMRA, is a mathematical system for assessing infectious risks caused by pathogens which are hazardous for human health. The procedure can help to reveal and regulate risks related to microorganisms transferred with water, in particular, as regards sporadic diseases. It can be applied to analyze specific risk factors, for example, quality of water taken from a recreation zone or drinking water from centralized water supply systems; it can also become a part of complex research [8].

Virological monitoring over environmental objects involves exploring drinking water taken from centralized water supply systems aimed at detecting viruses in it; usually, a virological trap is used with subsequent application of polymerase chain reaction (PCR). However, this procedure doesn't allow to quantitatively assess infectious agent contents in water as it can only detect whether virus DNA or RNA are present in a sample without distinguishing between live or inactivated viruses in water [9]. Therefore, this procedure for detecting viruses in drinking water can't be applied to identify hazards; given that, data on microbe contamination indicators are the only available microbiological data that can be applied to characterize water quality.

In spite of some uncertainties in estimating pathogen concentrations quantified as per detected microbiological parameters of drinking water quality, literature contains some examples how to use a ratio of a pathogen to quality parameters in order to quantitatively determine pathogen concentrations in the environment for QMRA. If we take into account epidemiology (regularities in microbe prevalence and sources) and environmental context (relative resistance and transfer), than we can see that fecal indicators data are greatly significant for QMRA. For example, QMRA model utility was applied in research based on limited data, and a conclusion was reached that coliphages quantity can be considered equivalent to quantitative contents of a virus in water (notably, rotavirus)[2] [7, 14]. Results of examinations performed on a wide range of bacterial and virus parameters related to fecal water contamination indicate that coliphages contents in water are more closely related to gastrointestinal tract diseases than detection of any other indicators showing microbiological contamination of water, such as coliform bacteria [15]. It is shown that when unpurified sanitary sewage penetrate a city water supply network and beach zones aimed for swimming, it leads to a growth in incidence with norovirus infection detected via parallel epidemiologic research [16].

In Arkhangelsk region 82% population living in Arkhangelsk, Novodvinsk, Kotlas, Koryazhma, and Severodvinsk are provided with water from centralized water supply systems which is taken from surface sources. All water sources that provide drinking water for people living in Arkhangelsk, Novodvinsk, Kotlas, and Koryazhma, belong to Severnaya Dvina water basin. A river called Solza is used as a water source for Severodvinsk; the river doesn't belong to the above-mentioned water basin. In 2017 only 35% population living in Arkhangelsk region were provided with qualitative drinking water [17] and it proves that it is necessary to examine microbe risks caused by population consuming low-quality drinking water.

Our research goal was to assess microbiological risks of intestinal infections for Arkhangelsk region population caused by consuming water from centralized water supply systems.

Data and methods. According to the QMRA procedure, the examination included

[1] MG 2.1.10.0031-11. Risks of bacterial enteric infections caught from water: a complex assessment / approved by the RF Chief Sanitary Inspector on July 31, 2011) [web-source]. – URL: http://base.garant.ru/70105056/(date of visit October 20, 2018).

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four stages: 1) hazard identification, or selecting a specific microbe agent and possible outcomes caused by it; 2) exposure assessment depending on a type, scope, and duration of influence exerted on a human body by a microbe agent; 3) assessment of "dose - response" relationship; and 4) risk characteristics [18].

At the first stage, when hazards were identified, we selected focus territories, infectious diseases groups, and indicators related to environmental factors; then we performed a descriptive epidemiologic examination of sporadic incidence with acute intestinal infections (AII) among the total population as per three nosologic groups: rotavirus, norovirus, and enterovirus AII with the data taken from a statistical report No. 2 "Data on infectious and parasitic diseases" collected over 2009-2017 in 5 cities in Archangelsk region (Arkhangelsk, Novodvinsk, Kotlas, Koryazhma, and Severodvinsk). Spatial analysis of incidence was performed with average long-term incidence value, and average long-term incidence among the total population in Severodvinsk was taken as a reference level. Severodvinsk was chosen as a reference territory due to an alternative water supply source as it is provided with water taken from Solza River which doesn't belong to Severnaya Dvina water basin; as for four other cities, they are all provided with water which comes basically or even solely from Severnaya Dvina. We compared average incidence values on different territories with the reference level as per a fraction of differences in indicators and indicators ratio. Differences were considered to be epidemiologically apparent if a fraction of differences in indicators exceeded 20%, and indicators ratio was higher than 1.25.

When identifying hazards, we examined quantitative contents of infectious agents in drinking water taken from centralized water supply systems as per social-hygienic monitoring data collected in 2006–2017. To solve the task, we performed a sanitary-hygienic assessment of water quality as per coliphages contents for water taken from centralized water supply systems in 5 cities in Arkhangelsk region – Arkhangelsk, Novodvinsk, Kotlas, Koryazhma, and Severodvinsk. To describe an examined parameter, we applied a specific weight of samples which deviated from standards, a median (Me), 75-th and 95-th percentiles (P_{75} and P_{95}).

Microbe agent dose was calculated as per the following formula (1):

$$\text{dose} = C*V, \quad (1)$$

where C is microbe agent concentration in 1 liter of consumed water, V is water consumption volume.

To calculate a dose, we applied coliphages contents at P_{95} level. Coliphages are more resistant to the environment than their host bacteria and it makes them able to indicate there is long-term fecal contamination. Researchers have proven there is a dependence between coliphages contents in water and contents of enteroviruses that are hazardous for human health [19, 20]. To calculate doses, we took water consumption as being equal to 0.743 l per day[3], which included tap water only and didn't take into account bottled water or other finished food products or drinks that contained water.

Exposure was assessed on the basis of openly published research results[4] [9, 10]. To assess probability of catching rotavirus and enterovirus infections, we applied "dose - response" exponential relationship model calculated as per the following formula (2):

$$P_{\text{infection probability}} = 1 - \exp(-\text{dose} * k), \quad (2)$$

where k quotient amounted to 0.00374 for enterovirus [22]; 0.173 [9], for rotavirus.

To assess probability of catching norovirus infection, we applied confluent hypergeometric function equation where α and β are Poisson beta-distribution parameters (3):

[3] Exposure Factors Handbook – Update (2009, External Review Draft) [web-source] // United States Environmental Protection Agency, Washington (DC), EPA/600/R-09/052A, 2009. – URL: https://cfpub.epa.gov/ (date of visit October 11, 2018).

[4] On sanitary-epidemiologic welfare of the RF population in 2017: the State Report. – M.: The Federal Service for Surveillance over Consumer Rights Protection and Human Well-being, 2018.– P. 105.

$$P_{\text{infection probability}} = 1 - {}_1F_1(\alpha, \alpha + \beta, -\text{dose}), \quad (3)$$

where α and β values for norovirus amount to 0.04 and 0.055 respectively [10]. To calculate equation values, we applied Wolfram Mathematic online, a software package used to calculate mathematical functions.

Probability of a diseases occurrence was calculated as per the following formula (4):

$$P = 1 - (1 - P_{\text{infection probability}})^n, \quad (4)$$

where n is a quantity of samples that deviate from the standard as per coliphages contents registered over the whole period under examination.

Results obtained for risk levels were assessed as per three ranges: R lower than 0.047 meant a risk of infectious disease occurrence among population was low (acceptable); R from 0.057 to 0.6095, average risk; R from 0.619 to 1 meant a risk was high [11].

Results and discussion. 48,931 AII cases with different etiology were registered in 5 cities in Arkhangelsk region in 2009-2017 among overall population living in them. Etiology remained unknown in 65.6% AII cases among all the registered ones and 34.4% had clear etiology. Ratio of AII with virus etiology to AII with bacterial etiology was 3:1.

Rotavirus infection took the first place among virus AII (86.9%); norovirus infection, the second one (7.7%); and enterovirus infection, the third one (3.7%). The lowest specific weight in virus intestinal infections structure belonged to hepatitis A (1.7%).

We analyzed AII incidence among population living in the examined cities and revealed that the highest incidence with rotavirus infection was registered in Kotlas ($299.4^0/_{0000}$) and Novodvinsk ($288.8^0/_{0000}$) as compared with other territories (Table 1). The highest frequency of norovirus infection and enterovirus infection was registered in Arkhangelsk and Novodvinsk, $19.4^0/_{0000}$ and $16.8^0/_{0000}$; as well as enterovirus infection, $11.0^0/_{0000}$ and $12.4^0/_{0000}$ respectively.

Rotavirus infection developed most rapidly in Novodvinsk and Kotlas where its advance ratios were 2.6-2.9 times higher than in Severodvinsk. Incidence with rotavirus infection developed among population in Koryazhma and Arkhangelsk 1.5-1.6 times faster than in Severodvinsk. As for incidence with norovirus infection, it developed in Novodvinsk and Arkhangelsk 10.3 and 9.1 times faster respectively than in Severodvinsk. No enterovirus infection cases were registered in Kotlas and Koryazhma; bearing in mind a persistent growing trend for this nosology in the country in general, we may assume that in these cities there is no epidemiologic suspicion as regards enterovirus infection. Thus, long-term incidence with enterovirus infection, including recent years (2015–2017) grew 1.5 times, and taken in comparison with long-term average level measured over previous ten years, it was 3.3 times higher [12].

Table 1

Spatial and time characteristics of incidence with virus AII among overall population living in the examined cities in Arkhangelsk region

Parameters	Territories (cities)				
	Arkhangelsk	Novodvinsk	Kotlas	Koryazhma	Severodvinsk
Rotavirus infection					
Average incidence, ($^0/_{0000}$)	161.8	288.8	299.4	159.2	104.0
Average advance ratio*, (times)	1.6	2.8	2.9	1.5	–
Norovirus infection					
Average incidence, ($^0/_{0000}$)	19.4	16.8	2.7	9.4	13.6
Average advance ratio*, (times)	1.4	1.1	0.1	0.7	–
Enterovirus infection					
Average incidence, ($^0/_{0000}$)	11.0	12.4	–	–	1.2
Average advance ratio*, (раз)	9.1	10.3	–	–	–

* means in comparison with Severodvinsk.

So, epidemiologic analysis of sporadic incidence with AII allowed to reveal the most widely spread infectious agents at the hazard identification stage. We performed a comparative analysis of spatial distribution and long-term dynamics of incidence with AII probably caught from water and revealed high frequency of rotavirus infection and norovirus infection in Arkhangelsk, Novodvinsk, Koryazhma, and Kotlas.

Specific weight of samples which deviated from hygienic standards as per coliphages contents in drinking water amounted to 6.2% and 7.0% after water treatment in Koryazhma and Kotlas respectively (Table 2). We didn't detect any microbiological abnormalities in drinking water at the median and 75-th percentile levels. Specific weight of samples taken from water pipelines in Arkhangelsk and Koryazhma that contained coliphages in quantities higher than fixed in hygienic standards amounted to 6.2% and 7.0% respectively. We detected samples deviating from hygienic standards as per coliphages contents at P_{95} in Arkhangelsk and Koryazhma; coliphages contents exceeded hygienic standards by 1.4 and 2.2 times respectively.

Quantitative risk of virus AII occurrence was assessed in the present research only for Arkhangelsk and Koryazhma as water samples taken from water supply systems with coliphages contents that exceeded hygienic standards were registered only in these two cities over the whole examined period.

We quantitatively assessed microbiological risks of AII occurrence related to centralized water supply systems and revealed that rotavirus, norovirus, and enterovirus infections were highly likely to occur in Arkhangelsk (R = 0.97–0.99); rotavirus infection, in Koryazhma (R = 0.95) (Table 3). Average probability of norovirus and enterovirus infection caused by consuming drinking water from centralized water supply systems was detected in Koryazhma (R = 0.58 and R = 0.43 respectively).

Conclusions. The performed research allowed to establish a structure of sporadic incidence with AII probably caught from water; it indicated that virus AII were the most widely spread with rotavirus, norovirus, and enterovirus infections prevailing among them.

Table 2

Quality of drinking water taken from centralized water supply systems assessed for a period 2006–2017 as per coliphages contents in pipelines

Territory	Number of samples			Me	P_{75}	P_{95}	X_{max}
	total	Higher than HS*	%**				
Arkhangelsk	1,382	86	6.2	0	0	1.4	16.1
Severodvinsk	337	0	0.0	0	0	0	0
Kotlas	153	0	0.0	0	0	0	0
Koryazhma	129	9	7.0	0	0	2.2	16.1
Novodvinsk	155	0	0.0	0	0	0	0

Table 3

Microbe risk related to occurrence of intestinal infections which are probably caught from water

Nosology	Infection probability	Disease probability	Probability characteristics
Arkhangelsk (dose = 10.4)			
Rotavirus infection	0.16	0.99	high
Norovirus infection	0.09	0.99	high
Enterovirus infection	0.04	0.97	high
Koryazhma (dose = 16.4)			
Rotavirus infection	0.24	0.95	high
Norovirus infection	0.11	0.58	average
Enterovirus infection	0.06	0.43	average

We assessed microbiological quality of drinking water taken from centralized water supply systems and detected there were deviations from hygienic standards in tap water as per coliphages contents in Arkhangelsk and Koryazhma.

Consumption of drinking water taken from city pipelines caused high risks of rotavirus, norovirus, and enterovirus infections for population in Arkhangelsk; average risks of norovirus and enterovirus infections and high risk of rotavirus infection for population in Koryazhma.

The performed research substantiates a practical feasibility of implementing Quantitative Microbial Risk Assessment, or QMRA, into sanitary-epidemiologic surveillance over water treatment and indicates the necessity to work out practical recommendations aimed at improving a system of virological monitoring over centralized water supply, raising quality of drinking water, and preventing incidence with acute intestinal infections caused by infectious agents caught from water.

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EPIDEMIOLOGY OF PROSTATE CANCER In KURSK REGION

I.L. Kiselev, A.A. Pol'shin

Kursk Regional Clinical Oncology Hospital, 1Eliseeva Str., Kislino, Kursk District, 305524, Russian Federation

The paper dwells on analysis performed on 2,238 male patients with primary incidence and 999 patients who died due to prostate cancer over 2007–2016 in Kursk region.

Data were processed with "Statistica for Windows" software package (ver. 12.5). The authors applied intensive overall and sex-age (per 100 thousand males) parameters. Overall incidence and mortality were compared with the data collected in the Central Federal District. Sex-age incidence and mortality due to prostate cancer were compared with the same parameters in the Russian Federation as a whole. To test validity of discrepancy between data sets, Student's parametric test (t) was applied with 95 % significance. Incidence and mortality due to prostate cancer were predicted for a period up to 2021 with an autoregressive integrated moving average (ARIMA).

Prostate cancer occupies the fifth place among malignant neoplasms in Kursk region and the sixth place in the overall mortality structure. In Kursk region a growth in incidence with prostate cancer amounted to 100.3 % in 2016 against 2007; a growth in mortality, 85.6 %. By 2021 incidence with prostate cancer can reach 71.24, and mortality, 30.79. Incidence among males in Kursk region has grown due to an increase in number of male patients aged 60–64 and 80 and older. Mortality caused by prostate cancer has grown due to an increase in number of deceased males aged 50 and older.

In the nearest future Kursk region can take a leading place among other regions in the Central federal District as per incidence and mortality due to prostate cancer. The research results are being applied for planning and developing a regional target program "Oncology".

Key words: *malignant neoplasms, epidemiologic analysis, incidence, mortality, prostate cancer, sex-age parameters, target program.*

Prostate cancer (PC) is one of the most widely spread malignant neoplasms in men [1–3]. Incidence with PC has increased significantly over recent years [4–5]. Researchers revealed that incidence with PC was higher in highly developed countries, and the lowest incidence with the disease was detected in the least developed countries [6].

Incidence with PC varies worldwide and discrepancies between different population groups can be more than 50 times [7, 8]. The highest incidence with PC is observed in Australia / New Zealand (111.6), in North America (97.2), and in Western Europe (85.8); the lowest one is registered in South and Central Asia (4.5) [7]. Regions where incidence level is substantial in comparison with other regions located on the same continent include South Africa (61.7), Western Asia (28.0), North America, Western Europe (85.8), and Australia / New Zealand (111.6) [8].

Ageing of population leads to a growth in life expectancy and causes risks of PC in future [7]. Risk of the disease is equal to approximately 1:10,000 among people younger than 40; still, each sixth man will have PC diagnosed in him over his life span. Men younger than 50 account for only 0.1% of patients with PC. Risk of the disease increases substantially only among those who are older than 60. Average age of male patients with PC amounts to 72–74 [9].

There are several risk factors causing the disease; primary ones are low physical activity, overweight, alcohol intake, consumption of red meat, inflammatory diseases in the kidneys and urinary bladder etc. [9, 10, 11]. 20.3 million new PC cases are predicted to be

Igor' L. Kiselev – Candidate of Medical Sciences, Associate Professor at the Oncology Department, Chief physician (e-mail: igor.leonidovich.kiselev@ya.ru; tel.: +7(4712)73-19-43; ORCID: https://orcid.org/0000-0001-6632-1753).

Aleksei A. Pol'shin – Oncologist, urologist (e-mail: ligrigam@yandex.ru; tel.: +7 (910) 316-02-64; ORCID: https://orcid.org/0000-0001-8266-9725).

detected by 2030, and 13.2 million patients form them will not survive [5, 7].

An issue related to PC screening is rather controversial [9, 12, 13]. Primary PC treatment usually involves eradication of the prostate, radiotherapy and hormonal therapy, but sometimes it can be only medical supervision. Choice on a therapy depends on age, cancer stage, comorbidity, preference of a patient and an urologist etc. [14].

Mortality caused by PC remains the highest and occupies the second rank place after mortality caused by lung cancer [1, 2, 7]. Frequency and prevalence of PC differ in different world regions [7, 8]. In 2012 PC was diagnosed in 1.1 million men, and 70% out of them (795,000 cases) were registered in developed countries [6, 8, 9].

In the Russian Federation incidence with PC grew from 30.74 per 100,000 men in 2007 to 59.91 per 100,000 in 2017; the growth amounted to 100.5%. Average age at which a male patient was diagnosed PC became younger in 2017 as it fell to 69.6 while in 2007 it was equal to 70.5 [15, 16, 17]. In the Russian Federation PC occupied the second rank place among malignant neoplasm in men in 2017 after malignant neoplasms in the trachea, bronchial tubes, and lungs (17.4%) and accounted for 14.5% [15].

Mortality caused by PC grew from 13.54 pr 100,000 men in 2007 to 18.46 per 100,000 men in 2017 in the Russian Federation; the growth amounted to 34.27%. PC occupied the second rank place in mortality caused by malignant neoplasms and accounted for 8.1%; the first rank place belonged to malignant neoplasms in the trachea, bronchial tubes, lungs, and stomach (10.7%) [15].

Mortality caused by malignant neoplasms is a basic indicator of public healthcare efficiency. But still, statistical data are often distorted due to necessity to encode death causes of oncologic patients. It happens due to migration, errors related to filling in death certificates, incorrect determination of the primary death cause, incompetent personnel filling in medical death certificates etc. [18, 19].

Our research goal was to analyze PC epidemiology in a specific Russian region (by the example of Kursk region).

Data and methods. To accomplish the research, we took data from the cancer register collected over 2007-2016by the Kursk regional cancer clinic on 2,238 patients who had PC diagnosed for the first time and on 999 patients who died from PC. According to official statistic data, male population in Kursk region decreased from 531,102 in 2007 to 509,615 in 2016. Data were statistically processed with «Statistica for Windows» software (ver. 12.5). We applied "crude" (total and age-sex) intensive (per 100,000 men) incidence and mortality values according to the Methodical guidelines developed by P.A. Herzen's Moscow Scientific Research Oncologic Institute [20]. Total "crude" incidence and mortality values were compared with data collected in the Central Federal District (CFD) over 2007–2016. «Crude» age-sex incidence and mortality values were compared with the same official statistic data in the overall country over the same period [15]. To test validity of discrepancy between data arrays, we applied parametric Student's t-test with significance level being equal to 95%. Incidence and mortality caused by PC were predicted with autoregressive integrated moving average (ARIMA) [21] for a period up to 2021.

Results and discussion. PC accounted for 5% in the structure of incidence with malignant neoplasms in Kursk region and occupied the 5th rank place after skin neoplasms (13.1%), lung and breast cancer (10.9% each), stomach cancer (7.1%), and colon cancer (5.1%).

Average incidence with PC amounted to 41.03 per 100,000 in Kursk region over 2007–2016 and was authentically lower than in the CFD (51.63 per 100,000 men) over the same period of time, $t=2.12$, $p=0.05$.

But still, a growth in incidence with PC in 2016 against 2007 was more apparent in Kursk region (100.3%) than in the CFD (84.5%). Incidence with PC in Kursk region grew from 28.55 per 100.000 men in 2007 to

57.19 per 100,000 men in 2016; in the CFD it grew from 31.99 per 100,000 men in 2007 to 59.03 per 100,000 men in 2016. As predicted, by 2021 incidence with PC will have grown up to 71.24 per 100,000 men in Kursk region (Figure 1).

Kursk region occupied the 13th place among all the region in the CFD as per primary incidence with PC over 2007–2016 (Table 1). Therefore, in the nearest future Kursk region will take a leading place as per incidence with PC among CFD regions.

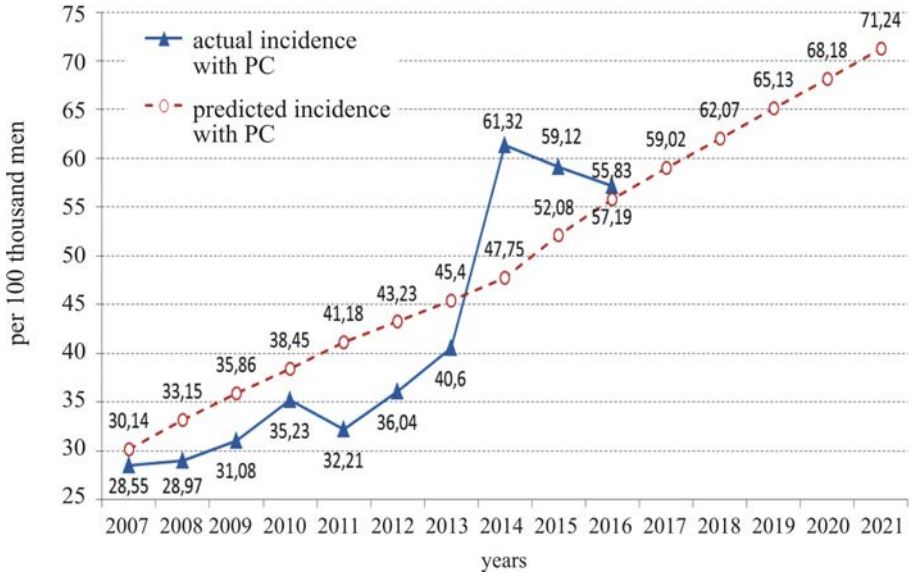

Figure 1. A prediction for incidence with PC in Kursk region

Table 1

Dynamics of incidence with prostate cancer in the Central Federal District regions

Regions	Incidence (per 100 thousand men)			
	2007	2016	average (2007–2016)	A growth in 2016 against 2007 (%)
CFD	31.99	59.03	51.63	84.5
Belgorod region	28.94	45.73	40.52	58.0
Bryansk region	31.94	40.44	43.96	26.6
Vladimir region	25.6	50.75	40.32	98.2
Voronezh region	36.55	59.56	51.38	63.0
Ivanovo region	28.02	77.49	46.57	176.6
Tver region	31.82	58.82	47.73	84.9
Kaluga region	44.19	83.28	62.99	88.5
Kostroma region	26.76	39.95	37.31	49.3
Kursk region	28.55	57.19	41.03	100.3
Lipetsk region	35.85	64.04	44.92	78.6
Moscow city	66.12	68.39	70.78	3.4
Moscow region	33.44	51.82	45.04	55.0
Orel region	29.21	61.43	46.86	110.3
Ryazan region	28.74	57.36	47.12	99.6
Smolensk region	18.68	35.63	28.17	90.7
Tambov region	19.22	41.73	35.34	117.1
Tula region	33.76	53.95	44.31	59.8
Yaroslavl region	29.67	62.62	47.83	111.1

In Kursk region men who had PC diagnosed in them for the first time were 35–39 years old (0.54 per 100 thousand men), a peak in incidence (367.49 per 100 thousand men) was detected among those aged 75–79 with subsequent fall among those aged 80 and older (316.42 per 100 thousand men). It was quite consistent with data collected in the country in general (Table 2). Authentically ($p<0.05$) lower sex-age incidence with PC in Kursk region than in the RF on average was detected among men aged 55-59 (46.02 per 100 thousand men against 60.40 per 100 thousand men in the RF on average, $t=3.23$, $p=0.05$); men aged 60–64 (112.50 per 100 thousand men against 154.95 per 100 thousand men in the country on average, $t=3.7$, $p=0.002$); and among men aged 70–74 (303.39 per 100 thousand men against 383.50 per 100 thousand men in the RF on average, $t=2.24$, $p=0.04$).

In Kursk region dynamics of sex-age incidence with PC was multidirectional among men aged 35–44 and there was no growth detected in it. Sex-age incidence decreased among men aged 45–59, with the most significant drop (–70,0%) being detected among men aged 50–54. Sex-age incidence with PC grew among men aged 60-80 and older, its growth being higher among those aged 80 and older (123.6%).

As for the RF in general, sex-age incidence with PC grew practically in all age groups, excluding 35–39 and 55–59 years; the most significant growth was detected among men aged 65–69 (70.3%); the least significant (8.3%), among those aged 80 and older (Table 2).

In Kursk region prostate cancer took the 6th place (4.5%) in the structure of mortality caused by malignant neoplasms after lung cancer (17.9%), stomach cancer (11.5%), breast cancer (7.2%) colon and rectal cancer (5.8% each), and pancreas cancer (4.6%).

We didn't detect any authentic discrepancies in mortality caused by PC in Kursk region (its average value over 10 years being equal 18.89 per 100 thousand men) and in the CFD (18.51 per 100 thousand men), $p>0.05$. However, mortality growth in 2016 against 2007 was equal to 85.8% in Kursk region and it was higher than in the CFD (35.24%). In Kursk region mortality caused by PC grew from 13.43 per 100 thousand men in 2007 to 24.96 per 100 thousand men in 2016; in the CFD it grew from 15.38 per 100 thousand men in 2007 to 20.80 per 100 thousand men in 2016.

Unless the situation changes, mortality caused by PC can reach 30.79 per 100 thousand men in Kursk region (Figure 2).

Kursk region occupied the 18th place as per PC-caused mortality over 2007–2016 among all the CFD regions (Table 3).

Table 2

Comparison of sex-age incidence with prostate cancer (per 100 thousand men of a relevant age) (average values for 2007–2016)

Age	Kursk region	Growth/decrease in 2016 against 2007 (%)	The RF	Growth/decrease in 2016 against 2007 (%)	t	p
	$\bar{x} \pm б$		$\bar{x} \pm б$			
35–39	0.54 ± 1.13	0	0.25 ± 0.1	0	0.76	0.46
40–44	0.28 ± 0.90	0	0.91 ± 0.13	26.9	2.06	0.06
45–49	2.54 ± 1.58	-30.0	4.16 ± 0.46	38.8	2.95	0.01*
50–54	14.79 ± 9.72	-70.0	19.21± 2.86	47.9	1.31	0.21
55–59	46.02 ± 10.83	-22.5	60.40 ± 8.16	-6.0	3.23	0.01*
60–64	112.50 ± 29.33	42.8	154.95 ± 18.97	39.7	3.7	0.002*
65–69	240.05 ± 65.68	61.5	279.07 ± 59.22	73.1	1.35	0.19
70–74	303.39 ± 91.39	50.5	383.50 ± 58.48	53.6	2.24	0.04*
75–79	367.49 ± 140.97	54.8	434.49 ± 70.29	55.0	1.29	0.22
80 and older	316.42 ± 108.90	123.6	346.89 ± 23.02	8.3	0.83	0.42

**note*: $\bar{x}$ is average value, б is standard deviation; t is Student's test, p is significance level.

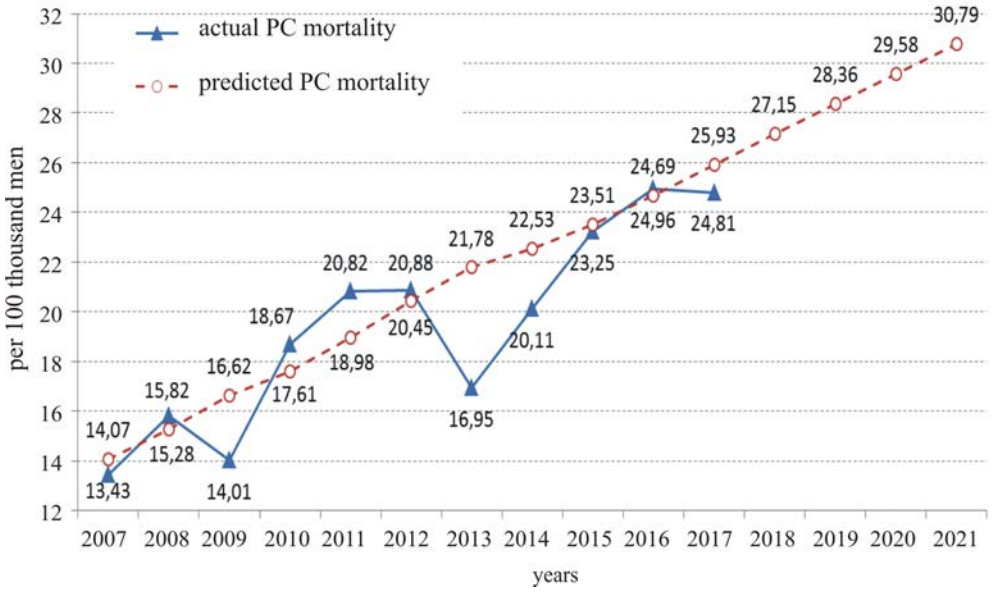

Figure 2. Predicted mortality caused by prostate cancer in Kursk region

Table 3

Dynamics of mortality caused by prostate cancer in the Central Federal District regions

Regions	Mortality (per 100 thousand men)			
	2007	2016	average (2007–2016)	growth/decrease in 2016 against 2007 (%)
CFD	15.38	20.80	18.51	35.2
Belgorod region	11.23	18.60	16.14	65.6
Bryansk region	17.48	20.94	18.08	19.8
Vladimir region	16.55	20.30	19.20	22.7
Voronezh region	14.81	19.04	18.13	28.6
Ivanovo region	12.24	20.56	16.30	68.0
Tver region	15.02	24.85	18.18	65.5
Kaluga region	19.91	24.83	20.14	24.7
Kostroma region	16.37	21.15	17.51	29.2
Kursk region	13.43	24.96	18.89	85.9
Lipetsk region	12.95	17.24	15.29	33.1
Moscow city	17.79	23.31	19.76	31.0
Moscow region	17.62	16.59	18.57	-5.9
Orel region	16.35	20.18	19.19	23.4
Ryazan region	15.13	23.33	19.68	54.2
Smolensk region	11.03	18.38	14.45	66.6
Tambov region	14.86	13.50	14.25	-9.2
Tula region	17.38	23.59	21.49	35.7
Yaroslavl region	16.62	22.10	18.87	33.0

Table 4

Comparison of sex-age mortality caused by prostate cancer
(per 100 thousand men of a relevant age) (average values for 2007–2016)

	Kursk region	Growth/ decrease in 2016 against 2007 (%)	The RF	Growth/ decrease in 2016 against 2007 (%)	t	p
	$\bar{x} \pm б$		$\bar{x} \pm б$			
40–44	0.55 ± 1.17	-	0.3 ± 0.06	-21.4	0.65	0.52
45–49	1.42 ± 2.04	-	1.18 ± 0.17	-11.5	0.35	0.73
50–54	8.9 ± 6.08	618.9	4.72 ± 0.29	13.4	2.06	0.06
55–59	18.24 ± 7.78	-8.1	14.99 ± 0.79	-6.0	1.24	0.23
60–64	51.69 ± 14.08	42.8	38.61 ± 1.28	2.1	2.77	0.01*
65–69	90.32 ± 27.8	96.4	75.81 ± 2.67	11.2	1.55	0.14
70–74	166.63 ± 85.69	209.3	130.19 ±7.22	14.7	1.27	0.22
75–79	197.48 ± 76.71	113.7	188.79 ± 11.15	20.6	0.34	0.74
80 and older	142.38 ± 51.98	26.5	243.05 ± 14.98	18.6	5.59	0.00003

**note*: $\bar{x}$ is average value, б is standard deviation; t is Student's test, p is significance level.

40–44 was the youngest age at which mortality caused by PC was detected in Kursk region (0.55 per 100 thousand men); it reached its peak among men aged 75-79 (197.48 per 100 thousand men) and then went down among those aged 80 and older. Authentically high discrepancies between mortality caused by PC in Kursk region against data collected in the RF were detected among men aged 60–64 (51.69 per 100 thousand men against 38.61 per 100 thousand men in the RF, t=2.77, p=0.01). Mortality caused by PC among men aged 80 and older was 2 times higher in the country on average (243.05 per 100 thousand men) then in Kursk region (142.38 per 100 thousand men), t=5.59, p=0.00003.

Mortality caused by PC grew in 2016 against 2007 practically in all age groups of men in Kursk region; the highest growth was among those aged 50–54, by 618.9%, 70–74, by 209.3%, and 75–79, by 113.7% (Table 4); growth rates were not so high in all the age groups as regards the country in general.

Conclusion. So, incidence with prostate cancer in Kursk region, just as in most regions in the CFD grows due to an increase among men aged 60–64 and 80 and older. In Kursk region, risk for men younger than 60 to die from prostate cancer amounts to $1.45*10^{-4}$, and it is almost 3 times higher than the same parameter in the RF in general ($5.3*10^{-5}$).

As per experts' forecasts, unless prevention activities are implemented, Kursk region can soon become one of those regions in the country where incidence with prostate cancer and mortality caused by it are the highest, an increase in mortality among men aged 50–65 being a reason for it as well.

All the data obtained in the research are being applied to plan and develop «Oncology» regional program in Kursk region.

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UGT1A1 GENE MUTATION AS A MARKER INDICATING THERE IS A HIGH RISK OF GILBERT'S SYNDROME: THEORETICAl AND APPLIED ASPECTS

A.N. Volkov[1,2], E.V. Tsurkan[2]

[1]Kemerovo State Medical University, 22A Voroshilova Str., Kemerovo, 650056, Russian Federation
[2]Kemerovo Regional Clinical Hospital, 22 Oktyabr'skii avenue, Kemerovo, 650000, Russian Federation

Gilbert's syndrome is a widely spread multi-factor pathology which is to a great extent genetically determined. Its basic etiological factor is lower activity of a liver enzyme, UDP-glucuronosyltransferase A1, caused by mutations in UGT1A1 gene. Functional disorders in the liver cause dyspepsia and concurrent acute and chronic diseases in the digestive system. The research goal was to substantiate the necessity and possibility to conduct mass examinations of population with molecular and genetic analysis of UGT1A1 gene in order to reveal Gilbert's syndrome. The authors performed molecular and genetic examination of UGT1A1 gene rs8175347 marker in 132 people living in Kemerovo region (population sampling) as well as in 71 patients who were supposed to have Gilbert's syndrome (clinical sampling).

*Frequency of *28/*28 mutant genotype of UGT1A1 gene associated with Gilbert's syndrome amounted to 13.6 % in the population sampling and it is quite consistent with previously published data. Therefore, a considerable rate of population includes people with potential or already revealed Gilbert's syndrome. Age structure of patients in the clinical group with *28/*28 genotype revealed there was a wide spread of an age at which the diseases was first detected due to its apparent manifestation; age varied from 4 to 71 years with its modal value being equal to 15 years. Basing on the obtained data, it is suggested to implement mass examinations aimed at revealing Gilbert's syndrome at its prenosological stage; such examinations can be based on molecular-genetic technologies. When children aged 7-10 are comprehensively examined, they can also undergo genetic diagnostics aimed at revealing any mutations in UGT1A1 gene. Obtained genetic data can be taken into account by medical personnel with relevant medical specializations when they determine strategies aimed at preventing and curing Gilbert's syndrome.*

Key words: *Gilbert's syndrome, UDP-glucuronosyltransferase A1, UGT1A1, rs8175347, mutations in a gene, genotype, molecular and genetic examination, genetic diagnostics.*

Gilbert's syndrome (GS) is the most frequent type of genetically determined pigmentary hepatosis. Icteritiousness of skin, sclera, and mucous tunics is its typical external evidence. The disease can also lead to various dyspeptic occurrences and asthenovegetative syndrome. Symptoms of the pathology are usually caused by physical overstrain or infectious diseases, and they can appear after starvation or low-calorie diet as well as after taking certain medications [1, 2]. Increased bilirubin concentration in blood is a basic laboratory parameter; it occurs primarily due to an indirect fraction. Both physical and biochemical indicators of the disease are variable, therefore they can't be considered reliable and sufficient to put a diagnosis, especially when it comes to children who haven't reached adolescence [3].

Basic GS signs are transitory and aren't considered to directly lead to grave damage to the liver. But still GS is often accompanied with other gastrointestinal tract diseases [4], and cholelithiasis can be a probable remote outcome of the syndrome in some patients [5, 6]. This trend becomes even more

Alexey N. Volkov – Candidate of Biological Sciences, Associate Professor (e-mail: volkov_alex@rambler.ru; tel.: +7(3842)734-856; ORCID: http://orcid.org/0000-0003-1169-715X).

Elena V. Tsurkan – Biologist (e-mail: volkov_alex@rambler.ru; tel.: +7(3842)396-023; ORCID: https://orcid.org/0000-0002-6268-6242).

apparent in case of any concomitant diseases. Thus, when GS is combined with sickle-cell anemia, it makes cholelithiasis much more probable [7, 8]. Gilbert's syndrome in infants with hemolytic states causes much greater risks of complications and death in the neonatal period [2].

Biochemical and genetic basics of GS are now well-established. Lower activity of UDP-glucuronosyltransferase A1 in case of the disease is determined either by changes in relevant *UGT1A1* gene expression or by structural modifications in the enzyme itself. In the first case there is usually a change in number of dinucleotide repeats *TA* in the gene promoter section (*rs8175347* polymorph marker). Thus, if a "wild type" allele **1* is characterized with six tandem repeats *TA*, then in case of GS-related mutations their number increases up to 7 (allele **28*) or 8 (allele **37*). Another variation in the promoter section is characterized with fewer repeats *TA*, usually 5 (allele **36*) and leads to increased *UGT1A1* activity without any pathological signs [9, 10]. It was detected that genotype **1/*28* carriers and especially **28/*28* carriers tended to have on average higher bilirubin concentrations in their blood serum than homozygotes **1/*1* [11–13].

There are very scarce data on *UGT1A1* genetic polymorphism as well as on its physiological and biochemical signs in population in Russia [14, 15]. Besides, Gilbert's syndrome frequency is unknown and there are no data on how many people or what specific population groups run risks of this pathology. It makes additional population-genetic research on *UGT1A1* truly vital.

This research can be considered relevant and practically significant as there is a necessity to work out efficient medical and diagnostic algorithms aimed at detecting multi-factor pathologies, such as Gilbert's syndrome, on the basis of the latest achievements in genetics and allied sciences.

Our research goal was to substantiate the necessity and possibility to perform mass health examinations among country population aimed at detecting Gilbert's syndrome via molecular and genetic analysis of *UGT1A1* gene.

Data and methods. We performed our examinations on two groups of people living in Kemerovo region; all the examined people were Caucasians (predominantly Russians). The population group was made up of personnel and patients who underwent a prophylactic medical examination at Kemerovo regional clinical hospital, both sexes (68 females and 64 males), the data are given in the Table. We didn't take the examined people's health into account. This sampling was applied to perform population-genetic analysis of *rs8175347* polymorphic marker in *UGT1A1* gene.

Table

Characteristics of the examined groups

Group	n	Age, years			
		M ± S.E.	Me	Mo	min-max
Population	132	36.4±0.61	36	38	23–51
Clinical	71	34.3±2.30	30	15	4–71

Note: n is a group volume; M is a simple mean; S.E. is a standard error of the mean; Me is median; Mo is mode; min-max are variation limits.

The clinical sampling included patients who were treated at Kemerovo regional clinical hospital (38 females and 33 males) and had been previously sent to a medical-genetic consultation to have a medical-genetic examination in relation to supposed Gilbert's syndrome. Polymorph section *rs8175347* in *UGT1A1* gene was analyzed in all patients and a conclusion on homozygosity as per a mutant allele **28* was made; it confirmed a preliminary diagnosis was correct. This group allowed to examine certain age aspects related to GS manifestations and detection.

Venous blood samples were taken from all the examined people in an in-patient department and EDTA was applied as an anticoagulant. DNA was extracted out of whole blood on "K-Sorb" columns produced by "Syntol" company according to the manufacturer's instructions. PCR-amplification of polymorph section *rs8175347* in *UGT1A1* gene was performed with a commercial reagent kit manufactured by "Litech" LLC. We detected allele**1* (a wild type, 6 *TA*-repeats in a promoter section of

UGT1A gene) and **28* (mutation, 7 *TA*-repeats). To detect PCR results, we applied horizontal electrophoresis of amplification products in 3% agarose gel with ethidium bromide used as a colorant.

We statistically analyzed initial data and calculated basic selective parameters for quantitative variables. Frequencies of marker rs8175347 alleles and genotypes in *UGT1A1* gene were calculated as fractions of their overall number in a sampling. Qualitative variables distributions were compared with χ^2 criterion in "STATISTICA 6.0". A discrepancy was considered to be authentic at $p<0.05$.

Results and discussion. When discussing genetic factors that cause GS risks, we should first of all assess prevalence of mutations in *UGT1A1* gene which are associated with the pathology. **1/*1* (47.0%) prevailed in the examined population group that included Caucasians living in Kemerovo region (Table 2). Frequency of minor allele **28* associated with a pathologic state amounted to 33.3%. A fraction that belonged to the rarest genotype **28/*28* associated with Gilbert's syndrome was equal to 13.6%. The established ratio of the genotypes didn't have any authentic discrepancies from the expected one in accordance with Hardy-Weinberg equilibrium ($\chi^2 = 0.674$; $p = 0.714$). It proves there is no apparent deadaptation in any genotype and a selection against this genotype in the examined population.

Table 2

Population and genetic peculiarities of marker *rs8175347* in *UGT1A1* gene in different Caucasian ethnic groups

Ethnic group	*UGT1A1* genotypes *(rs8175347)*, %			Allele frequency **28*, %	Source
	**1/*1*	**1/*28*	**28/*28*		
Russians	47.0	39.4	13.6	33.3	Own data
Russians	40.4	50.0	9.6	34.6	[14]
Russians	42.4	51.7	5.9	31.8	[15]
Croats	39.9	49.8	10.2	35.1	[16]
Italians	43.9	39.8	16.3	36.2	[17]
Dutch	44.2	43.7	11.9	33.7	[18]
Spaniards	40.0	51.0	9.0	34.5	[19]
Caucasians in the USA	46.6	43.1	10.0	31.6	[20]

Pathologic genotype frequency which we detected is rather high and it requires further confirmation based on other independent research. Data on population and genetic peculiarities of *UGT1A1* gene in population in Russia are scarce. Recently some research results have been published on *UGT1A1* polymorphism in a sampling which included people living in the south of Russia (Rostov-on-Don) [14]. According to the obtained results, **28/*28* genotype fraction amounted to 9.6%, and allele **28* frequency was even higher than we detected in our research. We should also note that this examined sampling, strictly speaking, can't be considered a population one as it was made up of oncologic patients who suffered from colorectal cancer. In other research, Shatalova E.G. et al. [15] detected frequency of *rs8175347* alleles and genotypes in healthy Russian females. Frequency of **28/*28* homozygotes amounted to 5.9% only, and a fraction of **28* allele was equal to 31.8%.

We analyzed some other works that focused on examining *UGT1A1* polymorphism; according to them, a fraction of a clinically significant allele **28* usually exceeds 30% but doesn't reach 37% among Caucasians [16–20]. It corresponds to a homozygote genotype **28/*28* frequency being, as a rule, about 10% or a bit higher. Such frequency of a minor allele is close to its critical value when fractions of homozygotes **1/*1* and heterozygotes are practically the same. Therefore there was a common regularity detected in different examined groups, that is, comparable frequencies of two genotypes, **1/*1* и **1/*28*, usually with insignificant prevalence of **1/*1* variant.

We can conclude that our data in general are consistent with results obtained in previous research. A fraction of potential or already detected patients with Gilbert's syndrome among Caucasians who are carriers of *UGT1A1 *28/*28* genotype usually exceeds 10%.

High frequency of homozygote *UGT1A1* mutation carriers detected in various research requires developing and implementing specific algorithms into medical practices that will allow to perform mass health examinations in order to detect population groups that run ele-

vated GS risks; such algorithms should be based on high-precision pathology markers. As it was stated previously, Gilbert's syndrome symptoms usually become apparent when adolescence begins and vary significantly in different patients depending on specific combinations of external influences. In such a situation conventional diagnostic techniques turn out to lack precision and make for a longer period during which the disease is diagnosed.

But at the same time, all the contemporary knowledge on Gilbert's syndrome etiology and a role played by mutations in *UGT1A1* gene allow to propose a reliable algorithm for GS diagnostics based on molecular and genetic approaches. Timely detection of mutant **28/*28* genotype carriers, ideally as early as at the prenosological stage already, enables adjusting a life style which a patient pursues in order to prevent the pathology from its manifestation or making possible harm to a patient's health as minimal as its only possible.

To determine an optimal age at which a diagnostic examination should be accomplished, we studied the sampling that comprised patients with GS with the diagnoses being given on the basis of clinical manifestations and detection of **28/*28 UGT1A1* genotype (Figure).

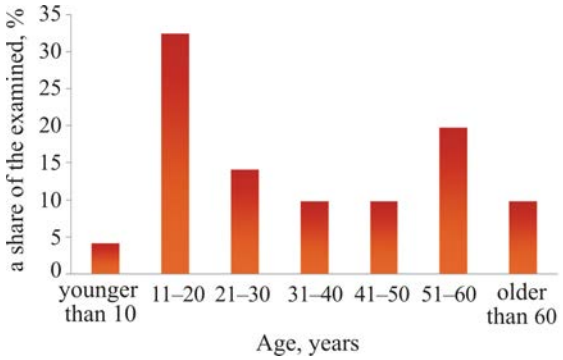

Figure. Age distribution of the examined patients suffering from GS

An age at which GS was diagnosed for the first time can vary significantly, starting from 4 and up to 71 (Table). But at the same time, more than 30% of the examined patients were people younger than 20, and a modal age as per the clinical sampling amounted to 15. It is consistent with previously published observations, according to which basic GS manifestations usually appear when pubescence starts [3, 21]. A fraction of patients in older age groups was slightly lower. The second peak related to GS diagnosis was detected among people older than 50 which can be caused by a decrease in body functional reserves at an older age and combined effects produced by pathogenic factors which appeared in previous years.

Obviously, there are people with GS being diagnosed for the first time in all age groups. The disease manifestations are known to be associated with an individual combination of pathogenic factors, peculiarities of a body, lifestyle, diet, etc., and they are un predictable in terms of the ontogenetic aspect [1–3]. Nevertheless, we can assume that an inherited decrease in *UGT1A1* function in most cases will sooner or later become apparent due to impacts exerted by either endogenous or exogenous factors. Besides, we can also assume there are individuals in a population with various manifestations of the pathology caused by **28/*28 UGT1A1* genotype carrying who haven't ever applied to a medical organization.

As it was mentioned above, when GS is diagnosed too late and there is no relevant therapy, it can cause elevated health risks for a patient [5–8, 21]. In our opinion, an age before pubescence starts is an optimal one at which GS should be detected as any symptoms of the pathology are usually absent thus early. Such check-ups can be performed during regular mass health examinations among school children.

According to the Order issued by the RF Public Healthcare Ministry "On a procedure for medical examinations of minors including those performed before entering an educational establishment and during studies"[1] minors are to be examined on a regular basis at a certain age. Schoolchildren usually undergo

[1] On a procedure for medical examinations of minors including those performed before entering an educational establishment and during studies: The Order by the RF Public Healthcare Ministry issued on December 21, 2012 No. 134bn [Web-source] // Garant: information and legal database. – URL: https://www.garant.ru/products/ipo/prime/doc/70255102/ (date of visit February 14, 2019).

a comprehensive medical examination at 7, 10, as well as 14, 15, 16, and 17 years. Notably, the existing system of medical examinations for minors doesn't include a consultation and examination by a gastroenterologist; given that, GS can be detected only when its first symptoms become apparent and a patient starts to complain about health.

A child usually starts school at 7 and additional risk factors that can cause GS appear at this moment. They are physical and psychoemotional strain and organized meals at a school. Before a child starts school, his or her parents usually can control the timetable, a child's diet and how regularly he or she eats, but when a child goes to school, the task becomes rather complicated. Children undergo preventive medical examinations at 7 and 10 and visit some medical specialists during them. They also have their blood analyzed; it could be an optimal moment to diagnose GS via examining *UGT1A1* gene mutations. Small aliquots of whole blood could be a DNA source.

A methodical part in a molecular-genetic examination can be implemented on the basis of the previously described approaches with a domestic instrumental base and test-systems so that requirements related to replacing imports of medical materials and equipment are met. A diagnostic procedure is going to be cheap and easy to perform thus being available for mass application [22].

Mass genetic examinations aimed at detecting Gilbert's syndrome will allow to divide all people into 2 groups. **1/*1* и **1/*28* genotypes carriers will be assigned into "conditional standard"; **28/*28* genotype carriers, into "a risk group as per Gilbert's syndrome". A "standard" can be only "conditional" as we can't completely exclude other, rarer mutations among people from this group; these mutations can appear in *UGT1A1* gene sections which haven't been examined so far and they can also lead to GS symptoms occurrence. But still, there are too few such people as it was detected in previous research on various ethnic groups [11, 20].

People from "risk group as pert Gilbert's syndrome" should be observed thoroughly by a gastroenterologist and their genetic data should be included into their overall case history. As genetic diagnostics is supposed to be performed among children, most of them can have no apparent GS signs. And public healthcare specialists should first of all communicate health risks to potential patients and perform efficient activities aimed at preventing any GS manifestations. It primarily concerns making children have responsible attitudes towards their lifestyle and diet. It is necessary to exclude factors that stimulate the pathology development; such factors include nervous and physical strain, acute chills, abundant low-calorie, spicy, or fried food in a diet as well as starvation or irregular meals etc.

Specific medical activities chosen for each individual patient will depend on peculiarities related to the pathology manifestations. Apart from relevant therapeutic measures, a physician should thoroughly analyze reasons that caused the disease manifestations in order to eliminate them completely. Obviously, knowledge on genetic predisposition to Gilbert's syndrome, preventive activities and timely relevant therapy can lead to a substantial improvement in life quality for most people from the risk group.

Conclusion. We conducted population-genetic research on *rs8175347* marker in *UGT1A1* gene among people living in Kemerovo region; the research revealed high frequency of **28/*28* mutant genotype which was associated with Gilbert's syndrome (13.6%). Obviously, a significant share of the population are potential patients or people with already diagnosed GS. It requires immediate measures to be taken to provide early detection of people with GS among those who run genetic risks and to implement prophylaxis and therapeutic activities in order to prevent the disease manifestations in them.

We proposed an algorithm for mass health examinations aimed at detecting GS at its prenosological stage; such examinations can be based on molecular-genetic technologies. When children aged 7 or 10 undergo their obligatory comprehensive medical examination as it is fixed in the existing regulatory documents, they can additionally have *UGT1A1* mutations diagnosed in them. To do

that, medical experts can use laboratory equipment and test-systems produced in Russia, and it will allow to accomplish mass health examinations among children in a shortest period of time.

All obtained results can be taken into account by relevant medical specialists so that they can determine further necessary actions aimed at preventing and curing Gilbert's syndrome. Correct determination or necessary adjustment of a lifestyle, behavior, and diet will allow to significantly improve life quality for those people who have genetic predisposition to GS.

The suggested algorithm is a feasible translation medicine model. The same procedure can be applied in relation to other multifactor diseases with well-known genetic component and significant genetic predisposition. Ideally, such activities should underlie a future system of personified medicine which focuses on individual genetic and physiological peculiarities of a particular patient.

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PECULIARITIES RELATED TO COMBINED EFFECTS PRODUCED BY CHEMICAL ALLERGENS MIXTURE

V.V. Shevlaykov, S.I. Sychyk

Scientific-Practical Hygiene Center, 8 Akademicheskaya Str., Minsk, 220012, Belarus

Workers employed at chemical productions run risks of occupational allergic diseases with chemical etiology. These risks should be assessed and relevant prevention activities are to be substantiated; in this respect it is vital to reveal essence, peculiarities, and mechanisms of hyperimmune response when a body is exposed to a mixture of chemicals with various allergenic and toxic properties. We performed a series of experiments to examine allergenic properties of 57 various chemical mixtures and their separate components; the experiments involved modeling of intradermal introduction in standard doses into albino guinea pigs (ears) or into white mice (bases of tails), the substances were mixed with complete Freund's adjuvant. The obtained results allowed to reveal that intensity of allergenic activity shown by chemical mixtures was determined both by intensity of allergenic properties possessed by specific components in a mixture and by additivity of their immunomodulating effects under combined exposure. These effects naturally depend on a combination of various allergens in a mixture, their contents and quantitative ratios, as well as occurrence of substances with non-specific adjuvant and toxic properties in a mixture. And combined allergenic effects produced by a mixture of chemicals that contains strong chemical allergens in high quantities tend to enhance hyperergic immune response (potentiating) to weaker allergenic components. Predominantly potentiating combined allergenic effects produced by a mixture of chemicals on a body occur due to interrelated mechanisms of specific (elimination of tolerance to conjugate epitopes and modified carrier protein, occurrence of cross-reactive antigen determinants etc.) and non-specific immune modulation (adjuvant, irritating, and immune-toxic effects produced by chemical components in a mixture).

Key words: *a mixture of chemicals, allergens, allergic disease, hyperimmune response mechanisms, essence of allergic processes occurring in a body as a response to exposure to a mixture of chemicals, specific and non-specific immune mechanisms of combined effects produced by chemical allergens.*

Assessment of risks related to occupational allergic diseases (OAD) of chemical etiology, their prevention and hygienic standardization involve a necessity to solve a lot of significant theoretical and practical tasks. One of them is determining essence, peculiarities and mechanisms of hyperimmune response under exposure to a mixture of chemicals (MC) with their allergic and toxic properties having various intensity. Despite its relevance, the issue has been given too little attention over recent years, probably, due to high labor and material costs necessary to perform experimental research.

It is known that when two or more complete or artificial antigens (Ag) are simultaneously introduced into a body, immune modulation between them has intramolecular or intermolecular competitive character; it frequently leads to an immune response to one or several Ag being inhibited and a response to a dominant Ag being restored or inhibited [1]. Chemicals as haptens become complete Ag only after their conjugation with auto-protein carriers that are modified with hapten but still preserve their natural tolerance to a greater or lesser extent [2]. Therefore, immune mechanisms that realize a combined allergenic ef-

Vitalii V. Shevlyakov – Doctor of Medical Sciences, Professor, Chief Researcher at the Industrial Toxicology Laboratory (e-mail: shev-vitaliy@mail.ru; tel.: +375 17 284 13 96, +7 (029) 180-50-26; ORCID: https: //orcid.org/0000-0001-8226-9350).

Sergei I. Sychik – Candidate of Medical Sciences, Associate Professor, Director (e-mail: svkasul@mail.ru; tel.: +375 17 284 03 87, +7 (029) 304-32-11; ORCID: http: //orcid.org/0000-0002-5493-9799).

fect can differ from Ag significantly under simultaneous exposure to several chemical allergens (CA) in MC that happens in real-life environment. It has been confirmed by multiple experimental and clinical data, mostly on enhanced body allergization under simultaneous exposure to several CA. But it still remains unclear what hyperergic immune response develops in a body under exposure to MC depending on a combination of chemicals with different sensitizing activity and toxicity as well as on their fractions in a mixture; possible mechanisms that realize a combined CA effect in a mixture also need to be clarified.

Our research goal was to perform experimental research and detect peculiarities of combined effects produced on a body by CA in a multi-component mixture and to theoretically substantiate possible immune mechanisms that realize it.

Data and methods. We performed experimental research with standardized techniques in order to examine irritating and allergic properties of 57 MC with various composure and their separate components[1]. Sensitization was modeled via intradermal introduction into a ear of an albino Guinea pig in a standard dose equal to 200 μg of MC and their separate components; another way was intradermal introduction into a tail base of a white mouse in a standard dose equal to 100 μg mixed with a surface-active substance (SAS). Essence and intensity of hyperergic immune response was determined via a set of allergy-diagnostic techniques *in vivo* (challenge direct and cross epicutaneous tests, intradermal tests of a swelling in a paw or ear) and *in vitro* (reaction of specific mast cells degranulation, micro-precipitations, blast-cell lymphocytes transformation etc.) that reflected induction of delayed and instant hypersensitivity (DHS or IHS) in a body.

Examined MC and their separate components were assessed as per their allergenic activity and hazard (4 categories overall); to do that, we applied criteria related to DHS intensity and detection, namely frequency of positive integral parameter of challenge intradermal tests in animals from the test group given in scores (in %), validity of discrepancies in average group values in integral test parameters in the test and reference groups as per Student's or Fischer's test, significance levels fixed at $p<0.05$ or $p<0.01$ as per "X" criterion (Van der Waerden criterion).

Results and discussion. Allergic activity of a MC is predominantly determined by strong or intensive allergenic components but sensitizing effects produced by MC are much more intense than detected allergic reactions to cross tests on animals performed with their separate allergic components. Allergy-diagnostic reactions to those components were detected mostly at the same level or a bit lower when they were introduced into animals separately though standard sensitizing doses were 10-100 times higher than their quantitative contents in sensitizing MC doses. Therefore, combined effects produced by MC are determined less by additivity of sensitizing effects produced by separate components than by their mutually modulating interrelations in a body that could have various character depending on sensitizing capacities and quantitative contents in a MC. To prove it, below we describe specific results of examining glass fiver greases (GFG) that contained components with their allergic activity differing in its intensity.

GFG-78 contained strong allergenic components (belonging to the 1st allergic activity category) in equal and quite high quantitative contents, namely TEG-1 epoxy resin and Di-cyan-diamide formaldehyde vinegar (DCV) resin; we detected an inhib-

[1] Requirements to experimental research on examining allergenic properties and substantiating maximum permissible concentrations of chemical allergens in working area air and the atmosphere: methodical guidelines No. 1.1.11-12-5-2003 / V.V. Shevlyakov [et al] / Belarus Public Healthcare Ministry // Collection of official documents on occupational medicine and industrial sanitary / Republican Center for Hygiene, Epidemiology, and Public Health, Republican scientific and Practical Center for Hygiene; edited by V.P. Filonov, S.M. Sokolov. – Minsk: PCHUP "Biznesofset", 2004. – Part 14: Industrial toxicology. – P. 133–156.

ited immune response to the latter component, especially as per DHS, while an intensity of a challenge dermal test showing response to TEG-1 was even a bit higher (average score was equal to 1.7±0.36) than when the component was introduced into animals separately (average score was equal to 1.5±0.4 while its dose was 12.5 times higher) with a simultaneous decrease in a humoral hyperimmune response. Consequently, TEG-1 is a dominant inhibiting immune modulator as regards DCV that is as strong an allergen. However, a bit higher DHS to GFG-78 as a complete mixture (average score is equal to 2.0±0.36) in comparison with its primary allergenic components proves that epoxy resin has a potentiating effect on other, weaker components in the mixture (Massil Ameo, the 3rd allergic activity category). When TEG-1 was replaced in GFG-78 with a weaker allergen TEG-10 (its fraction being the same), it resulted in a decrease in overall allergenic activity of the mixture (average score was equal to 1.7±0.36) against GFG-78, but components had the same immune modulation as the leading allergenic component TEG-10 dominantly inhibited predominantly cell hyperimmune response to another strong allergen DCV simultaneously potentiating sensitizing effects produced by other components.

L.A. Dueva, V.G. Chmut [3] introduced a simple combination of strong chemical allergens, chromium chloride and urea-formaldehyde resin or epoxy (ED-20 + DEG-1 plasticizer), their contents being proportionate, in substantial doses into animals; they also detected mutual reinforcement of a hyperimmune response that was more significant to chromium, that is, they observed a potentiating effect.

If contents of a strong allergenic component are reduced in a mixture, than a component that prevails in its quantitative contents becomes a dominating one as regards inhibiting allergenic effects produced by other components. Thus, epoxy resin ED-5 in GFG-483 inhibited a hyperimmune response to DEG-1 (its contents being 2.5 times lower) mostly as per DHS, but not IHS, with simultaneous reinforcement of effects produced by weaker allergens (KZOT, ES-1) that didn't lead to a substantial decrease in allergenic effects produced by the mixture.

Nevertheless, when a strong or intense chemical allergen is contained in a mixture in low quantity, for example, PR-4 in GFG TZ (0.3%) or PR-3 in GFG-76 (0.15%), and a mixture contains other weaker allergenic components (the 3rd category) in prevailing quantities (PN-M resin in GFG TZ or politerpens in GFG-76), we observed inhibition of a hyperimmune response predominantly as per IHS to a DCV resin, a leading allergenic component, with simultaneous highly active allergic effects produced by PN-M resins and politerpens. Potentiating immune modulations led to high allergization of animals to the whole mixtures GFG-TZ and GFG-76. Similarly, lower contents of DCV leading allergenic component (0.5%) and higher contents of PN-M resin (moderate allergen, 4.0%) in a mixture resulted in mutually enhancing immune modulation. We detected a similar effect when we examined some complex pesticides. Thus, when a strong allergen thiuram D (the 2nd category) was introduced into 53-90 pesticide (up to 14%), it led to an increase in sensitizing activity of another strong allergen, namely Tiabendazole (average score was equal to 0.8±0.27, $p<0.05$ against the control) and the overall mixture (average score was equal to 1.4±0.28, $p<0.01$) against 48-90 pesticide (average score was equal to 0.9±0.24, $p<0.05$). Although Tiabendazole contents were 3 times higher in the latter, average dermal test score in a challenge test performed on animals was lower (0.6±0.13, $p< 0.05$).

A bit different immune modulation in MC was detected when only one strong or apparent chemical allergen was combined with one or several chemicals with weak or moderate allergenic activity. Thus, in spite of DCV component concentration being rather high (2.5%) in GFG TZ-1 and TZ-2,

cellular and humoral hyperimmune response to it was a bit lower than in case of GFG-TZ and GFG-mTZ, but we detected a significant increase in allergenic effects produced by weak allergenic components. such as OkADA, stearox, and oxalen. And when DCV concentrations fell by 2-4 times in GFG-14 and GFG-1k, an immune response to a leading allergenic component became even less intense, especially as regards IHS, and allergy-diagnostic reactions to other components didn't enhance. As a result, detected DHS to the whole mixtures of the above mentioned GFG were significantly lower than to other DCV-containing mixtures.

This mutual weakening of a hyperimmune response was detected under exposure to a mixture of chromium chloride with intense and moderate allergens, for example, naphthalene-formaldehyde resin or divinyl styrene latex SKS-65, while a mixture of the former with a weak allergen (melanin-formaldehyde resin) drastically increased an immune response to the polymer [3]. E.S. Smirnova, G.V. Lomonova [4], S.A. Ashirova et al. [5] obtained similar results reflecting potentiating immune modulation of a strong allergen (toluene diisocyanate) as regards one or two weak sensitizers (dimethyl cyclohexalamine and dimethyl ethanolamine) under combined inhalation exposure to the mixture even at their Lim_{ch} levels. Researchers also detected an enhanced allergenic effect produced by styrene due to a combination with greatly allergenic formaldehyde or similar potentiating of allergenic effects produced by weakly allergenic methyl methacrylate due to strongly allergenic acrylonitrile under inhalation exposure to binary mixtures in comparison with their separate effects at Lim_{ch} levels [6, 7].

At the same time, we didn't detect immune-modulating competitive relations (additive effects) between moderate-weak chemical allergens in MC in intradermal sensitization models regardless of their quantitative contents and ratios in a mixture with relevant relatively weak allergenic activity of the latter (for example, such mixtures as GFG-1, 4-88, 6, 25, Shl-1, 2).

On the other hand, we should take into account influences exerted on immune-modulating relations by allergens and MC components with adjuvant effects and irritating properties. Indeed, when GFG mPE and TZ-1 that didn't contain mineral oils were introduced into animals, we detected not only a decrease in hypersensitivity induction to DCV leading allergen but also less apparent potentiating of effects produced by weaker allergenic components accompanied with a substantial decrease in allergenic activity of these mixtures.

We detected greater allergenic activity of those MC that had more apparent irritating properties. For example, GFG-2 (average score as per local dermal effects is 3.3±0.27) contained DCV in the same quantity as GFG-1k (average score as per local dermal effects is 1.3±0.27) but detected DHS and especially IHS to DCV were 2.5 times higher for the former mixture with simultaneous distinct increase in allergic dermal test-reactions to other components (DBS, ADE-3). Accordingly, GFG-2 had higher allergenic activity (average test-reaction score was 1.9±0.27, $p<0.01$ against the control and the 2nd test) than GFG-1k (average score was 0.75±0.27, $p<0.05$ against the control).

Therefore, allergenic activity of a MC is determined by immune-modulating relations between its components that can induce competitive (inhibiting), independent, or, much more frequently, potentiating hyperimmune response to chemical allergens. Intensity and predominant type of chemical immune modulation mostly depend on a combination of allergens with various intensity, their quantitative contents and ratios, occurrence of substances with non-specific properties in a mixture that influence immune processes. Accordingly, essence and mechanisms of immune modulation that induce allergenic reactions in a body under exposure to a mixture of chemicals with different toxic, irritating, and allergenic effects, will differ from

competition between complete or artificial Ag. And if competitive inhibition of the latter is mostly specific and is similar to immunologic tolerance induction [1, 8], then immune modulation to MC components is implemented via several interrelated and mutually correlated specific and non-specific mechanisms.

In this case specific mechanisms of immune response potentiating are quite similar to immunologic tolerance failure [1, 2, 9, 10]. Let us explain why. Firstly, simultaneous introduction of haptens with different functionally active groups determined their conjugation with different auto-protein carriers, and as T-helpers have non-specific receptors to a carrying section in an Ag, that is, belong to different clones, there is no competition between complex Ag [2, 10].

Secondly, tolerance and, consequently, competitive inhibition of hyperimmune effects is easily eliminated via immunization with cross-reacting Ag [8]. Thus, it was detected in experiments, that immunization of a body with bovine serum albumin (BSA) with two different adjugate groups (acetyl and picrite or arsenilate and sulfanilate) eliminated tolerance to BSA conjugate with one of these haptens. And the higher was an extent of replacement with hapten, the more efficient this conjugate was an agent that eliminated areactivity to a carrier protein [12]. Elimination of tolerance results in occurring anti-bodies and sensitized lymphocytes to new conjugate epitopes and to epitopes of a modified carrier protein and initial auto-protein which tolerance had been to [2, 11].

We should also take into account that haptens immune dominants are specific but a considerable part of ligand dominant created by a modified auto-carrier, especially in case of identical or similar active chemical groups of haptens, has relative specificity [2]. Cross-reacting antigen determinants stimulate activated T-helpers ($CD4^+CD28^+$), which, due to specific and non-specific helper factors, activate b-lymphocytes, macrophages, and effector T-lymphocytes, and it is accompanied with an enhanced antibody and/or cellular hyperimmune response to a combination of haptens [2, 13–15]. Therefore, the stronger haptens modify a protein matrix (that is, have high sensitizing capacities) and the higher their dose is, the more intensely a balance immune regulation mechanism is violated with prevailing IHS reinforcement; on the contrary, weak chemical allergens with preserved tolerance to their carrier auto-proteins will not induce an enhanced hyperimmune response under joint effects.

Indeed, experiment with MC revealed that the stornger allergens a mixture contained, the more intense a potentiating effect was, especially as regards enhanced IHS. With relatively low contents of strong allergens in a mixture, one of them dominantly inhibits predominantly DHS, but not IHS, to a weaker one or that contained in smaller quantity. Simultaneously, other, less active allergens are also activated, mostly as per IHS. If a mixture contains one or even several strong chemical allergens but in low concentrations, it doesn't induce potentiating immune modulation as it is confirmed by data collected by L.A. Dueva et al [16] in their research on combined veterinary medications.

At the same time, non-specific factors make a significant contribution into potentiating of allergenic effects produced by MC. Some research revealed that substances with irritating or adjuvant effects stimulated the macrophage system with adequate activation of cooperation between immune-competent cells and non-specific cytokine regulating network; it led to a greater humoral and cellular immune response to chemical allergens. We should also take into account that multiple industrial chemical mixtures (greasers, cutting lubricants, synthetic detergents, etc.) contain emulsifying and antistatic substances that are mostly synthetic SAS. Many SAS are known to not only have irritating, allergenic, and auto-allergenic properties, but also promote penetration of other chemicals into a body through natural barriers (skin and mucous tunic) [17, 18]. Besides, it has been proven recently, that cation, non-ionic, and especially

poly-cation SAS have apparent dose-dependent activating immune-modulating effects due to activation of macrophages, enhanced migration and cooperation between T- and B-cells with significant stimulation of antibodies formation [1, 19].

It is also quite appropriate to mention violation of cognate identification and idiopathic regulation of an immune response due to immunotoxic effects produced by chemicals on immune-competent cells which are especially sensitive to any metabolic failures, predominantly, on proliferating cytotoxic suppressor T-cells ($CD3^+CD8^+$). This process is significantly involved into potentiating immune modulation of haptens. A deficiency in activated $CD8^+CD28^+$ lymphocytes leads to tolerance failure and is accompanied with polyclonal activation of B-lymphocytes and selection of activated effector T-lymphocytes clones [1, 20, 21].

Therefore, realization of allergic effects by a mixture of chemicals that contains components with different toxic, irritating, and allergenic properties, simultaneously involves multiple interrelated mechanisms of specific immune modulation (elimination of tolerance to epitopes of conjugate and a modified carrier protein, occurrence of cross-reacting antigen determinants, etc.) and non-specific one (adjuvant, irritating, and immunotoxic effects on immune-competent cells) that determine intensity and a predominant type of a hyperergic immune response to a MC and, consequently, a probable clinical course of an OAD.

Conclusion. We analyzed the results obtained in experimental research and it allowed us to make the following conclusions.

1. Intensity of allergenic activity a MC has is determined not only by how intense allergenic properties of its separate components are but also by immune modulating relations between them that can have additive or, more frequently, potentiating effects on a hyperergic immune response.

2. Essence and predominant type of immune modulation effects produced by MC components naturally depend on a combination of allergens with different intensity, their quantitative contents and ratio, occurrence of substances with non-specific adjuvant, irritating, and immunotoxic properties in a mixture:

– if a mixture contains several strong chemical allergens in high concentrations, modulation induces an enhanced immune response between them and to weaker allergenic components;

– if a mixture contains strong and intense chemical allergens, a stronger one or a component that is contained in prevailing quantity dominantly inhibit predominantly DHS of another simultaneously potentiating an immune response mostly as per IHS to other, weaker allergenic components, especially if they are contained in a mixture in high quantities;

– If a strong chemical allergen is contained in a mixture in low quantities, or a mixture contains only moderate and weak allergenic components, a hyperimmune response is additive;

– If a mixture contains chemical allergens and components with adjuvant and/or irritating properties (greasers, SAS, polyelectrolytes), it leads to a significantly enhanced immune response predominantly as per humoral hypersensitivity.

3. Predominantly potentiating effects produced by combined exposure to a mixture of chemical allergens are determined by several interrelated mechanisms of specific and non-specific immune modulation; it should be taken into account when developing and implementing activities aimed at correcting and reducing allergenic activity of MC, their hygienic regulation, and assessing risks related to OAD development in workers and their prevention.

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UNFAVORABLE CLINIC COURSE OF ULCERATIVE COLITIS: RISK ASSESSMENT WITH DETERMINING GENE POLYMORPHISM OF TUMOR NECROSIS FACTOR ALPHA

Y.I. Tretyakova[1], A.P. Shchekotova[1], I.A. Bulatova[1], A.V. Krivtsov[2]

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

The research goal was to assess functional significance that belonged to TNF-α gene polymorphism in the region -308G/A (rs1800629) in ulcerative colitis development.

The authors examined 70 patients with ulcerative colitis when the disease was in its active phase and 50 healthy donors. They focused on TNF-α concentration in blood serum and TNF-α gene polymorphism in the region -308G/A.

It was detected that TNF-α level was authentically higher in patients who suffered from grave ulcerative colitis and had high endoscopic activity than in people with low endoscopic activity and milder forms of the disease. It allows to apply these tests as additional non-invasive markers to assess apparent damages done to the mucous tunic in the large intestine in a patient suffering from ulcerative colitis. Frequency of allele variations in TNF-α gene -308G/A (rs1800629) was assessed; the assessment revealed that unfavorable homozygote AA significantly more frequently occurred in patients with ulcerative colitis than in donors. Besides, ulcerative colitis might be accompanied with different endoscopic activity; the major allele G and the allele pair GG significantly more frequently occurred among patients with mild and average disease attack and 1–2 degree of endoscopic activity than among patients with 3-4 degree of endoscopic activity and grave ulcerative colitis (χ^2=14.19; p=0.000). The authors also detected a mutant allele A and the unfavorable homozygote AA associated with grave progressive ulcerative colitis. Mutant allele occurrence causes 5 times higher risk that a patient will have to face the disease in its grave form (OR 5.03; CI 12.07-12.21).

Therefore, risks related to unfavorable clinical course of ulcerative colitis with frequent recurrences and fast progressing are associated with the allele A of TNF-α gene in the region -308G/A. It should be taken into account when making forecasts on the clinical course o f the disease and choosing treatment strategies.

***Key words**: damages to the mucous tunic in the large intestine, ulcerative colitis, TNF-α, gene polymorphism, assessment of gene variations occurrence, TNF-α, degree of attack severity, endoscopic activity.*

Ulcerative colitis (UC) is a chronic disease in the colon that involves immune inflammation in its mucous tunic, usually without any granulomas detected with biopsy [1, 2]. Undoubtedly, diagnostics, treatment, and prevention of UC are vital issues nowadays. It is confirmed with absence of complete data on the disease etiology, incidence growth among workable population, recurrences, development of life-threatening complications, necessity to perform long-term, often lifelong and expensive therapy, and unfavorable medical and social forecasts [3–5].

Yuliya I. Tretyakova – Candidate of Medical Sciences, Associate Professor at the Polyclinic Therapy Department (e-mail: tretyakovayi@gmail.com; tel.: +7 (342) 217-10-31; ORCID: http://orcid.org/0000-0002-7411-6204).

Alevtina P. Shchekotova – Doctor of Medical Sciences, Professor, head of the Clinical Laboratory Diagnostics Department (e-mail: al_shchekotova@mail.ru; tel.: +7 (342) 217-10-31; ORCID: http://orcid.org/0000-0003-0298-2928).

Irina A. Bulatova – Doctor of Medical Sciences, Professor at the Clinical Laboratory Diagnostics Department (e-mail: bula.1977@mail.ru; tel.: +7 (342) 217-10-31; ORCID: http://orcid.org/0000-0002-7802-4796).

Aleksandr V. Krivtsov – Candidate of Medical Sciences, Head of Immune Genetics Laboratory (e-mail: krivtsov@fcrisk.ru; tel.: +7 (342) 236-86-99; ORCID: https://orcid.org/0000-0001-7986-0326).

Immune-inflammatory process with cytokines imbalance is a leading mechanism in UC development and progression [6, 7]. Tumor necrosis factor-alpha (TNF-α) is the strongest anti-inflammatory cytokine that participates in UC pathogenesis [8]. It is produced by macrophages in the colon and can directly damage its mucous barriers causing inflammatory changes [9, 10]. Bacterial lypopolysaccharides are considered to be a significant TNF-α inductors; when UC occurs, they can be found in great quantities in the colon lumen. Research revealed that that 77.8% patients suffering from UC had increased TNF-α level in the colon mucous tunic in the acute phase of the disease [11].

UC is a disease with genetic heterogenic complex predisposition [12]. Recently a lot of works have been accomplished that revealed correlations between variants of alleles in genes of regulatory inflammation molecules, relevant proteins production, and predisposition to this or that disease. Knowing their role in pathogenesis of many diseases allows, on the one hand, to predict risks related to pathology development or how grave this pathology is going to be; on the other hand, to make a choice on a specific therapy for a concrete patient [13]. There is active search for genes that are responsible for predisposition to UC [14]. When *TNF-α* gene polymorphism was examined in patients with UC in comparison with healthy people, the highest statistically significant changes were detected in -308 G/A position [15, 16]. As per data obtained by F. Wang [et al.] there were no statistically significant discrepancies in frequency of allele variations in *TNF-α* -308 G/A gene between patients with UC and healthy people, either among Europeans, or Asians. However, researchers revealed GA and GA + AA genotype prevalence over GG one among Asians [17]. According to some other data, frequency of A and G genotypes and alleles in *TNF-α* gene (-G308A) among patients with UC didn't have any discrepancies from that detected among population in general [18, 19].

Therefore, literature data on assessing a contribution made by *TNF-α* -308 G/A gene polymorphism into immune inflammatory development in the colon in case of UC are rather controversial. There are not enough data on a correlation between polymorphism of the said gene and UC attack gravity and endoscopic changes; thus, a basic trend of our research was identified.

Our research goal was to assess functional significance of *TNF*-α gene polymorphism in -308G/A (rs1800629) region in UC progression (on the example of patients living in Perm region).

Data and methods. We examined 70 patients with UC with the disease being in its active inflammatory phase. Average age amounted to 36.21±11.69, 34 were males, and 36, females (48% and 52% respectively). Duration of the disease was on average equal to 7.24±6.56 years. Patients were examined at gastroenterology and surgical departments in the Clinical Medical-Sanitary Hospital No. 1 and City Clinical Hospital No. 2 (Perm). UC was diagnosed on the basis of clinical recommendations on diagnostics and treatment of patients with UC [1]. We assessed endoscopic activity (EA) and clinical and endoscopic activity indexes (CAI and EAI). A control group was made up of 50 practically healthy people and was comparable with the test group as per age and sex.

We measured TNF-α concentration in blood serum of all the examined people with ELISA test applying ELISA test kits produced by "Vektor Best" LLC (Novosibirsk); results were registered with "Stat-Fax" photometer (The USA). To detect single nucleotide polymorphisms of *TNF-α* gene in region -308G/A in patients with UC and healthy people, we applied PCR in real time mode; whole DNA was extracted out of whole venous blood that had been preliminary stabilized with ethylenediaminetetracetic acid with "DNA-Sorb-V" set ("Inter Lab Service" LLC, Moscow). We examined polymorphism of -308G/A in *TNF-α* gene with Real-time "CFX-96" amplifier ("Bio-Rad Laboratories, Inc.", the USA) applying allele-specific PCR "SNP-Screen" ("Syntol" LLC, Moscow) with product detection in real time mode.

All the obtained results were statistically processed with Statistica 7.0 (Stat Soft). To describe obtained quantitative signs, we presented data as medians (Me) and 25th and 75th percentile. To assess significance of discrepancies between independent groups, we applied non-parametric Mann-Whitney test. We quantitatively assessed a linear correlation with Spearman correlation coefficient (r). We applied $\chi 2$ technique to describe ratios of genotypes and alleles frequencies in the examined genes and determined odds ratio (OR) as a ratio of a probability that an event occurs to a probability that it doesn't occur. A dependence between examined qualitative signs was determined as per contingency tables (cross tabulation)[1]. We measured how strong a correlation between signs was with Pearson's contingency coefficient (Ki). Discrepancies between samplings were considered to be authentic at $p<0.05$.

Results and discussion. Our research allowed us to reveal that there was a significant increase in TNF-α concentration in blood serum of patients with UC in its active phase against practically healthy people (3.38 (0.85–4.90) and 0.00 (0.00–0.00) pg/ml respectively; $p = 0.02$), which was well in line with literature data [6, 9, 11]. Values of the factor were higher than average in 86.2% (62 people) patients against reference values. Therefore, most patients with UC during the disease attack had TNF-α anti-inflammatory cytokine content in blood serum that was several times higher than reference values and it proved that the nosology involved an apparent immune-inflammatory component.

Correlation analysis revealed a significant strong direct correlation between TNF-α contents in blood serum and UC attack gravity ($r = 0.77$; $p = 0.000$), CAI ($r = 0.4$; $p = 0.04$), EAI ($r = 0.31$; $p = 0.01$), and duration of erosive-ulcerative processes in the colon ($r = 0.3$; $p = 0.04$). That is, TNF-α adequately assesses prevalence and intensity of

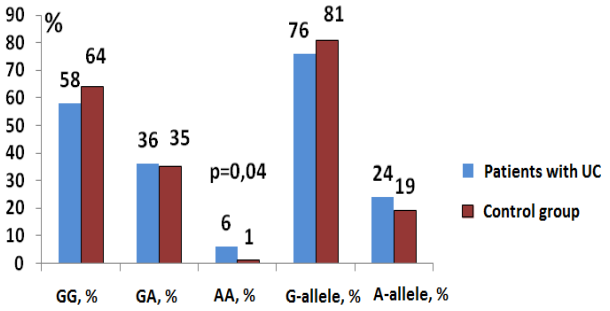

Figure 1. Frequency of detected allele variants in *TNF-α* gene, -308G/A (rs1800629) region, among examined healthy people and patients with UC. Y-axis shows a number of patients given in %. X-axis shows genotypes and alleles in *TNF-α* gene, (rs1800629) section

inflammation in the colon. Assessment of TNF-α contents in blood serum can be applied to stratify UC attack gravity, determine endoscopic activity, and to discuss therapy adjustments as it was confirmed by some researchers [8, 10, 11].

We examined what polymorphism genotypes and alleles prevailed in region rs1800629 of *TNF-α* gene among patients with UC and healthy people in Perm region and revealed that GG genotype prevailed (Figure 1).

We didn't detect any authentic discrepancies in frequency of protective allele G (76.43% and 81.48% respectively; $\chi 2 = 1.16$; $p = 0.28$; $OR = 0.74$) and minor allele A (23.57% and 18.52% respectively; $\chi 2 = 1.16$; $p = 0.28$; $OR = 1.36$) prevalence in *TNF-α* gene, -308G/A region, in the examined groups, which was consistent with other research data [17, 18]. However, unfavorable homozygote AA was significantly more frequently detected among patients with UC than among practically healthy people (5.71% and 1.23% respectively; $\chi 2 = 2.35$; $p = 0.04$; $OR = 4.85$) (Figure 1). Therefore, if a person has homozygote AA in *TNF-α* gene (rs1800629), it can be considered a sign that he or she is genetically predisposed to UC.

To perform comparative analysis of TNF-α level and to assess functional significance that

[1] Shelud'ko V.S., Poluzhnaya M.Ya Theoretical aspects of medical statistics: a procedure and recommendations. – Perm, 2001. – 36 p.

Table 1

TNF-α level and frequency of allele variants occurrence for *TNF-α* gene in -308G/A (rs1800629) region in patients with UC depending on attack gravity (AG) and EA degree

Factor/genotype/alleles		UC, n = 70		OR(95%CI)	p
		Attack gravity (1–2) and I–II degree EA; (n = 37); % ± m	Attack gravity (3) and III–IV degree EA; (n = 33); % ± m		
TNF-α, pg/ml		0.90 (0.20–2.55)	8.70 (7.10–12.00)	–	0.0000
TNF-α -308G/A	GG. %	78.38±6.77	36.36±8.37	6.34 (2.21–18.24)	0.001
	GA. %	21.62±6.77	51.52±8.7	0.26 (0.09–0.73)	0.001
	AA. %	0±0	12.12±5.68	0.0 (0.00–0.00)	0.001
Alleles	G-allele. %	89.19±3.61	62.12±5.97	5.03 (12.07–12.21)	0.000
	A-allele. %	10.81±3.61	37.88±5.97	0.20 (0.08–0.48)	0.000

Note: TNF-α is tumor necrosis factor alpha; OR is odd ratio, 95%CI is confidence interval (CI) for OR, p is significance of discrepancies; AG (1, 2, 3) and EA (I, II, III, IV) are degrees of attack gravity and endoscopic activity.

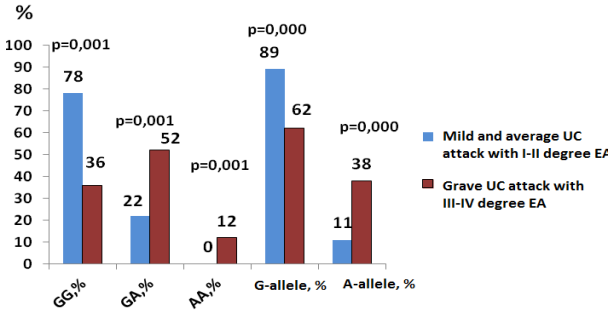

Figure 2. Frequency of allele variants occurrence in *TNF-α* -308G/A (rs1800629) in patients with UC under various attack gravity and EA degree. Y-axis shows number of patients given in %. X-axis shows genotypes and alleles in *TNF-α* (rs1800629)

gene *TNF-α* polymorphism in -308G/A region had in UC development, we divided patients with UC into two sub-groups taking into account attack gravity (AG) and EA degree as per data obtained via sigmoidoscopy (SS) and fiber colonoscopy (FCS). The first sub-group (n = 37) included patients suffering from UC with mild and average attack, rare recurrences, and with 1–2 degree EA. The second group (n = 33) was made up of patients with grave continuous UC with frequent recurrences and 3–4 degree EA.

TNF-α levels in patients with UC who had 3–4 degree EA were authentically higher than in patients with 1–2 degree EA (p = 0.0000) (Table 1). Assessment of TNF-α contents in blood serum in patients with UC can be quite informative for determining degree of EA and discussing adjustments to therapy.

We assessed frequency of allele variations in *TNF-α*-308G/A (rs1800629) under different endoscopic activity and revealed that major allele G and allele pair GG significantly more frequently occurred among patients with mild or average attack and 1–2 degree EA than among those who had 3–4 degree EA and suffered from grave UC attack ($\chi 2 = 14.19$; p = 0.000; OR = 6.34). Minor or mutant allele A, on the contrary, significantly more frequently occurred among patients with 3–4 degree EA and grave UC attack, in 37.88% ($\chi 2 = 14.19$; p = 0.000; OR = 0.20) (Table 1, Figure 2).

Unfavorable homozygote AA was detected in 12.12% patients with UC and high EA and didn't occur in patients with low or average EA ($\chi 2 = 14.11$; p = 0.001; OR = = 0.00) (Table 1, Figure 2). Consequently, occurrence of mutant allele A and homozygote AA in *TNF-α*-308G/A gene predict grave recurrent UC course with proneness to progress.

We examined dependences between examined qualitative signs with contingency tables (cross tabulation) among patients with

UC and found an apparent correlation between polymorphism of promoter region -308G/A in *TNF-α* gene and an increase in production of the same TNF-α factor, attack gravity ($K_i = 0.75$; $p = 0.000$ and $K_i = 0.84$; $p = 0000$ respectively), clinical and endoscopic activity indexes ($K_i = 0.85$; $p = 0.000$ and $K_i = 0.78$; $p = 0.000$ respectively) and a length of damage areas in the colon as per endoscopy data ($K_i = 0.89$; $p = 0.000$). We also revealed a strong dependence on C-reactive protein contents ($K_i = 0.73$; $p = 0.000$), blood leukocytes ($K_i = 0.59$; $p = 0.000$), sedimentation rate ($K_i = 0.67$; $p = 0.000$), and α_1,α_2 and γ-globulins ($K_i = 0.46$; $p = 0.017$; $K_i = 0.72$; $p = 0.000$; $K_i = 0.61$; $p = 0.000$), which are conventional laboratory markers of an acute inflammation phase, and albumin contents in blood ($K_i = 0.73$; $p = 0.000$).

Therefore, this determined correlation between polymorphism of promoter region -308G/A in *TNF-α* gene and increased TNF-α expression in blood of patients with UC can make a contribution into immune-inflammatory syndrome activation among carriers; our results confirm data collected in other research [11–13]. The revealed correlation between polymorphism of this gene and UC attack gravity, EAI, blood parameters characteristic for an acute phase, as well as occurrence of mutant allele A and homozygote AA in patients with high EA degree and grave exacerbations, can indicate that there is a contribution made by *TNF-α* gene polymorphism into predisposition to unfavorable UC course. We assume this risk factor for grave UC course to be a significant one (homozygotes as per a normal allele predict milder disease course), OR 6.34 (CI 2.21-18.24).

We recommend to perform a preventive examination in order to detect a significant polymorph allele in *TNF-α* gene, -308G/A region (OR 5.03; CI 12.07-12.21); it will help create additional examination and therapeutic procedures for patients with UC as mutant allele occurrence results in 5 times higher risk of graver disease course.

Conclusion. We detected higher TNF-α contents in blood of patients with UC in its acute phase against the control group. Concentration of this cytokine was significantly higher in patients with grave disease course, frequent recurrences, and high EA degree; it can be applied to stratify UC attack gravity and to determine EA degree.

We examined distribution of genotypes and alleles in -308G/A region of *TNF-α* gene, rs1800629 position, among patients with UC and health people living in Perm region and revealed that GG genotype prevailed in them. We didn't detect statistically significant discrepancies in frequency of protective allele G and minor allele A occurrence between the examined groups. Nevertheless, unfavorable homozygote AA significantly more frequently occurred among patients with UC than among healthy people. We can assume that if a person carriers this homozygote, he or she is genetically predisposed to UC.

Besides, as we assessed frequency of allele variations occurrence in *TNF-α* gene under different UC attack gravity and endoscopic activity, we detected mutant allele A and unfavorable homozygote AA to be correlated to grave and progressing UC course.

Consequently, a risk related to unfavorable UC course with frequent recurrence and progressing is associated with carrying mutant allele A of *TNF-α* -308G/A gene. Given that, a genetic examination can be recommended when UC is diagnosed for the first time; this genetic examination should involve determining polymorphism of *TNF-α* -308G/A gene in order to assess risks of unfavorable disease course and to work out individual therapeutic and prevention procedures.

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Conflict of interests. the authors state there is no any conflict of interests.

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TOXICOLOGICAL AND HYGIENIC ASSESSMENT OF TITANIUM DIOXIDE NANOPARTICLES AS A COMPONENT OF E171 FOOD ADDITIVE (REVIEW OF THE LITERATURE AND METAHANALYSIS)

I.V. Gmoshinski[1], O.V. Bagryantseva[1,2], S.A. Khotimchenko[1,2]

[1]Federal Research Center for Nutrition, Biotechnology and Food Safety, 2/14 Ust'inskiy proezd, Moscow, 109240, Russian Federation
[2] I.M. Sechenov First Moscow State Medical University (Sechenov University), Bldg. 2, 8 Trubetskaya Str., Moscow, 119991, Russian Federation

The review focuses on exposure values, biological availability, toxic effects, and risks caused by nanoparticles of TiO_2 under their oral introduction into a body as a food coloring agent or E171 food additive, or as a significant component in its structure. According to toxicological assessment performed by JECFA in 1969, TiO_2 is considered to be insignificantly hazardous. However, at present experts employed by several foreign and international organizations that deal with food safety believe that the assessment should be reviewed as there are new scientific data on adverse effects produced by nano-sized TiO_2 on a human body. Overall intake of TiO_2 by people with food products, cosmetics (tooth pastes) and medications can vary from 0.5 to 5 mg a day; children aged 3–9 and teenagers aged 10–17 are the most exposed population groups. Despite insignificant intestinal absorption of TiO_2 nano- and micro-sized particles, a lot of scientific works revealed their overall toxic effects produced on a body under oral and intragastric introduction. Detected effects produced by TiO_2 include organotoxic (mostly hepatotoxic) ones, genotoxicity, immune toxicity, reproductive toxicity, and neurotoxicity. Still, there haven't been any data on carcinogenic effects produced by TiO_2 when it is introduced into the gastrointestinal tract. Presumably, some effects produced by TiO_2 nanoparticles are mediated by their local impacts on the lymphoid tissue associated with an intestinal mucosa as well as on the structure and activity of intestinal microbiocenosis, and nanoparticles are not necessarily absorbed in the intestines in the process. We performed meta-analysis of 64 articles (published over 2007–2019) which complied with criteria related to scientific authenticity and completeness; the meta-analysis revealed that a probable NOAEL for nano-sized TiO_2 amounted to less than 10 mg/kg of body weight a day, and a daily reference safe dose of the substance is estimated as being equal to 0.1 mg/kg of body weight. Given all the above-mentioned, a risk caused by TiO_2 intake as E171 food additive depends on nanoparticles fracture in its composition and it can be unacceptably high if this fracture exceeds 10 % of the overall TiO_2 mass. Therefore, it is necessary to control and regulate TiO_2 nanoparticles contents in the structure of E171 food additive that is applied in food industry.

Key words: *titanium dioxide, food additive, nanoparticles, exposure, biological availability, toxicity, intestinal microbiocenosis, risks.*

Titanium dioxide (TiO_2) is widely used in various food products as a coloring agent E171 due to its intense white color. Production volumes of the substance exceeded 5 million tons in 2008 [1]. Both Russian and international legislation allows using E171 in food products manufacturing (The Customs Union Technical Regulations "Requirements to safety of food additives, flavoring agents and technological auxiliary substances" (TR CU 029/2012)[1],

Ivan V. Gmoshinski – Doctor of Biological Sciences, leading researcher at the Laboratory for Food Toxicity and Nanotechnologies Safety Assessment (e-mail: gmosh@ion.ru; tel.: 8 (495) 698-53-71; ORCID: https: //orcid.org/0000-0002-3671-6508).

Olga V. Bagryantseva – Doctor of Medical Sciences, Professor (Republic of Kazakhstan), leading researcher at the Laboratory for Food Toxicity and Nanotechnologies Safety Assessment (e-mail: bagryantseva@ion.ru; tel.: +7 (495) 698-54-05; ORCID: https: //orcid.org/0000-0003-3174-2675).

Sergey A. Khotimchenko – Doctor of Medical Sciences, Professor, Head of the Laboratory for Food Toxicity and Nanotechnologies Safety Assessment (e-mail: hotimchenko@ion.ru; tel.: +7 (495) 698-54-05; ORCID: https: //orcid.org/0000-0002-5340-9649).

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"Codex General Standard For Food Additives" (CODEXSTAN 192-1995)[2], Regulation (EU) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives[3]). Significant amounts of TiO_2 can obviously be found in covers of medications or in cosmetics (toothpastes). Besides, according to the Eurasian Union legislation, TiO_2 as a component of titanium enamels can be used in manufacturing package and materials that contact food (the Customs Union Technical Regulations "On safety of package", TR CU 005/2011[4]). Therefore, population exposure to TiO_2 via the gastrointestinal tract can be rather considerable. And there is a question here related to TiO_2 safety for consumers and health risks that can be probably caused by it. It is especially true for nanoparticles (NPs) that can be contained in E171; that is, particles with their sizes being less than 100 nm [2]. In all cases that are practically significant these NPs are represented by two alternative crystal forms of TiO_2, namely anatase and rutile. Anatase NPs are, as a rule, spherical or oval and their sizes vary from 10 to 100 nm (most typically 20-30 nm); rutile NPs often have irregular shape or are rod-like with their cross section being less than 10 nm and their length being 40-50 nm or more. Both NPs tend to agglomerate or aggregate significantly in water suspensions and as components in food products; these processes depend on concentrations of a nanomaterial and disperse medium structure. Particle size analysis performed on widely spread E171 trademarks revealed that they often had anatase or rutile in their structure [3]. Due to their small size these NPs have potentially much greater penetrability than their micro-analogues (microparticles or MPs) and it allows them to penetrate through biological barriers including those located in the gastrointestinal tract [4]. They also possess considerably greater chemical potential per a mass unit, greater catalytic activity and greater solubility as well. It means that we can't neglect toxic effects possibly produced on a human body by TiO_2, NPs contained in food products, cosmetics or medications.

Our research goal was to analyze data taken from scientific literature on exposure values, biological availability, toxic effects, and risks caused by TiO_2 nanoparticles when they were introduced into a body via the gastrointestinal tract as a food additive E171 or as a significant component in its structure.

Exposure volumes and scenarios

TiO_2 is used in food products manufacturing as a coloring agent that makes a product white due to a decrease in a volume of the "grey" component in a spectrum of light radiation that is back-scattered by this product [2]. In 1969 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) developed the first and still the only one officially accepted and valid toxicological assessment of TiO_2 as a food additive [5]. An issue related to probable negative impacts exerted by NPs contained in titanium dioxide was not considered within that assessment procedure. There was a conclusion that it was not necessary to fix safe daily levels of consumption for this substance, mostly due to its extremely low solubility. According to European Food Safety Authority, pigment

TiO_2 (or so called "titanium white") with its particles sized 01-1.0 μm is safe for a human body due to its poor solubility in water and biological media and total absence of its absorption in the gastrointestinal tract [6,7].

In the USA TiO_2 was allowed as a coloring agent in 1966 [8]. US FDA* allows using TiO_2 in a food product in a quantity not exceeding 1% of its total mass [9]. Besides, in

[2] CODEX STAN 192-1995. General Standard For Food Additives [web-source]. – URL: http://www.fao.org/gsfaonline/docs/CXS_192e.pdf (date of visit March 23, 2019).

[3] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance) [web-source].– URL: https://eur-lex.europa.eu/eli/reg/2008/1333/oj (date of visit March 23, 2019).

[4] TR CU 005/2011. "On safety of package" (last edited on October 18, 2016): The Customs Union Technical Regulations [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL: http://docs.cntd.ru/document/902299529 (date of visit March 23, 2019).

* United States Food and Drug Administration.

* Joint FAO/WHO Expert Committee on Food Additives.

the USA TiO_2 is allowed as "a material that contacts food" (as a component in food products packages). In Japan there are no limits imposed on TiO_2 application as a coloring agent [10]. In India a quantity of TiO_2 that can be added to a food product is limited to 1% in chewing gums and to 0.01% in dried mixtures used to make drinks [11]. As per data provided by X.-X. Chen [et al.], if any standard for TiO_2 contents in chewing gums is not fixed in a country, its quantity can reach up to 0.2% of a total mass of a product, and 93% out of this quantity can be a nanoform of the substance [12]. In the European Union [13] and in Russia, according to TR CU 029/2012[1], TiO_2 is allowed in manufacturing all types of food products in conformity with technical specifications, excluding products enlisted in Appendix 9. TiO_2 can also be applied to dye covers of medications (The RF Public Healthcare Ministry Order issued on March 19, 1998 No. 80[5]) and in cosmetics (toothpastes).

In 2013 EU Scientific Committee on Consumer Safety (EU-SCCS) published an expert opinion on a food additive E171 consisting of NPs [14]. This opinion being based on results of only two publications [15, 16], the Committee concluded that a minimal dose corresponding to LOAEL for nano-TiO_2 amounted to 5 mg/kg of a body weight a day. Experts also concluded that the assessment made by JECFA* in 1969 was no longer valid due to new scientific data being discovered and it was necessary to perform a new examination on TiO_2 safety when the substance was applied as a coloring agent in food products.

Therefore, it is crucial to determine which part of orally introduced TiO_2 is represented by NPs if we want to assess exposure and risks related to it. As per data provided by A. Weir [et al.], [17] an average diameter of particles contained in popular pigment TiO_2, applied in food manufacturing can be equal to 110 nm; and according to electronic microscopy, at least 36% out of overall number of particles have their diameter varying from 30 to 100 nm. However, as a mass of a particle grows proportionally to a cube of its diameter, an overall mass of a nano-sized component in food TiO_2 will be significantly lower. According to recommendations issued by the European Commission, to be called a nano-material, a substance should contain nanoparticles in a quantity not lower than 50% out of their total number [18]. If this criterion is applied, most E171 trademarks that are currently used can be considered a nanomaterial.

It was shown that when water extractions from such products as sweets, confectionary, chewing gums, and toothpastes were put through membrane filters, only about 5% Ti passed through pores with their diameter being 0.45 or 0.5 μm [17]. The result can have two interpretations; first of all, it can occur due to practical absence of nanoparticles in a sample, or it can be due to a massive aggregation or agglomeration of NPs contained in it or their adsorption on matrix components with their size being much greater than that within a nano-range.

According to research results provided by R.J. Peters [et al.] [19], most E171 samples obtained from food products contained NPs sized less than 100 nm in a quantity equal to 10-15% out of total particles number. TiO_2 was detected in 24 out of 27 types of examined food products and products for personal hygiene in significant quantities ranging from 0.02 to 9.0 mg/g, and 5-10% particles in these products were within a nano-range and it was qualitatively consistent with analysis performed on pure E171 samples.

Titan (Ti) as chemical element is known to be widely spread in the Earth crust; it is a natural component in animal tissues though it is found there in minimal quantities [20]. There are no data on any specific biological role played by Ti or the element being essential for animals. Nonetheless, data on background Ti levels in biological objects should be taken into account when assessing results of research that focused on attempts to determine NPs contents in food products and biological

[5] On application of coloring agents in medications: The RF Public Healthcare Ministry Order issued on March 19, 1998 No. 80 [web-source]. – URL:http://www.consultpharma.ru/index.php/ru/documents/drugs/374-80?showall=1 (date of visit March 23, 2019).

materials basing on data obtained via elemental analysis. Introduction of overall Ti being a natural food component into a human body can amount to approximately 300-400 μg a day, while introduction with drinking water is considered insignificant. A human body is exposed to significantly greater quantities of Ti due to various consumer products containing TiO_2 (E171) which was deliberately added to them both as microparticles (MPs, diameter is 100-2,500 nm) and as NPs.

As per early data provided by Dietary intake of food additives in the UK [21], in the UK average daily TiO_2 consumption amounted to 5.4 mg/kg of body weight. Later Weir [et al.] [17] performed more precise estimation and obtained another value varying from 0.2 to 2 mg/kg and it is rather close to the above-mentioned assessment for LOAEL without taking into account percentage of nanoparticles in TiO_2 contained in food products.

According to M.B. Herringa [et al.] [22], in Western European countries up to 57% of orally introduced TiO_2 came from toothpastes. There are some other products that can also be a source of TiO_2 NPs, for example, chewing gums (14%), coffee creamers (13%), dried milk for coffee (8%), glazed chocolates (3%), mayonnaise (7%), spicy sauces (5%), and instant cappuccino (3%). Younger children obviously face additional exposure with toothpaste as they tend to swallow it and it is quite significant for this age group. According to these data, TiO_2 was consumed as E171 coloring agent in the following quantities: 0.67 mg/kg b.w. by children aged 2-6; 0.17 mg/kg b.w. by people aged 7-69; and 0.06 mg/kg b.w. by people older than 70. Average estimated consumption of TiO_2 NPs amounted to 0.19 μg/kg b.w. by people older than 70; 0.55 μg/kg b.w.by people aged 7-69; and 2.16 μg/kg b.w. by children aged 2-6. 95%-percentile of consumption amounted to 0.74, 1.61, and 4.16 μg/kg b.w. in these age groups respectively. The authors didn't estimate quantities in which this coloring agents was consumed by children younger than 2. When performing their calculations, the authors assumed that average NPs contents in commercial samples of E171 amounted to approximately 0.31% as per a sample mass [1].

A work published by EFSA* [6] focused on exposure to TiO_2 as a component in covers of medications and biologically active additives to food (BAA) sold as pills or capsules. In such cases TiO_2 contents can reach up to 3% out of the overall mass of a pill, and up to 12.5% out of this quantity can be rutile. Average daily consumption of medications usually amounts to approximately 20-200 mg; BAA, 10-1,000 mg. Daily exposure to TiO_2 can therefore be estimated as 15-37.5 mg respectively, or 0.625 mg/kg b.w. The same work contained another estimation of exposure to rutile in confectionary and it varied from 0.071 to 0.495 mg/kg b.w. a day.

M.-H. Ropers [3] stated that in the USA amount of TiO_2 consumed with food amounted to approximately 0.2-0.7 mg/kg of b.w. a day while in the UK, Holland, and Germany it could reach 1 mg/kg b.w. These data were obtained via taking into account all scenarios of introduction with food. For example, in Holland median estimation of E171 introduction varied from 1.1 to 1.4 mg/kg b.w., and the upper introduction limit for children amounted to 3.2 mg/kg b.w. Similar results were obtained in Germany. In spite of all the differences between analyzed exposure scenarios, data obtained in all the research works coincide about the most exposed populations groups and these are children aged 3-9 and teenagers aged 10-17. EFSA experts believe that a contribution made by chewing gums is insignificant in comparison with other confectionary should we take into account consumed breath fresheners in pills, spicy sauces, and salad seasonings [7]. A research work conducted in Germany contained a conclusion on food products that made maximum contribution (up to 75%) into consumed TiO_2 quantities; they were spicy sauces, salad seasonings, soft drinks, and cheese [23].

As per data obtained via Monte Carlo method in the USA population consumed TiO_2 in all its forms in quantities being equal up to 1 mg/kg b.w. a day [17].

* European Food Safety Authority.

Table 1

Estimated daily oral exposure to titanium dioxide used as a component in food products, cosmetics, medications, and BAA

Reference	Exposure, mg/kg b.w.	Region, country	Population group	Note
MAFF, 1993	5.4	Great Britain	All groups	Median
Weir [et al.], 2012	1.0 2.0	The USA Western Europe	All groups – « –	Median Median
EFSA, 2004	1.1	EU countries	All groups	Median
Rompelberg [et al.], 2016	0.59 1.29 0.08 0.50	Holland – « – – « – – « –	Children aged 2-6 – « – Children aged 7–16 and adults – « –	Median 95%-percentile Median 95%-percentile
Ropes [et al.], 2017	0.7 1.4 3.2	The USA Western Europe Western Europe	All groups All groups Children younger than 16	Median Median Median

Therefore, oral exposure of population to TiO_2 NPs was estimated by different authors and the results were not quite similar depending on a chosen scenario, selection of examined products, probable TiO_2 contents in them, and, above all, uncertainties related to a question which fraction of a pigment was represented by NPs. Completely nano-sized TiO_2 products are assumed to be scarcely used as a food additive E171 because maximum whitening (light-diffusing) effects are produced by the substance with its average particle size being equal to approximately 200 nm [1].

Table 1 contains basic results as regards estimating oral daily exposure of adults and children to all TiO_2, forms.

Biological availability, distribution and accumulation

There are few works with attempts to directly examine penetrability of intestinal mucous tunics for TiO_2 NPs and MPs. To do this, some researchers applied *in vitro* models of intestinal epithelium that applied monolayers with cells being functionally and morphologically identical to enterocytes, for example, Caco-2 line cells. Z.M. Song et al [24] showed in their work that when TiO_2 NPs influenced a cellular monolayer in a concentration equal to 10-100 μg/ml (which corresponded to an oral introduction into a human body equal to approximately 1-10 mg/kg b.w.), they penetrated through the epithelial layer in small quantities, both via transcytosis and paracellular transport through inter-cellular contacts due to damaged γ-catenane structure. As per data provided by B.A. Koeneman [et al.] [25], NPs were able to accumulate in Caco-2 cells but they didn't penetrate through a monolayer in significant quantities. G. Janer [et al.] [26] applied models for mucous tunics of rats' intestines and Caco-2 cells monolayer to show that NPs penetrated epithelial cells in minimal quantities excluding specialized M-cells of Payer's plaques. G.E. Onishchenko et al. [27] applied transmission electronic microscopy (TEM) to examine effects produced by rutile NPs on a mucous tunic of a rat's intestine when they were introduced in a quantity equal to 50 mg into an isolated loop of the ileum with preserved blood supply and innervations. The authors detected massive NPs deposition on the apical surface of enterocytes and their penetration into apical cytoplasm in small quantities. So, there are data confirming TiO_2 NPs ability to penetrate through intestinal mucous tunics in rather limited quantities.

It is rather difficult to quantitatively estimate TiO_2 absorption in the gastrointestinal tract; the task can probably be solved by detecting NPs or MPs of the substance with analytical TEM or via elemental analysis of Ti contents in tissues. Historically, P.U. Jani [et al.] were the first to do that [28], as they applied histological and chemical procedures to detect TiO_2 MPs sized 500 nm in the liver, spleen, and lymphoid tissue of the intestines taken from female Sprague Dawley rats after a single oral introduction in a dose equal to

12.5 mg/kg b.w. MPs were not detected either in the heart or kidneys.

TiO_2 MPs (150 nm) and NPs (27 and 80 nm) were introduced into male and female mice in a dose equal to 5,000 mg/kg b.w.; after this single introduction Ti was detected in the liver, spleen, and kidneys [29]. Y.Cui [et al.] also highlighted that Ti quantities grew in the liver of mice that had been orally exposed to TiO_2 NPs during 60 days [30]. X. Sang [et al.] detected growing Ti contents in the spleen [31, 32] and thymus of mice [33] under longer exposure (90 days) to TiO_2 NPs in a dose equal to 10 mg/kg b.w. Anatase NPs accumulated in the spleen and ovaries of female Sprague-Dawley rats under 5-day introduction in a dose equal to 1-2 mg/kg b.w. As per data provided by G.E. Onishchenko [et al.] [27], when rutile was introduced during 28 days into Wistar rats in a dose equal to 100 mg/kg b.w., it resulted in its accumulation in the liver. Data obtained by R. Shrivastava [et al.] [35] indicate that TiO_2 NPs are likely to penetrate through the blood-brain barrier and enter cytoplasm and nucleuses of brain cells after a single intragastric introduction in a dose equal to 500 mg/kg b.w. Y. Ze [et al.] [36] also detected these NPs in mice's brains after 90-day introduction in a dose varying from 2.5 to 10 mg/kg of b.w. TiO_2 NPs accumulation in the stomach mucous tunic of mice was detected after their introduction in a dose equal to 500 mg/kg b.w. during 5 days [37]; the authors believed it could probably result in gastritis. Similar data were obtained regarding accumulation in mucous tunics of rats' small intestine [38]. There was a sub-acute 14-week experiment [39] when anatase NPs were introduced into mice's stomachs in doses up to 320 mg/kg b.w.; Ti was biologically distributed in the liver, spleen, small intestine, kidneys, and pancreas. F. Hong [et al.] [40] introduced TiO_2 NPs in a dose equal to 25-100 mg/kg b.w. into female mice during their pregnancy (17 days); after that TI accumulation was detected in fetuses and placenta [40]. A recent work by J. Yang [41] contains data on profoundly described anatase NPs sized 21 nm and their accumulation in mice's lives after 14-day oral introduction.

Some other researchers failed to detect TiO_2 NPs absorption in the gastrointestinal tracts and their biological accumulation at detection limits of analytical procedures which they applied in their experiments. Thus, Cho [et al.] applied ICP-MS and didn't detect Ti accumulation in the liver, kidneys, spleen, brain, and urine excretion after a mixture of anatase and rutile NPs (80:20) had been orally introduced into rats in a dose equal to 1,000 mg/kg b.w. during 13 days [42]. Geraets [et al.] performed a 5-day experiment on male and female Wistar rats orally introducing TiO_2 NPs (with diameter 38-67 nm) or MPs (with diameter 132-267) into them. They revealed trace Ti quantities (not more than 0.001% of an introduced dose) only in the liver and spleen and it was rather close to a detection limit for this analytical procedure [43]. L. Martins [et al.] didn't reveal any Ti accumulation in the liver, kidneys, or blood after NPs had been introduced into Wistar rats during 45 days in a dose equal to 0.5 mg/kg b.w. [44]. E.M. Donner [etal.] [45] also stated they didn't reveal any biological accumulation in internal organs in their research during which rats were exposed to a single introduction of 6 different anatase or rutile NPs or MPs in a dose varying from 500 to 2,000 mg/kg b.w.; the same results were obtained by N. Gu[etal.] [46] after TiO_2 MPs had been introduced into mice in a dose equal to 64 mg/kg b.w.

Therefore, chemical analysis procedures, even highly sensitive ones (ICP-MS) didn't allow obtaining unambiguous results as regards probable TiO_2 NPs absorption in the gastrointestinal tract and their accumulation in organs and tissues. It can partly be due to artifacts associated with natural background Ti contents in biological objects and, owing to it, its excessive introduction into organs and tissues as a component in NPs can only be measured with great inaccuracy. On the other hand, part of negative results that have been described above can be explained by an incorrect choice on a dose of a nanomaterial (too low or, on the contrary, too high a dose resulting in total NPs aggregation), or by exposure duration being insufficient. This uncertainty could

be eliminated via applying NPs with a radionuclide marker; however, Ti is a chemical element without any long-living radionuclides. An attempt to mark TiO_2 NPs with iodine isotope [23] failed as this marker easily detached from NPs in a biological medium. Yu.P. Buzulukov [et al.] [47, 48] tried to mark rutile NPs with scandium radionuclide via bombarding with fast neutrons with an energy exceeding 1.9 MeV on a cyclotron as per the following reaction: $_{22}Ti^{47}(n,p)_{21}Sc^{47}$. Marked NPs were introduced into male Wistar rats; there was a single introduction in a dose equal to 400 mg/kg b.w. Registered radioactivity in internal organs didn't allow detecting markers in the spleen, pancreas, gonads, kidneys, lungs, heart, brain, or urine. The authors detected only trace quantities of markers (approximately 0.002% out of an introduced dose) in the liver and blood. More than 99.9% of radioactivity was excreted with feces, and about 0.06% of the introduced radioactivity was detected in bone-muscular-skin carcasses; the authors explained it by fur near anuses being probably contaminated with feces. Therefore, a single result obtained via a radioisotope procedure is consistent with research works where it is stated that TiO_2 NPs have very insignificant (very close to zero) biological availability when they are introduced into the gastrointestinal tract.

Hazard characteristic

1. General effects and target organs.

Obviously, TiO_2 NPs and MPs really have very insignificant biological availability but still there is a great number of works containing data on revealed overall toxic effects produced by them on a human body under oral and intragastric introduction. Chronologically, the first work was published by Wang [et al.] [29]; they introduced NPs (25 and 80 nm) or MPs (150 nm) into male and female mice; there was a single introduction in a dose equal to 5,000 mg/kg b.w. The authors didn't detect any signs of acute toxicity (lethality or drastic changes in animals' behavior) but they revealed perivascular degeneration and localized necrosis of hepatocytes, shifts in LDH activity and AST/ALT ratio in blood plasma, increased urea contents, and pathological changes in the kidneys. A single intragastric introduction of TiO_2 NPs in a dose equal to 160-1,000 mg/kg b.w. into Wistar rats resulted in higher contents of taurine, citrate, hippurate, histidine, trimethylamine-N-oxide, citrulline, α-ketoglutarate, phenyl acetyl glycine and acetate in urine and in lower contents of lactate, betaine, methionine, threonine, pyruvate, 3-D-hydroxybutyrate, choline and leucine. Similar metabolome shifts were also detected in blood plasma [49].

When anatase NPs were orally introduced into female CD-1 (ICR) mice for 30 days in doses from 62.5 to 250 mg/kg b.w., it caused higher ALT, AST and alkaline phosphatase activity, higher concentrations of nitrogen oxide, bilirubin, and interleukin-s, shifts in blood leukogram and contents of CD3(+), CD4(+), CD8(+) cells, populations, NK-lymphocytes, and B-cells populations as well [15].

R.V. Raspopov [et al] [50] introduced anatase NPs, rutile NPs, and TiO_2 pigment MPs into growing male Wistar rats during 30 days; the substances were introduced intragastrically in doses equal to 1 and 100 mg/kg b.w. The lowest dose caused greater penetrability of intestinal walls for protein macromolecules, greater excretion of 8-oxo-G (a product of DNA oxidative destruction), lower contents of reduced thioles and CYP2B1 activity, greater overall activity of glutathione-S-transferases in the liver, and lower alkaline phosphatase activity in blood plasma. Effects revealed in the research were both those specific for NPs only and those produced by both particles types, MPs included. Other researchers applied proteomics to examine Wistar rats after exposure to anatase NPs in doses equal to 1-10 mg/kg b.w. and revealed abnormal isoform of glutathione-S-transferase expressed in the liver as well as occurrence of 53 protein spots and disappearance of 19 others that were not exactly identified [51].

Y.Cui [et al.] introduced TiO_2NPs into mice during 60 days and revealed enhanced hepatocytes apoptosis, oxidative stress development, lower expression of metallothionen, heat shock protein HSP70, P53, and trasnferrin [52]; transcriptome changes in expression

of TLR2 и 4 , IKK1, IKK2, NF-κB, NF-κBP52, NF-κBP65, TNF-α, NIK, IκB and IL-2 genes [30].

In another research Wistar rats were exposed to TiO_2 NPs in a dose equal to 300 mg/kg and it resulted in greater lipoperoxides contents in the liver, greater ALT and AST activity in blood plasma, and lower antioxidant enzymes activity. Histological picture of damages done to the liver included apoptosis, centrilobular necrosis, and inflammation cells proliferation [53]. Changes in expression of p53, BAX, caspase-3 и -9 and Bcl-2 and signs of oxidative damage to DNA were observed in the liver of mice exposed to TiO_2 NPs in a dose equal to 100 mg/kg b.w. for 14 days [54].

Anatase NPs (21 nm) turned out to be hepatotoxic for mice in a dose equal to 150 mg/kg b.w.; it became obvious in a 14-day experiment through greater transaminase activity in blood plasma, liver edema, malonic dialdehyde accumulation in liver tissues, liver macrophages activation, greater TNF-α and IL-6 production, expression of nucleus erythroid-2-related factor 2 and NF-κB together with inhibited expression of Bcl-2[55]. Yang [et al.] revealed disorders in metabolic functions of the liver caused by exposure to orally introduced anatase NPs sized 21 nm [41].

Y. Wang [et al.] [56] showed that introduction of TiO_2 NPs sized 75 nm into young male Sprague-Dawley rats caused shifts in mineral metabolism that became apparent via lower Mo, Co, Mn and P contents in the liver as well as lower Rb and Na contents in the kidneys.

TiO_2 NPs introduced in a dose equal to 2-50 mg/kg b.w. turned out to be cardiotoxic for Sprague-Dawley rats in 30-day or 90-day experiments; the effects were a smaller interval between systolic and diastolic blood pressure, leukocytosis, greater activity of lactate dehydrogenase, a-hydroxybutyrate dehydrogenase, and higher TNF-α and IL-6 contents in blood plasma [57]. Functional disorders in the cardiovascular system were observed not only under exposure to NPs, but also to pigment TiO_2 that contained NPs in a small fraction only and was introduced into mice orally in a dose up to 500 mg/kg b.w. Not only cholinergic vasorelaxation intensified in coronary artery also, but also did serotonergic vasoconstriction that competed with it [58].

Some researchers assessed influence exerted by TiO_2 nanoforms on carbohydrate-energy metabolism in animals. 14-week anatase NPs introduction (64-320 mg/kg b.w.) into mice caused hyperglycemia, insulin resistance, increased ARS1 phosphorilation and decreased Akt under effects produced by JNK1 and p38 MAPK respectively. The process was accompanied with oxidative stress and greater anti-inflammatory cytokines concentrations [59].

TiO_2 NPs introduction into female mice in a dose up to 50 mg/kg b.w. during 14 days caused oxidative stress, hyperglycemia, and shifts in levels of thyroid hormones, estradiol, and prolactin [60]. A probable mechanism of influence exerted by TiO_2 NPs on carbohydrate metabolism was examined in research works [59, 61]; the authors applied trascriptomics to determine metabolic pathways (KEGGs) that were a target for such influence. Thus, multiple oral introductions of these NPs into mice in a dose equal to 50-200 mg/kg b.w. influenced a xenobitoics transformation system and caused a stress in endoplasmatic liver reticulum.

An extent to which TiO_2 NPs influence animals can depend on their sex and age. Specifically, increased glycemia level and higher glutathione peroxidase activity were detected in young male Sprague-Dawley rats (initial age being 3 weeks) that were orally exposed to TiO_2 NPs in a dose equal to 30 mg/kg b.w. during 30 days; no such effects were detected in older animals (6 weeks) [62]. Changes in reduced glutathione, lipid peroxidation products, IL-1α, IL-4, and TNFα caused by exposure to TiO_2 NPs in a dose up to 50 mg/kg b.w. in a 90-day experiment were more apparent in female mice than in male ones, animals' age in both sex groups being comparable [63].

We should pay special attention to some research works where authors didn't reveal any toxicity of various TiO_2 forms under oral introduction. Gu [et al.] [46] exposed mice to pigment titanium white (MPS size being greater than 100 nm) multiple times but didn't

reveal any signs of hyperglycemia or intensified lipid peroxidation. Warheit [et al.] [64] declared their research work to be an arbitration one and performed it in full conformity with OECD test guidelines TG 407, 408, 425. Growing male rats were exposed to a single introduction of NPs (73 nm) or MPs (145; 173 nm) (an acute toxicity test) in a dose equal to 24,000 mg/kg b.w. or to multiple introductions (28 or 29 days) in doses equal to 1,000 mg/kg b.w. or more. The authors tested integral parameters and internal organs morphology and didn't reveal any signs of toxicity. Despite the research having strict methodological substantiation, later on it was criticized [22] mostly due to a limited set of examined parameters as well as inadequate choice on a dose of a nanomaterial; chosen doses were extremely high and it could presumably lead to its massive aggregation and formation of extended three-dimensional structures (gelation) in the gastrointestinal tract lumen.

2. Genotoxicity

TiO_2 NPs (33 nm) and MPs (160 nm) were introduced intragastrically into CBAB6F1 mice in doses varying from 40 to 1,000 mg/kg b.w. for 7 days; researchers detected occurrence of micronucleuses in the bone marrow and liver cells. Both MPs and NPs caused an increase in mitotic index in glandular mucous tunic of the stomach and large intestine epithelium. NPs introduction also caused apoptosis of cells in the stomach mucous tunic and multi-nucleus spermatids occurrence in the testicles [65]. A 5-day TiO_2 NPs introduction into mice in a dose equal to 5-500 mg/kg b.w. led to apoptosis, DNA fragmentation, and mutations in gene p53 exons together with biochemical signs of oxidative stress in the stomach mucous tunic [37]. Anatase NPs introduction into Wistar rats (100-200 mg/kg b.w., 60 days) caused various disorders in erythrocytes system in blood, including occurrence of cells with a micronucleus, together with damage to chromosomes in bone marrow cells and DNA fragmentation revealed via a comet assay [66]. A micronucleus test performed on lymphocytes culture revealed genotoxicity of anatase NPs in their concentration being equal to 1.6 μg/ml, while MPs became genotoxic only in concentrations equal to 40 μg/ml and higher [67].

There were some data on TiO_2 NPs having no genotoxicity; they were obtained via an experiment on male Sprague-Dawley rats exposed to anatase NPs (10-200 mg/kg b.w., 30 days). No chromosome aberrations or mitosis disorders in bone marrow cells were revealed under those experimental conditions [68]. Rats were exposed to a single introduction of 6 various NPs and MPs types in doses equal to 500-2,000 mg/kg b.w. and there was no increase in number of cells with a micronucleus in their bone marrow and blood reticulocytes [69]. And finally, according to Martins [et al.] [44], there were no genotoxicity signs revealed in male Wistar rats after a 45-day exposure to NPs in a low dose (0.5 mg/kg b.w.).

Therefore, data on TiO_2 NPs genotoxicity are rather controversial at present. Effects do not directly correlate with particles size and introduction duration, or a type of experimental animals, and it indicates that further research on the subject is required.

3. Immunotoxicity

When considering specific or remote effects produced by oral TiO_2 NPs introduction, one should linger on their interaction with the immune system. Tassinari [et al.][34] reported there were disorders in white pulp structure in the spleen in female (but not male) Sprague-Dawley rats after a 5-day intragastrical introduction of anatase NPs (1-2 mg/kg b.w.). These disorders were also accompanied with changes in morphology of the thyroid gland, adrenal cortex, and ovaries; in the authors' opinion, it indicated that there was a systemic endocrine disorder developing in experimental animals.

Male Wistar rats were immunized with chicken ovalbumin during a 28-day experiment and then exposed to rutile NPs in a dose equal to 100 mg/kg b.w. It resulted in lower number of immature cells and B-lymphocytes and growing phagocytic activity of neutrophils in peripheral blood. IgG antibodies level increased in animals exposed to NPs and the authors thought it to be accelerating B-lymphocytes maturing into plasmatic cells caused by this exposure [70].

Anatase NPs were introduced into mice during 90 days in a dose equal to 2.5-10 mg/kg; it intensified lymphocytes apoptosis, caused greater production of macrophage inflammation factors MIP-1α, MIP-2, Eotaxin, MCP-1, as well as IFN-γ, VCAM-1, IL-13, IFN-inducible protein-10, greater expression of CD69, tyrosine protein kinase and phosphatase, basic growth factor for fibroblasts, Fasl and GzmB with simultaneous decrease in expression of NKG2D, NKp46, and 2B4 [33]. The same model was applied to demonstrate necrosis and oxidative stress developing in the spleen, expression of cyclooxigenase-2 with simultaneous growth in production of prostaglandin PGE2, higher levels of mRNA for ERK, AP-1, CRE, Akt, JNK2, MAPKs, PI3-K, and c-Junи c-Fos [32]. The authors also reported there was an increase in TNF-α, macrophage migration inhibitory factor, IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, IL-1β, cross-reaction protein, TGF-β, expression of Bax and CYP1A1 together with suppression of Bcl-2 и HSP-70 in blood of the experimental animals [31].

30-day experiments that were similar in their procedure allowed revealing spleen edema in experimental mice accompanied with reinforced lipid peroxidation and expression of heme oxygenase via p38-Nrf-2 signal pathway [71].

4. Reproductive toxicity

There are experimental data, although rather few, on impacts exerted by orally introduced TiO_2 NPs on mammals' reproductive system. Male Kunming mice were introduced these NPs which were smaller than 50 nm in size during 52 days in a dose up to 250 mg/kg b.w. The exposure caused greater number of anomalies in sperm in the testicles and a decrease in number of sperm cells and bubbles in testicular tubes. There was a simultaneous decrease in circulating testosterone level and expression of 17β-hydroxysteroid dehydrogenase in the testicles [72]. Wistar rats were orally exposed to NPs in a dose equal to 50 mg/kg b.w. for 2 or 3 weeks and it resulted in greater expression of γ-glutamyl transferase and a decrease in c-kit and steroidogenic acute regulatory protein (StAR). Overall number of developing sperm cells reduced but a number of ones with defects only grew. There were signs of oxidative stress in the prostate and testicles, decrease in reduced glutathione contents, increase in TNF-α concentration, greater expression of Fas, Bax and caspase-3 together with falling Bcl-2 contents. Hormonal disorders included higher levels of gonadotropin and estradiol and lower testosterone concentration [73].

Pregnant female mice were exposed to TiO_2 NPs during 17 days (during pregnancy) in a dose equal to 25-100 mg/kg b.w.; it led to skeletal anomalies in fetuses with signs of cartilage being underdeveloped and lower ossification. There was a growth in number of fetuses with dysplasia; there were fetuses with exencephaly, spina bifida, twisted tales, scoliosis, underdeveloped ribs and breast bone [40].

But as opposed to the above-mentioned work, D.B. Warheit [et al.] introduced 6 types of TiO_2 NPs and MPs into pregnant female Wistar rats in a dose up to 1,000 mg/kg b.w. and didn't detect any anomalies in fetuses [74]. A hypothesis on reproductive toxicity TiO_2 NPs might require further research.

5. Neurotoxicity

There has been no reliable evidence on probable translocation of orally introduced TiO_2 NPs into the brain so far; still, some research shows that their impacts lead to functional disorders in the CNS. Thus, when ICR mice were introduced NPs during 60 days in doses 5-50 mg/kg b.w., researchers detected changes in their behavioral reactions and spatial recognition memory. NPs inhibited activity of metal-dependent ATPase (ion pumps for Na^+/K^+ и Ca^{2+}/Mg^{2+}), acetyl cholinesterase and nitrogen oxide synthase in the brain; There were disorders in metabolism of dopamine, norepinephrine, and their metabolites. According to the authors, detected changes could be related to shifts that occurred under exposure to NPs in mineral substances status, including contents of Ca, Mg, Na, K, Fe and Zn in the brain [16]. Probable effects produced by TiO_2 NPs on metabolism of dopamine and norepinephrine in the brain cortex were also mentioned by Shrivastava [et al.] [35] who performed their experiments on mice. There was a single introduction of NPs in a dose

500 mg/kg b.w. A significantly lower dose, 2.5-10 mg/kg b.w., introduced into mice for 90 days, caused grave morphological changes in the brain, loss of spatial orientation, weaker long-term memory together with a decrease in expression of NR2A sub-units, B-receptor of N-methyl-D-aspartate (NMDA), inhibited expression of CaMKIV, CREB-1, CREB-2 and FosB/DFosB in the hippocampus [36]. Very small anatase NPs (5-10 nm) introduced into Wistar rats in doses 50-200 mg/kg b.w. during 60 days inhibited acetyl cholinesterase activity in the CNS and caused greater IL-6 production by glia tissue. There was a growth in accumulation of glial fibrillar acidic protein GFAP in the brain cortex [75].

6. Carcinogenicity

Inhaled TiO_2 NPs are likely to be carcinogenic as IARC* assigns them into 2B group (substances that are presumably carcinogenic for humans) [76]. However, probable carcinogenic effects produced by these NPs when they are introduced into the gastrointestinal tract have not been examined in great details. There was a single research work [77] when TiO_2 coloring agent which was a mixture of rutile and anatase NPs and MPs was introduced as a part of ration (2.5–5% of total mass) into Fischer 344 rats and B6C3F142 mice during 103-104 weeks. There was a slight growth in frequency of tumors (adenoma and carcinoma) with various localization in female rats exposed to high NPs doses with a probability of the zero hypothesis being accepted p=0.043; it wasn't sufficient to substantiate carcinogenic effects according to Bonferroni criteria. Therefore, the authors didn't obtain any compelling data that TiO_2 could have carcinogenic effects when introduced into the gastrointestinal tract.

7. Effects produced by titanium dioxide in the intestine lumen

When we discuss probable reasons and mechanisms related to obvious oral toxicity of TiO_2 NPs, we should dwell on its effects in the gastrointestinal tract lumen; to become apparent, these effects do not necessarily require system translocation. We should linger on the following aspects: impacts exerted by NPs on intestinal uptake, interaction between NPs and mucous tunics in the gastrointestinal tract and gut-associated lymphoid tissue (GALT) in particular, and their influence on intestinal microbiome as well.

TiO_2 NPs didn't exert any impacts on fat acids absorption by intestinal cells in an acute experiment. However, in a chronic experiment researchers detected a considerable decrease in their absorption in the gastrointestinal tract. NPs are known to be able to influence mineral substances absorption in the gastrointestinal tract. First of all, NPs penetrate a food product matrix and form complexes with proteins, fats, and carbohydrates thus creating a "crown" on its surface. These changes in a product surface can influence nutrients biological availability, their solubility, and recognition by structural elements in a body that are responsible for intestinal uptake of nutrients; as a result, all these changes influence NPs toxicity [78].

Koeneman [et al.] applied *in vitro* model for Caco-2 cells monolayer and showed that NPs introduced in concentration equal to 10-100 μg/ml didn't cause any cells death, morphological disorders in micro-villi, or adhesion contacts failure [24]. Rutile NPs introduced in a concentration equal to 100 μg/ml didn't produce any effects on Caco-2 cells monolayer as it was shown by M. Fisichella [et al.] [79]. Similar results were obtained by M.R. Jo [et al.] [80] who performed comparative analysis of impacts exerted by much larger MPs (117 and 153 nm) on Caco-2 cells layers and rats' small intestine *in vivo*. G.E. Onishchenko [et al] [27] didn't detect any ultrastructural changes in erythrocytes of rats' ileum under intraintestinal introduction of rutile NPs.

Still, there are some research works where TiO_2 NPs ability to produce certain effects on the intestinal epithelium is described. For example, there was a research work [38] that described an effect produced by NPs, namely, an increase in villi length in small intestine mucous tunics of rats orally exposed to them. There was also an experiment on Caco-2 cells

* International Agency for Research on Cancer.

when researchers observed morphological disorders in the brush border under exposure to pigment TiO_2 (a mixture of 25% NPs and 75% MPs) in a low concentration (lower than 0.35 μg/ml) [81].

All the results obtained in the above-mentioned works are related to effects produced by NPs on "absorptive" epithelium of intestinal mucous tunics. However, when NPs interact with GALT of payer's plaques, a totally different effect can occur. Thus, when male C57Bl/6J mice were exposed to MPs (260 nm) and NPs (66 nm) in a dose equal to 100 mg/kg b.w. for 10 days, researchers detected an increase in CD4+ number in lymphoid tissue of the duodenum, empty intestine, and the ileum. There was an increase in production of IL-4, IL-12, IL-23, TNF-α, IFN-γ and TGF-β by Peyer's plaque cells against the control group [82]. The above-mentioned anti-inflammatory cytokines are known to act systemically; taking this into account, we can state that this single result is of great interest when toxic effects produced by TiO_2 NPs are explained as they have extremely low biological availability and absorbability in the gastrointestinal tract. However, to confirm these data, further research is required.

As per data provided by J.J. Faust [et al.] [83], impacts exerted by TiO_2 NPs in a chronic experiment caused a significant damage to barrier functions performed by intestines. The process was accompanied with active oxygen forms occurrence, intensified inflammation, and alkaline phosphatase being more active. There was also a decrease in transport of iron, zinc, and fat acids through intestinal mucous tunics due to weaker absorbing capacity of epithelial cells in the brush border after exposure to TiO_2 nanoparticles.

A possibility in principle that TiO_2 NPs can have mediated effects on a human or an animal body through influencing a structure and functions performed by components of the intestinal microbiome becomes obvious due to multiple data on biocide effects produced by these NPs on various groups of microorganisms *in vitro* (an overview of basic results is given in the work [84]). Besides, it was determined that NPs could increase pathogenic properties of opportunistic micro-pathogens which are parts of the intestinal microbiota [85]. And it is important that growth of various microorganisms or their groups is not inhibited in the same way and it can result in a nanomaterial interfering significantly with a subtle adjustment of symbiotic and competitive relations between multiple components in the microbiome. However, there are few data on direct ability of TiO_2 NPs to influence intestinal microbiocenosis under natural conditions.

Preparations containing anatase and rutile NPs as well as pigment TiO_2 were introduced into make Wistar rats during 30 days in doses equal to 1 and 100 mg/kg b.w. [86]. Under those conditions, exposure to both low and high doses led to an increase in quantity of hemolytic and common streptococci and staphylococci and a decrease in quantity of bifid bacteria. Changes in the immune background in animals became apparent via an increase in IL-10 production. These detected changes were not related to a crystal form or sizes of particles and were detected after exposure both to NPs and MPs. W. Dudefoi [et al.] [87] applied both MPs and NPs in their research and revealed that there were minimal changes in the microbiome state *in vitro* in gasification test and production of fat acids C16:0, C18:0, cisC15:1w5 and cisC18:1w9c under exposure to NPs in concentration being equal to 0.1-0.25 mg/ml; but there was a change in microbe populations structure as quantity of bacteroids dropped but that of *Clostridium sp* increased. Mice were orally exposed to anatase and rutile NPs during 28 days and it resulted in gradually developing changes in the intestinal microbiome including such phylums as *Proteobacteria, Prevotella, Rhodococcus* and *Bacteroides*. Intensity of an effect was different for two crystal forms of NPs and was not accompanied with apparent morphological changes in intestinal walls [88]. We can conclude that searching for changes in a structure and functional activity of the intestinal microbiome caused by exposure to TiO_2 NPs is a promising research trend that can allow establishing mechanisms of biological ef-

fects produced by this poorly absorbed nanomaterial; however, research works in the field are rather scarce.

8. Meta-analysis of data on toxicity

Therefore, we analyzed data from 64 sources (published in 2007-2019); all the analyzed data are scientifically authentic and complete according to MG 1.2.2522-09 (publications in reviewed scientific journals that contain detailed description of a research object and applied biological model; are accomplished with quantitative techniques; contain statistically authentic results; and are not questioned in later publications). Distribution of all the analyzed research works as per years of their publication is given in Figure 1a. Applied biological models included a single oral introduction (15 works), multiple sub-acute introduction (42), and chronic introduction (1) into female rats and mice from different lines as well as experiments *in vitro* on monolayers of intestinal epithelium cells and cultures of the gastrointestinal microbiota. 50 articles focused on TiO_2 NPs; 3 articles, on MPs; and 9 works, MPs and NPs being compared. 35 works out of the overall number contained data on adverse (toxic) effects produced on a body by TiO_2 doses varying from 0.1 to 1,000 mg/kg b.w.; 6 works contained no data on toxicity; data obtained in 27 works didn't allow unambiguous assessment of LOAEL, or a focus was on bioaccumulation only. Basic target organs were the liver (17 works); the gastrointestinal tract (9 works); the spleen and immune system organs (8 works); as well as the heart, kidneys, and brain. Special attention should be paid to data on effects produced by

TiO_2 (both in nano- and micro-form) on a structure and biological properties of microorganisms in the intestinal microbiota; such effects were revealed in 4 research works. Distribution of publications as per assessed LOAEL is given in Figure 1b.

As we can see from the data presented above, LOAEL was 10 mg/kg b.w. and even lower in 10 out of 41 revised publications; it roughly corresponds to a 25-percentile of the overall number of the analyzed sources. Remarkably, all works that reported absence of any toxic properties possessed by TiO_2 NPs were predominantly performed with introducing extremely high doses of the nanomaterial (1,000 mg/kg b.w. and even more) into experimental animals. It can possibly indicate that authors obtained false negative results due to NPs aggregation [22]. We should also note that toxic effects were less frequently observed after an acute (single) introduction of the nanomaterial into animals than after sub-acute one (30-90 days). Therefore, we can be almost certain to conclude that TiO_2 NPs, both anatase and rutile ones, when introduced in a dose not higher than 10 mg/kg b.w. can produce adverse effects on a body. Obviously, there are organs and tissues in a body that are primary targets for them, first of all, the spleen and immune system as well as mucous tunics in the stomach and small intestine, the liver, and the brain. Thus, NOAEL for TiO_2 in its nanoform is less than 10 mg/kg b.w. that is qualitatively consistent with previously performed assessments that were based on much smaller number of sources [14].

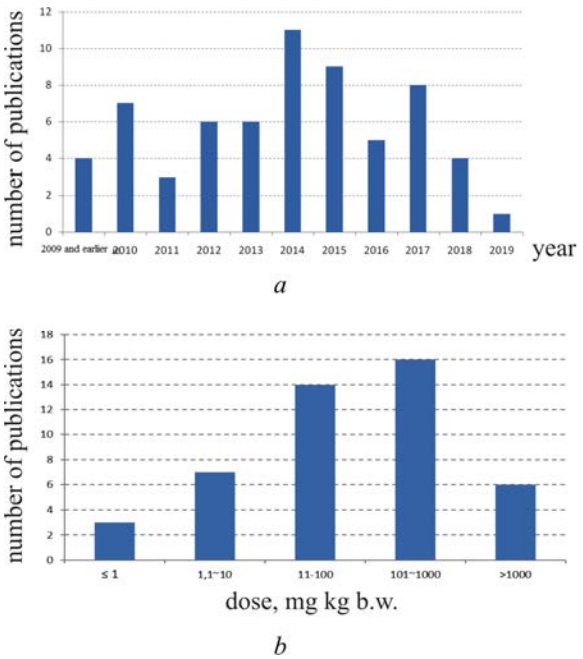

Figure 1. Distribution of publications (quantity) on oral toxicity of titanium dioxide nanoparticles
a) as per a year when they were published,
b) as per assessed LOAEL

9. Risk assessment

Non-carcinogenic risk caused by impacts exerted by a nanomaterial on a human body is estimated according to MG 1.2.0038-11 "Assessment of risks caused by impacts exerted by nanomaterials and nanoparticles on a human body"[6] via calculating a hazard quotient as per the following formula:

$$HQ = E_d/RfD$$

where RfD is a safe reference dose that is given in mg/kg b.w. * day; and E_d is assessed exposure in the same units. As we can see from Table 1, a dose equal to 1 mg/kg a day is a realistic estimation for TiO_2 consumption as a food additive E171 by most people. And also, as it was shown in the Section 8, NOAEL for TiO_2 in its nanoform was not higher than 10 mg/kg b.w. a day. If we want to determine RfD basing on this value, we should introduced two 10-fold assurance factors; the first one allows for uncertainty (discrepancies in experimental data obtained from various publications), and the second one is a similarity coefficient that is applied when data obtained via experiments on small animals (laboratory rats and mice) are transferred onto a human body. Therefore, RfD for TiO_2 in its nanoform should be equal to not more than 0.1 mg/kg b.w. a day

Table 2 contains results of assessing risks caused by oral TiO_2 NPs introduction taking into account 4 possible scenarios of NPs contents in E171. The first scenario is optimistic and means that NPs account for not more than 0.3% of the overall mass of a sample as it was shown in [2, 22]; the last one is extremely pessimistic and means that the overall TiO_2 sample was a nanomaterial.

These data prove that application of TiO_2 that contains NPs in an amount higher than 1% of an overall mass can result in such effects produced on a human body that cause unacceptable health risks.

10. Conclusion and recommendations

So, all the available literature data allow us to conclude that TiO_2 NPs in their two most widely spread forms (anatase and rutile) exert various adverse effects on a body both after a single introduction into the gastrointestinal tract (an acute experiment) and after multiple ones. The most frequent effects are produced on the liver as it becomes obvious via damage to liver tissues, oxidative stress signs, changes in biochemical parameters of blood plasma, as well as via shifts occurring in proteome and trascriptome of the organ. The immune system is another target, the bone marrow and spleen in particular, where researchers detected changes in genetic apparatus of cell nucleus, shifts in production of anti-inflammatory cytokines and growth factors. There were also signs that NPs had neurotoxicity and reproductive toxicity; however, LOAEL in those cases, as a rule, was substantially higher than 10 mg/kg b.w. Any toxic effects produced by TiO_2 NPs were not revealed in relatively small number of works. A reason for this can be extremely high doses of nanomaterials chosen by their authors; massive NPs aggregation that occurs in such a situation can be a factor that imposes certain limitations on biological effects occurrence. There is a well-known contradiction between various toxic effects produced by TiO_2 in its nanoform and data on the substance being obviously poorly absorbable and therefore poorly biologically available in the gastrointestinal tract. A key insight into this paradox can obviously be provided by data contained in relatively few works on effects produced by

Table 2

Assessment of risks caused by titanium dioxide as a component of E171 under oral exposure

No.	Scenario (NPs contents, % in overall mass)	Hazard quotient (HQ)	Risk (assessment)
1	0.3	0.03	Acceptable
2	1.0	0.1	Acceptable
3	10	1.0	Unacceptable
4	100	10.0	Extremely unacceptable

[6] MG 1.2.0038-11. Assessment of risks caused by impacts exerted by nanomaterials and nanoparticles on a human body [web-source] // KODEKS: an electronic fund of legal and reference documentation. – URL:http://docs.cntd.ru/document/1200097700 (date of visit March 23, 2019).

NPs on the intestinal microbiome. Impacts exerted by NPs can intensify pathogenic properties of opportunistic micro-pathogens that are part of the intestinal microbiome. Disorders in quantitative and qualitative structure of intestinal microflora can, in its turn, lead to functional disorders in intestinal lymphoid tissue (GALT), acid balance in the intestinal lumen, inflammatory cells proliferation, and putrefaction. Such impacts can cause significant shifts in production of pro- and into-inflammatory cytokines, and absorption of nutrients and biologically active metabolites in microflora; all these impacts combined can explain a lot of observed systemic effects produced by nano-sized TiO_2. However, getting better insight into these significant peculiarities related to effects produced by nano-sized TiO_2, will require further research; by analogy, it can be applied to other nanomaterials that are poorly absorbed or not absorbed at all under oral introduction (silicon oxide NPs, silica-alumina clays, and carbon nanotubes).

We assessed risks caused by TiO_2 NPs that could penetrate a body with food, cosmetics, or medications, basing on literature data on population exposure to all TiO_2 forms as a food additive E171 and experimental determination of LOAEL/NOAEL. The assessment revealed that a risk depended on a scenario for NPs contents in a product. In particular, when NPs contents amounted to 10% and more out of a total mass, a risk could become unacceptable. Given that, we can conclude that it is necessary to regulate and control nano-sized material contents in E171 that is applied in food industry. To do that, the following measures seem advisable.

1. A specification that describes E171 food additive should be revised; it should impose a limitation on mass contents of a nano-sized component in it that is not to exceed 1%.

2. When assessing conformity of a product that contains E 171, an applicant should provide obligatory data on its particles size distribution.

3. There should be new sanitary-epidemiologic surveillance techniques developed and implemented into every day practice; these techniques should allow estimating TiO_2 particles size, both in pure E17 samples and in food products containing it.

4. Application of E171 should be strictly prohibited by law in food products for preschool children, pregnant women and feeding mothers, as well as in specific food products aimed for preventive and therapeutic diets.

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LIPID ACCUMULATION PRODUCT OR LAP AS AN UP-TO-DATE CLINICAL BIOCHEMICAL MARKER OF HUMAN OBESITY

A.M. Kaneva, E.R. Bojko

Institute of Physiology, Komi Science Centre, the Ural Branch of the Russian Academy of Sciences,
50 Pervomaiskaya Str., Syktyvkar, 167982, Russian Federation

Obesity is a grave medical and social problem causing significant hazards for human health due to frequent occurrence of grave concomitant diseases. Visceral obesity is considered to be the most hazardous. Contemporary diagnostics which allows to reveal visceral obesity both during screening examinations of people who are considered to be conditionally healthy and in patients who suffer from cardiovascular diseases is becoming a very promising trend both in primary and secondary prevention.

The review focuses on an up-to-date marker used to detect visceral obesity, namely, lipid accumulation product or LAP. This index was first introduced by Kahn H.S. in 2005 and is calculated on the basis of two variables, waist circumference (cm) and fasting concentration of triglycerides (mmol/l). When a biochemical and an anthropometric parameter are applied simultaneously to calculate LAP, it allows not only to assess how fats are distributed in a body but also to reflect functional state of fat tissues.

LAP index is widely used as a marker showing there are metabolic disorders in a body (metabolic syndrome, diabetes, resistance to insulin, or nonalcoholic fatty liver disease) and as a cardiovascular diseases predictor. Besides, LAP index, when used to identify obesity phenotype, allows to assign people with overweight into a "metabolically healthy" category or "metabolically ill" one and to reveal patients suffering from metabolic obesity among people with normal body weight. A lot of research revealed that LAP index had a very good diagnostic and predictive potential as regards metabolic and cardiovascular diseases and was a more precise marker of cardio-metabolic risks than conventional anthropometric parameters. The review highlights LAP value ranges, its sex and age peculiarities, as well as a character and an extent to which LAP values change in case of certain diseases. It also dwells on advantages and drawbacks of LAP practical application.

Key words: *lipid accumulation product (LAP), waist circumference, triglycerides, body mass index, obesity, metabolic syndrome, cardiovascular diseases.*

The concept “lipid accumulation product” (LAP) was first mentioned in studies by Kahn H.S. [1, 2] where it was considered as a marker of excessive lipid accumulation in adults. LAP index calculation is based on two simple parameters, waist circumference and triglycerides contents in blood plasma on a fasting. Suggested formulas for LAP index calculation are as follows [3]:

$$\text{LAP for men} = (\text{WC} - 65)*\text{TG}$$

$$\text{LAP for women} = (\text{WC} - 58)*\text{TG},$$

where WC is waist circumference (cm), TG are triglycerides (mmol/l), 65 and 58 are minimal waist circumference for men and women obtained via population research.

A wish to implement LAP index into practices is based on previously published researches [4, 5] when it was determined that increased waist circumference together with elevated triglycerides contents indicated there was a risk of metabolic diseases. However, waist circumference and triglycerides contents were considered to be dichotomous parameters (standard and above standard) in these researches, whereas LAP index calculation allows to obtain qualitative (continuous) values and it gives wider possibilities for analytical processing and interpretation of all obtained data.

LAP index simultaneously describes anatomic and biochemical changes related to excessive lipids accumulation in adults [3].

Anastasiya M. Kaneva – Doctor of Biological Sciences, Chief researcher at the Ecological and Medical Physiology Department (e-mail: amkaneva@mail.ru; tel.: +7 (8212) 24-14-74; ORCID: https://orcid.org/0000-0002-7789-4300).

Evgenii R. Bojko – Doctor of Medical Sciences, Professor, Director (e-mail: boiko60@inbox.ru; tel.: +7 (8212) 24-14-74; ORCID: https://orcid.org/0000-0002-8027-898X).

An increase in fats contents in a body usually leads to obesity. Obesity is excessive body weight and it is usually assessed as per body mass index (BMI). However, BMI, being a ratio of a person's weight to his or her height, doesn't take into account somatotype, a ratio between fat and muscle weight in a body, and fat tissue distribution; all that significantly decreases its value for an objective overweight evaluation. Thus, a study that was performed on 1,400 people allowed to detect significant discrepancies between results of determining obesity with BMI and x-ray absorptiometry [6].

Unfavorable outcomes that obesity might have are less related to overall fat contents in a body than to peculiarities of its distribution. Basically, there are three types of fat tissue: subcutaneous fat, subfacial fat, and visceral fat. Excessive fat accumulation in the upper part of a body and on the stomach leads to abdominal obesity. In its turn, abdominal obesity is divided into visceral obesity and subcutaneous-abdominal one. Abdominal obesity is thought to be the most hazardous; it usually develops due to excessive increase in visceral fat contents. When such obesity occurs, excessive visceral fat exerts pressure on internal tissues, causes functional disorders in them, and deteriorates blood circulation and lymph flow. Besides, visceral fat tissue differs from subcutaneous fat as per adipocytes type, their endocrine function, lipolytic activity, and sensitivity to insulin and other hormones. Visceral fat tissue, due to its high lipolytic activity, promotes excessive input of free fat acids in big quantities into portal veins and peripheral blood flow; it, in its turn, causes hypertriglyceridemia and atherogenic dyslipidemia, resistance to insulin, hyperglycemia, and hyperinsulinemia [7, 8]. The simplest way to indirectly determine abdominal obesity is to perform anthropometric waist circumference measurement. However, waist circumference measurement doesn't allow determining abdominal obesity type [9, 10], while combine application of waist circumference and triglycerides contents in a formula for LAP index calculation gives an opportunity to apply this index as a marker of metabolic disorders, abdominal-visceral obesity and related diseases.

LAP index in widely used as a marker showing metabolic syndrome [11, 12], resistance to insulin [13, 14], diabetes [15], non-alcoholic fatty liver disease [16, 17], hormonal disorders [18, 19], as well as risk of hypertension [20, 21], stroke [22], renal dysfunction [23, 24], and cardiovascular diseases [25]. The index is also successfully applied to identify people who run high metabolic risks in case of certain diseases (psoriasis, polycystic ovarian disease, and hyposomatotropism) [18, 26, 27]. Besides, researchers revealed a correlation between LAP index and blood lipid profile atherogenicity and lipoproteins sizes [28], blood viscosity [29], blood pressure parameters [30, 31], left ventricle geometry [32], thyreotrophin concentration [33], alcohol intake [34, 35], smoking [36], physical activity [37, 38], alanine aminotransferase level [39], inflammatory biomarkers, and adiponectin [40], and overall mortality as well [41].

LAP index values vary within a wide range. Among population studies, the greatest LAP index variations were detected among Iran population (from 0.62 to 570.26 cm.mmol/l in men (n=3,682) and from 0.56 to 620.39 cm.mmol/l in women (n=4,989)) [42]. Significant variations in LAP index values were also detected among people living in Kenya (from 0 to 388.87 cm.mmol/l in men (n=255) and from 3.30 до 205.54 cm.mmol/l in women (n=273)) [43]; among elderly males in Poland (aged 50–75, n=313) (from 7.36 to 338.97 cm.mmol/l) [19]; and among young women (younger than 40, n=2,810) in Korea (from 0 to 252.0 cm.mmol/l) [44]. However, a significantly wider range of LAP index variations is detected when it comes to patients with cardiovascular pathologies (0.8–1,020 cm.mmol/l in men (n=3,604) and 0.7–1,020 cm.mmol/l in women (n=2,320) respectively) [41].

Table 1

Comparative assessment of LAP index among men and women as per literature data

References	Country	LAP index (cm.mmol/l)				p
		Men		Women		
		M±SD or Me (25%; 75%)	n	M±SD or Me (25%; 75%)	n	
Xia C. et al., 2012 [13]	China	25.96 (14.2; 41.97)	1,510	23.99 (13.09; 40.12)	1,014	0.074
Omuse G. et al., 2017 [43]	Kenya	29.52 (40.95) #	255	23.97 (29.69) #	273	0.055
Wakabayashi I., 2014 [47]	Japan	23.7 (12.1; 44.0)	35,684	16.4 (8.9; 30.1)	18,793	<0.01
Chen Y. et al., 2018 [33]	China	26.69 (34.72)	3,786	22.88 (29.46)	4,941	<0.001
Tripolino C. et al., 2017 [29]	Italy	42.7±28.5	193	29.1±16.1	151	<0.001
Abulmeaty M.M. et al., 2017 [46]	Saudi Arabia	62.17±54.64	167	77.37±60.78	223	0.044
Chiang J.K. et al., 2012 [48]	Taiwan	23.0±23.2	266	28.6±19.3	247	0.001
Wang H. et al., 2018 [32]	China	21.95 (10.71; 43.60)	5,179	28.31 (16.17; 50.95)	6,079	<0.001
Motamed N. et al., 2016 [45]	Iran	47.34±45.55	3,281	58.73±52.86	2,516	<0.001

Note: data are given either as a mean and standard deviation (M±SD), or median and 25% and 75% percentiles (Me (25%; 75%)); # means data are given as median and interquartile spread.

According to some research, LAP index values tend to be higher in men than in women [29, 33], whereas other studies, on the contrary, contain data on higher LAP index values to be more typical for women [45, 46] (Table 1). Some authors state there are no age-related differences in LAP index values [13, 43].

Bearing in mind that the above data differ greatly and are mostly inconsistent, it is impossible to reveal any age-related peculiarities in LAP index changes as per results obtained in various research. Meanwhile, there are only few studies that focus on age dynamics of LAP index changes; besides, they are significantly different as per researches design. I. Wakabayashi [47] performed his research on men (n=35,684) and women (n=18,793) who were divided into four age groups (aged 35–39, 40–49, 50–59 and 60–70). It was detected that LAP index increased with age among women, while its maximum values were detected among men aged 40-49 and then they went down. This ambiguous age dynamics of parameters among men and women resulted in LAP index values being lower among women than among men in younger age groups, but higher in older ones. H.S. Kahn [et al.] [49] gave average LAP index values for two age groups (18–44 and 45–64) that amounted to 28.8 (15.6; 56.6) and 50.2 (30.2; 84.9) cm.mmol/l in men, and 20.8 (11.5; 38.4) and 47.6 (25.3; 82.8) cm.mmol/l in women. Similar age-related dynamics of LAP index values was detected in studies by S.H. Fu [et al.] [50], and H. Joshi [et al.] [51]. Average LAP index among people living in China who were younger than 60 (n=694) amounted to 35.92 (20.33; 61.74) cm.mmol/l; and they significantly grew in people older than 60 (n=846) (p=0.001), up to 41.55 (25.90; 66.13) cm.mmol/l [50]. LAP index amounted to 21.43±14.34 cm.mmol/l in young people (younger than 40, n=1,180) living in India, and it was equal to 31.42±18.11 cm.mmol/l among those older than 40 (n=1,003) [51]. Therefore, basing on the results of all the researches outlined above, we can come to a conclusion that LAP index values, as a rule, tend to increase with age. Obviously, it is due to a fact that waist circumference and triglycerides contents applied for LAP index calculation also tend to go up with age.

A significant number of research that dwells on correlations between LAP index and various diseases focuses on metabolic syndrome. Researchers determined a diagnostic potential (sensitivity, specificity, positive and negative predictive value) of LAP index that could be applied to predict the disease and detected that it was an "ideal" marker to identify metabolic syndrome [45, 48, 52, 53]. Some studies revealed that LAP index predicted metabolic syndrome with greater precision in comparison with anthropometric measurements (BMI or waist circumference) [11, 13, 45, 54]. Nevertheless, some researchers recommend not to overestimate LAP index predictive value as regards metabolic syndrome. When it is calculated, waist circumference and triglycerides contents are applied, but they are also key components in metabolic syndrome and it can determine an internal relation between the index and the disease [55]. Besides, there are data on absence of any advantages LAP index might have against anthropometric parameters for metabolic diseases prediction [56, 57].

Diagnostic precision of LAP index seems to depend on age and sex. Some researchers revealed a greater correlation between LAP index values and risks of diseases among women than among men [47, 58]. Diagnostic precision of LAP index was significantly higher for young people of both sexes than for elderly ones when diabetes and non-alcoholic fatty liver disease were identified [17, 47]. On the contrary, LAP index precision as visceral obesity marker was higher among elderly people [59].

Despite LAP index is widely applied nowadays to assess cardiovascular and metabolic risks, there is no any conventional standard for this index. Researchers, as a rule, calculate optimal threshold LAP index values to predict diseases development basing on ROC-curves (Receiver Operator Characteristic). This procedure allows to determine a threshold value that is the most adequate point for separating one diagnosed group from another. However, optimal threshold LAP index values obtained for each particular disease are different in different studies; it is due to examined populations being different as per sex, age, ethnic groups, and health state of examined people.

Considerable variations are also observed for average LAP index values for different diseases (Table 2).

LAP index is rarely used in Russia. There was a large-scale study entitled "Epidemiology of cardiovascular diseases and risk factors causing thin in the RF regions" (ESSE-RF) that involved LAP index calculating. The study was accomplished in 2012-2014 in 13 regions in Russia by Drapkina O.M. et al, and Shal'nova S.A. et al. There were also some clinical studies (Khripun I.A. et al.; Kornoukhova L.A.). According to data collected via ESSE RF that comprised 20,878 people aged 25-64 (8,058 men and 12,820 women), average LAP index values amounted to 45.5±0.88 and 35.4±0.31 cm.mmol/l among men and women respectively who didn't have ischemic heart disease or pancreatic diabetes [63–66].

LAP index has an advantage over other anthropometric parameters as it provides a possibility to identify obesity phenotype. Thus, LAP index application allows to divide people with overweight into "metabolically healthy" and "metabolically ill" [67]. At the same time, LAP index enables detecting people with metabolic obesity among those with normal body weight [68].

If a person is healthy but still has metabolic obesity, then excessive fat accumulation in him or her doesn't lead to unfavorable metabolic effects such as resistance to insulin, disrupted tolerance to dextrose, dyslipidemia, or primary hypertension. As per literature data, prevalence of metabolically healthy obesity in Europe varies from 10% to 45% among people with obesity [69]. Precise diagnostics of obesity phenotype allows to make a correct choice on a set of therapeutic activities aimed at helping a patient to lose weight. At the same time, early detection of

Table 2

Average LAP index values for different diseases as per literature data

References, disease	Country	Sex	LAP index (cm mmol/l)			
			Control		Test	
			M±SD or Me (25%; 75%)	n	M±SD or Me (25%; 75%)	n
Li R. et al., 2018 [12]; metabolic syndrome	China	m+f	21.58±15.04	617	53.47±30.22	375
Cheng Y.L. et al., 2017 [60]; metabolic syndrome	Taiwan	m+f	23.61±18.57	21,233	64.22±43.52	8,564
Mazidi M. et al., 2018 [14]; resistance to insulin	The USA	m+f	53.3±0.6	17,403	127.2±0.5	915
Kavaric N. et al., 2018 [57]; II type pancreatic diabetes	Monte Negro	m+f	44.34 (38.14; 51.53)	119	82.87 (76.69; 90.73)	180
Dai H. et al., 2017 [17]; non-alcoholic fatty liver disease	China	m/f	23.7±22.0 16.6±15.5	10,266 18,043	62.4±59.7 49.4±41.2	8,070 4,080
Wang H. et al., 2017 [61]; ischemic stroke	China	m/f	35.24 (21.78; 42.93) 40.19 (15.93; 49.49)	5,087 5,904	43.49 (29.76; 50.27) 65.21 (31.51; 88.64)	166 188
Li R. et al., 2017 [58]; atherosclerotic stenosis of intracranial vessels	China	m/f	31.73±36.73 37.03±25.65	339 411	32.83±28.74 45.99±37.85	56 39
Wang H. et al., 2018 [21]; hypertension	China	m/f	17.10 (8.45; 34.35) 21.79 (12.86; 38.13)	2,326 3,130	27.46 (13.98; 51.38) 37.27 (21.78; 64.04)	2,732 2,952
Rashid N. et al., 2017 [62]; polycystic ovarian disease	India	m/f	25.77±14.13	45	40.37±22.17	95
Ganguly S. et al., 2018 [26]; psoriasis	India	m+f	23.79±13.02	42	46.42±27.2	40
Chan L. et al., 2016 [18]; hyposomatotropism	China	m+f	21.30 (10.35; 32.48)	75	43.93 (18.74; 69.31)	75

Note: data are given either as a mean and standard deviation (M±SD) or median and 25% and 75% percentiles (Me (25%; 75%)).

"metabolically unhealthy people" with normal body weight can make prediction and prevention of diabetes and cardiovascular diseases much more efficient.

So, LAP index is a marker that reflects joint anatomic and biochemical changes that are related to excessive lipid accumulation in a body. It is an appropriate instrument to detect predisposition to metabolic and cardiovascular diseases. LAP index is easy-to-use, doesn't require any expensive laboratory tests, and should be included into laboratory screening as an early, precise, and cheap metabolic risk predictor. Nevertheless, diagnostic significance, informative values, and possibilities of LAP index application in everyday practices to assess excessive lipids accumulation in a body require further exploration. LAP index has its drawbacks such as low comparability of results obtained in different research, absence of standards for its assessment, and difficulties related to interpreting data at an individual level.

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ENERGY POTENTIAL OF MITOCHONDRIA UNDER LED LIGHTING AND RISKS OF EYES DISEASES

V.A. Kaptsov[1], V.N. Deinego[2], V.N. Ulasyuk[3]

[1]All-Russian Research Institute of Railway Hygiene, 1 Pakgauznoe shosse Str., Bldg. 1, Moscow, 125438, Russian Federation
[2] «Biolumen» scientific and technical center, 8 Mira avenue, Fryazino, Moscow, 141195, Russian Federation
[3] «Platan Scientific Research Institute with In-House Plant» PLC, 2 Zavodskoy lane, Fryazino, 141190, Russian Federation

Disorders in refraction, myopia and other eye disorders lead to a decrease in efficiency of any activity and impose certain limitations on educational and working capabilities of economically active population. As light denaturation grows, fatigue caused by performing test visual efforts also increases. The lowest decrease in physiological and mental parameters occurs when a person works under natural luminance, and the greatest one, under completely artificial luminance. Artificial light sources, as opposed to an even sunlight spectrum, have peaks and notches in photon flow under specific wave lengths.

It is shown in the paper that a drastic decrease in spectral-energy characteristics occurs in red light area with wave length 670 nm as compared to sunlight spectrum. The authors consider how 670 nm red light deficiency influences visual analyzer cells and mitochondria in particular. A theory that focuses on mitochondria aging states that oxidative stress caused by DNA mutations in mitochondria is associated with a decrease in adenosine triphosphate (ATP) production leading to cell degeneration. A rate at which this degradation develops is related to metabolic demands of a body, progressing inflammation in the outer retina, macrophages penetration and cells loss; as a result, eye sight deteriorates. A mechanism of a decrease in efficiency of ATP-synthesizing structures is examined within "670 nm light – water structural properties – efficiency of mitochondria rotary engine operations" cause-and-effect chain. The authors substantiate the necessity to synthesize red 670 nm luminopfor and to optimize LED lighting in this spectrum area.

Key words: *red 670 nm light, water structure, ATP synthesis efficiency, energy potential of mitochondria, LED lighting.*

Influence exerted by light on human circadian rhythms and visual analyzer efficiency is a global issue arising when health risks are assessed. F.F. Erisman, who established hygiene as a branch of science, highlighted a correlation between lighting environment and myopia development in his research [1]; at present this disease is becoming an epidemic one as it is spreading fast all over the world [2–4].

At the same time, F.F. Erisman pointed out that «myopia is not to be considered an inevitable evil that is essentially caused by educational conditions at school; on the contrary, progressive myopia is an evil that is caused only by inadequate lay-outs at schools, and it could be easily eliminated should the society pay greater attention to arrangements provided for educational process» [5].

Leading Russian and foreign ophthalmologists had an opportunity to discuss international experience gained in efforts to control myopia at the XII All-Russian conference entitled «Public health as grounds for prosperity in Russia» [6]. The Conference was opened by its chairs, RF Public Healthcare Minister V.I. Skvortsova and RAS Academician and The President of the Public Health League L.A. Bokeria. There was a report delivered by T.V. Pavlova, Associate Professor at the Ophthalmology Department of N.I. Pirogov's Russian National Research Medical University; it was entitled «Issues and achieve-

Valerii A. Kaptsov – Doctor of Medical Sciences, The Corresponding Member of the RAS, Head of the Occupational Hygiene Department (e-mail: kapcovva39@mail.ru; tel.: +7 (499) 15-33-628; ORCID: http: //orcid.org/0000-0002-3130-2592).

Vitalii N. Deinego – Senior researcher (e-mail: vn-led@bk.ru; tel.: +7 (916) 530-68-82).

Vladimir N. Ulasyuk – Doctor of Physical and Mathematical Sciences, Chief scientific consultant (e-mail: v_n_uls@mail.ru; tel.: +7 (495) 70-29-658; ORCID: http: //orcid.org/0000-0002-1130-710X).

ments related to control over myopia. Role that belongs to a public organization in consolidating efforts aimed at fighting against myopia». She noted that refraction disorders, myopia, and other eyesight disorders led to a decrease in efficiency as regards any activity and imposed certain limits on educational and labor capacities of employable population. Shimidzu Tadashi, The President of Asia Networks JSC (Japan), noted that wide use of smartphones, pads, and other up-to-date technical devices was the primary cause for wide spread of myopia in Japan. As a result, according to experts' evaluation, the global economy suffers annual productivity losses that amount to approximately 269 billion dollars [7].

S.I Vavilov, a truly great scientist, cited J.W. Goethe in his book entitled «The Eye and the Sun»: «The Eye owes its existence to the light. Out of indifferent animal auxiliary organs, the light picks up an organ that could be its equal; thus the eye is formed only in the light, to see the light, to make internal light meet external one». Sergey Vavilov also pointed out that from a biological point of view «optimal illumination» should result from the evolutionary adaptation of the eye to average illumination created on the Earth by the Sun. As regards energy, the eye is adapted not to the Sun itself, but to sunlight diffused from all surrounding objects.

Starting from 2017, «NTS Svetotekhnika», the Scientific-Technical Council, has been functioning to solve global issues related to lighting technologies and their practical application. The Council unites public and state figures, authorities, representative from the RF State Duma and Federation Council, scientists, (academicians, professors, and doctors of science), representatives of big business and who are socially responsible, and leading experts in the lighting technology sphere. Gennadiy Onishchenko, RF State Duma Deputy, the first Deputy to the Head of the State Duma Committee on Education and Science, took part in The Council regular meeting. He stated that «in spite of this meeting being overwhelmingly technical, there is an interesting report entitled «Peculiarities related to impacts exerted by LED lighting sources on visual organs of children, teenagers, and adults» by I.E. Aznauryan and it means that the Council is heading to the right direction». At present public organizations and associations are trying to take on responsibility for negative outcomes that may occur due to artificial light sources being sold by them on the lighting devices market.

Here we should note that, starting from 70-ties last century, biological effects produced by natural and artificial illumination have been assessed on the state level by experts employed at the Illumination Hygiene Laboratory (Radiant Energy Laboratory) at the A.N. Syisin' s Institute of Common and Communal Hygiene that is now called «The Center for Strategic Planning and Management of Medical and Biological Health Risks». Research performed by those experts [8] at the Laboratory focused on analyzing to what extent fatigue of observers determined by the fucntional F [f_i, E] depended on overall illumination [E] calculated as per dynamics of each examined function f_i [E]. It was shown that as light denaturation grew, that is, a share of natural light in the overall light flow dropped, fatigue caused by accomplishing test visual work also increased. The most insignificant drop in physiological and mental parameters of examined observers' state was detected under natural illumination, and the greatest one, under completely artificial illumination. Figure 1 shows a relationship between fatigue and a ratio of natural and artificial light under illumination created by luminous LB type lamps (light flow is equal to 2,800–3,000 lm and 3,450 K–4,200 K when illumination level on a work surface was controlled).

Experts employed at A.N. Syisin's Institute determined hygienic minimum of natural light in working areas inside public buildings and it gave grounds for hygienic requirements to combined illumination that fixed 250–300 meter-candela of natural light, η.

When luminous illumination was implemented, experts employed at the USSR Gosstroy's Scientific Research Institute for Construction Physics also noted that visual working capacity was higher under natural light

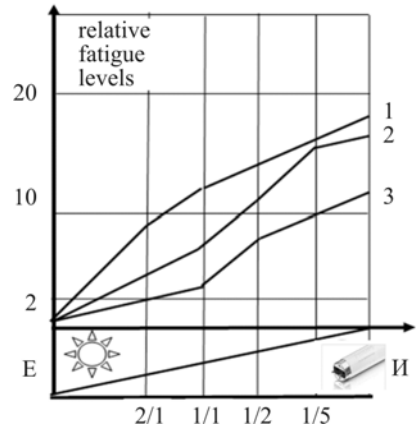

Figure 1. A relaitonship between fatigue and raito of natural and artificial light [8]
Note: under illumination being 300 meter-candela – line 1; 500 meter-candela, line 2; 1,000 meter-candela, line 3; E means natural light, И means artificial light.

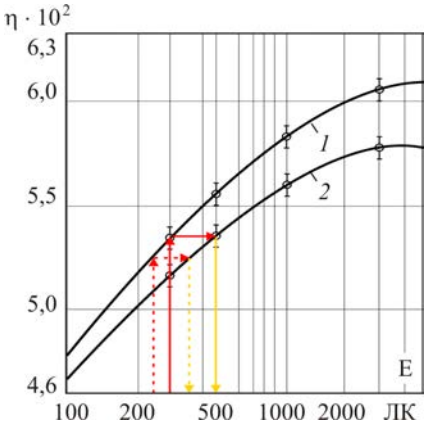

Figure 2. Relationship between visual working capacity and illuminaiton under natural light (curve 1) and artificial one (curve 2) [9]

than under artificial one. To make a comparison, they took luminous LB40 type lamps. Numerical values of visual working capacity parameters were determined as a product of discerning time and correct identification probability (Figure 2).

It is obvious that minimal level of luminous illumination that is 400–500 meter-candela at the given working capacity corresponds to hygienic minimum level of natural light being equal to 250–300 meter-candela. If we make a light spectrum of an artificial lighting source closer to the sunlight spectrum, we can not only save energy but also secure compatibility of these two light sources functioning; it will create safe lighting environment with a biologically adequate radiation spectrum.

Z.A. Skobareva and L.M. Teksheva, scientists working for A.N. Syisin's Institute, came to the following conclusions:

1. Examining biological effects produced by light on a human body is still a vital issue in illumination hygiene;

2. It was proven at cellular, biological, and psychophysiological levels, that natural and artificial light with the same intensity were still biologically inadequate; the difference remained when illumination by artificial light sources grew;

3. Natural light has great hygienic significance and it should be taken into account when developing illumination standards and new technical means for light environment optimization in places where people have to spend a considerable amount of time [8].

It is important to point out that experts at the Institute didn't perform a spectral evaluation of inadequacy between natural and artificial light.

Figure 3 shows spectra of luminous lamps, LEDs, and sunlight.

Artificial light sources, in comparison with the even sunlight spectrum, have spikes and dips in the photon flow at certain wave lengths, namely, spikes in the blue light area, dips at 480 nm wave length, and also dips in the red light area at 670 nm wave length.

Wide implementation of LED lighting with the blue light dose in its spectrum being considerably higher than in luminous lamps resulted in a great number of research on assessing health risks related to possible damage to the visual analyzer caused by «the blue danger».

But still, ophthalmologists and experts in light technologies didn't pay attention to the fact that 670 nm red light in modern white LEDs was lower than in the sunlight spectrum. A role played by the red light was underestimated due to this radiation range being beyond the spectral sensitivity curve of the human eye (luminosity curve). Experts in photobiological safety employed maximum values of light flows at specific wave lengths and considered that their minimum values couldn't do any significant damage to the eye. This concept was

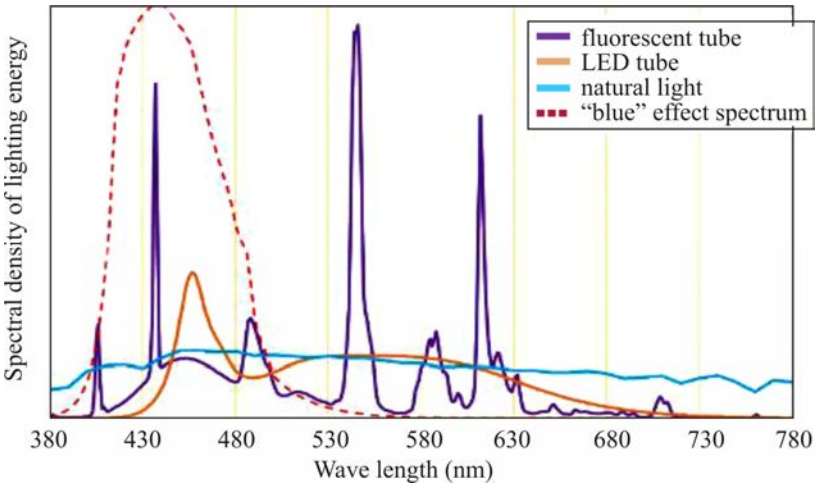

Figure 3. Spectra of luminous and LED lamps compared with the sunlight spectrum

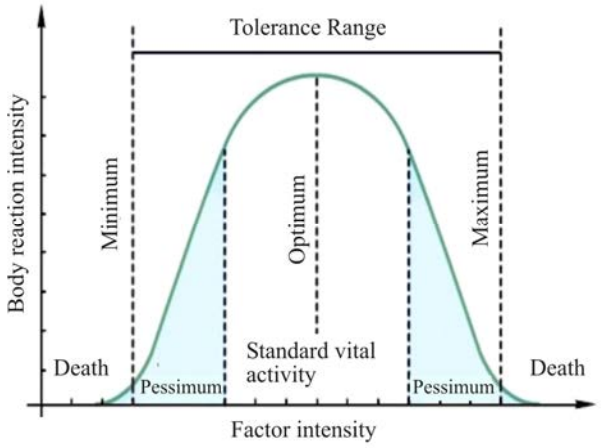

Figure 4. Optimum law (Shelford's Law) curve

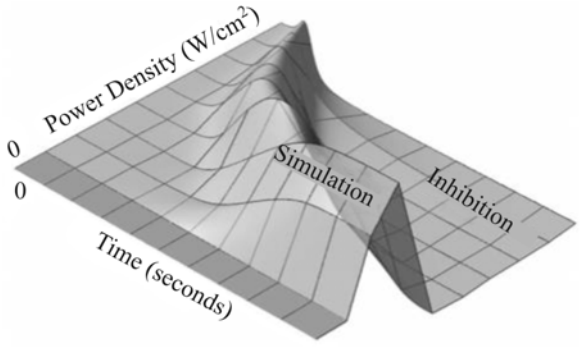

Figure 5. Three-dimensional model of Arndt-Schulz curve showing a possible two-phase dose reaction of radiation or illumination time when low-level laser therapy is applied [10]

determined by assessing how safe the visual analyzer was from impacts exerted by laser light sources. But hygienists and light therapists know that both excessive and insufficient light flow produce adverse effects on vital capacity of cells in a live biological object.

Hygienists assess risks caused by such impacts as per Shelford's Law of Tolerance (Figure 4).

The Law states that there are optimal values of an influencing factor and they secure normal vital activity of a cell as well as there are pessimum and death zones. This hygienic approach was further developed when laser therapy was implemented. Low-level laser therapy (LLLT) was first introduced in 1967; the technique involved applying visible light, as a rule, red or almost infrared one generated by a laser or a LED and was used to treat various pathologies in people and animals. That light usually had narrow spectral width between 600 and 1,000 nm. To select influence intensity, a three-dimensional model creased as per Arndt-Schulz rule was applied (Figure 5).

According to the quantum theory of light, each wave length in electromagnetic fluctuations has its corresponding energetic photon flow that creates its own chain of photochemical reactions. F.H. Grotthuss in Russia (1817) and Draper in the USA (1839) independently formulated a law stating that only that light which was absorbed by a reactive mixture could be chemically active. The basic photobiology law states that a biological effect can be produced only by spectrum waves with such a length at which they can be absorbed by molecules in cells. If a photobiological effect occurs, it unambiguously means that there are mole-

cules in cells that absorb quanta of light in the given spectrum area [11]. A relationship between absorbing capacity of a substance and a light wave length is determined by an absorption spectrum.

Light absorption by a substance is an intramolecular physical process. Light is absorbed by molecules (their complexes, atoms, radicals, or ions) and not by complex biological structures such as nucleus, mitochondria, cells, or eye retina. The only exemption is semiconductors that absorb light with combined energy levels created due to interaction between many centers (atoms, ions, or molecules). When a substance interacts with light absorbing it, both quantum (corpuscular) and wave properties of the latter become apparent [12].

Spectra of modern LEDs (a blue crystal covered with yellow phosphor) are based on the above mentioned regularities (Figure 6).

Previously we examined negative influence exerted by blue light spikes on the human retina and hormonal system as well as effects produced by a dip within light-blue 480 nm light range on the eye pupil response [14–17].

The present paper focuses on an influence exerted by a decrease in 670 nm red light dose on vital capacity of cell mitochondria. Mitochondria are basic free radicals producers in eukaryotic cells. Such free radicals as superoxide radical or hydroxide radical normally occur in mitochondria due to the respiratory chain functioning that provides synthesis of adenosine triphosphate (ATP synthesis) which is the primary energy «currency» of any cell. Mitochondria are also to a greater extent prone to damage by free radicals due to mitochondrial DNA, as opposed to nuclear one, not being protected by histons or any other DNA-binding proteins. And since mitochondria are important cellular organelles, any disorders in their proper functioning can lead to adverse outcomes, for example, apoptosis or programmed cell death.

G. Ling explains in his monograph entitled «Life at the cell and below-cell level, the hidden history of fundamental revolution in biology» that a cell is not a water solution in a lipid membrane but a protein-water-electrolyte structure that is held together due to many-layers of polarized water around full-scaled protein structures. Ling's theory is confirmed by practice and the lipid membranes theory contradicts these observations as a cell tends to absorb more water than can be explained with the membranes theory. Association-induction theory is an effort by the author to shift «gravity center» in getting an insight into vital functions of a cell from the cellular membrane to the cytoplasm via considering changes in electronic density in macromolecules caused by external signals as a basic mechanism for cellular functions regulation. These concepts are built on a close correlation between three basic «players» in the cellular cytoplasm, namely, proteins, structured water, and non-organic ions [18] such as hydrogen, sodium, iron, copper, and zinc ions.

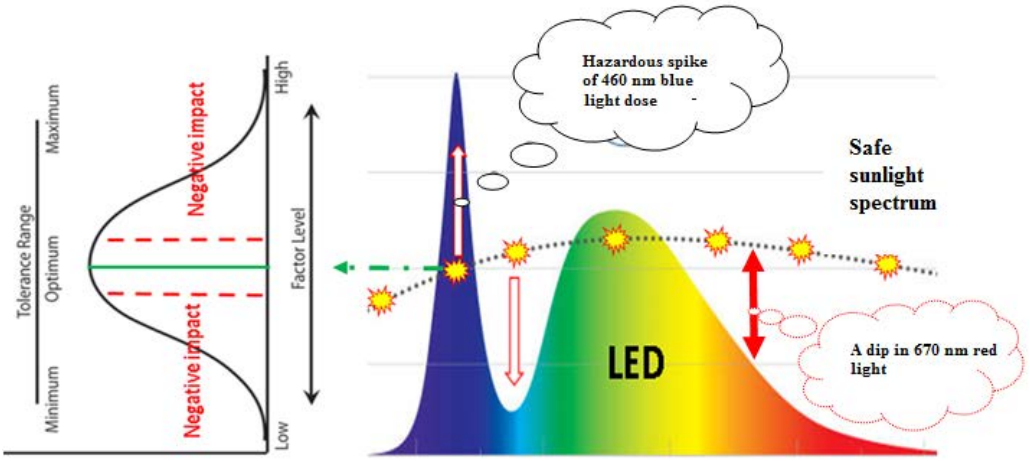

Figure 6. Spectral properties of sunlight and a traditional LED (a blue crystal covered with yellow phosphor) [13]

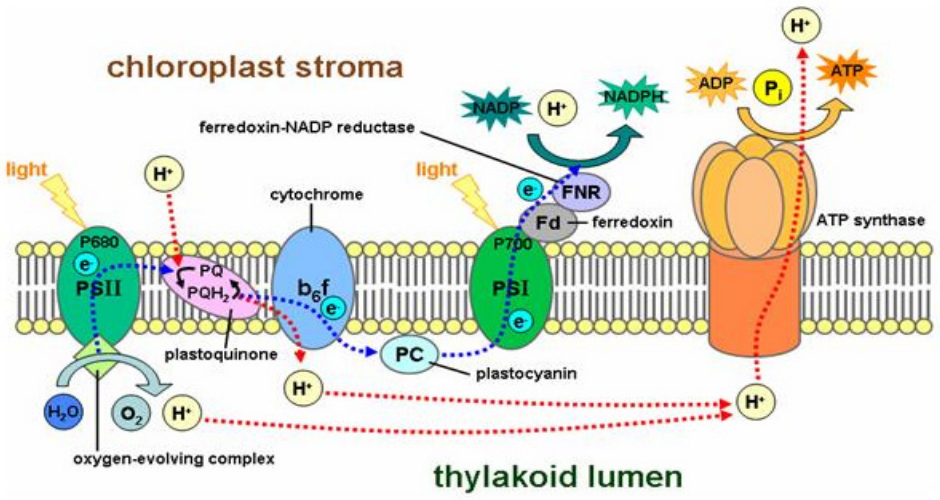

Figure 7. Photosynthetic transportation chain for thylakoid membrane electrons [19]

At present there are a lot of works on mitochondria functioning and mechanic-chemical models describing how mitochondrial structures produce ATP. The chemiosmotic theory developed by P. Mitchell, a British biochemist (1961) is the most recognized among them. He assumed that the electrons flow through a system of molecules-carriers was combined with carrying H+ ions through internal mitochondria membranes. As a result, electrochemical potential of H+ ions was created on membranes and it included both chemical (osmotic) and electric gradients (membrane potential). According to the chemiosmotic theory, it is electrochemical trans-membrane potential of H+ ions that provides energy for ATP synthesis due to H+ ion transportation through a proton channel in membrane H+ – adenosine triphosphate synthase (ATP). Mitchell's theory states that molecules-carriers lace up the membrane following one another in such an order that electrons and protons together can be transferred to one direction, but only electrons can be transferred to an opposite one. As a result, H+ ions accumulate on only one side of the membrane.

Electrochemical potential occurs between two sides of the internal mitochondrial membrane due to a directed motion of protons against a concentration gradient. Energy that is accumulated in this way is used to synthesize ATP as a result of membrane discharge at reverse (as per a concentration gradient) transportation of protons through ATP that functions as ATP-synthase in this case (Figure 7).

P. Dimroth et al. [20] described a mechanic-chemical model for induction of transmembrane sodium-driving force into torque (Figure 8).

The same mechanism will probably function in other F-ATP synthases including proton H^2-F-ATP synthase [21]. All the above mentioned mechanic-chemical models for obtaining ATP are based on nano-electric motor functioning due to H^+ or Na^+ ion flows; the motor has both a rotor and stator, that is, it's a mitochondrial rotary drive called ATP-synthase.

P. Dimroth et al. [20] also pointed out that electrostatic interaction between rotor sections and stator charge made transfers from state to

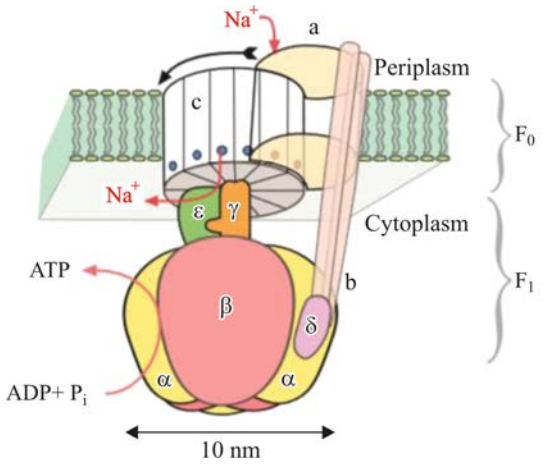

Figure 8. Basic diagram showing ATF synthase Na^+ FoF1 (An arrow shows rotating direction during ATF synthesis)

state depend on an angular position of a rotor denominated as θ. Chemical state of a rotor undergoes evolution that can be described symbolically with the following equation:

$$\frac{ds}{dt} = K(\theta)s, \qquad (1)$$

where K [θ] is a matrix of transition rates between chemical states. Movement of a rotor can be described via equating viscous resistance of a rotor to torques that influence it, to Brownian force that models thermal fluctuations of a rotor (that is, Langevin equation [22, 23]):

$$\underbrace{\zeta \frac{d\theta}{dt}}_{\text{Friction resistance}} = \underbrace{\tau_Q(\theta, s)}_{\text{Rotor-stator of interaction charge}} + \underbrace{\tau_{\Delta\psi}(\theta, s)}_{\text{Membranes with potential}} + \underbrace{\tau_{\Delta\varepsilon}(\theta, s)}_{\text{Dielectric barrier}} + \qquad (2)$$

$$\underbrace{\tau_{RS}(\theta)}_{\text{Rotor - stator passive interaction}} - \underbrace{\tau_L(\theta)}_{\text{F1 load torque}} + \underbrace{\tau_B(t)}_{\text{Brownian torque}},$$

There are the following addenda in the second part of the equation:

♦ **[*i*] τ_Q[θ,** s**]** is determined by electrostatic interaction between a stator charge (R227) and rotor components that are inside a hydrophilic line of rotor-stator. According to Coulomb's law, a charged (free) section will depend on a stator charge (R227) that corresponds to a dielectric and screening medium of a stator. Hydrophilia is a characteristic that describes how intensely a substance interacts with water, its ability to absorb water well, as well as high wettability of a surface. Together with water repellence, it can be applied both to solid objects in which it is a property of a surface and to separate molecules, their groups, atoms, or ions;

♦ **[*ii*] $\tau_{\Delta\psi}$[θ,** s**]** is determined by a drop in membranes potential via a horizontal segment between a periplasm channel and a boundary of a stator;

♦ **[*iii*] $\tau_{\Delta\varepsilon}$[θ,** s**]** is an electrostatic barrier that prevents a charged section from penetrating into a hydrophobic interface of a rotor-stator;

♦ **[*iv*] τ_{RS}[θ]** is passive interaction between a rotor and a stator;

♦ **[*v*] τ_L[θ]** is a load produced by F1 on a rotor via γ-shaft;

♦ **[*vi*] τ_B[*t*]** is a random Brownian torque caused by thermal fluctuations of a rotor.

Previously experts assumed that ATP-synthase (the smallest rotor drive ever known) functioned with 100%-efficiency; such a concept was based on somewhat idealistic assumptions, including those stating that viscosity of the medium that surrounded a drive was to be considered only in the bulk [24], while any viscosity gradients close to surface were not taken into account [10]. When this viewpoint is neglected, it has critical importance due to mechanical behavior of molecular machines being different from mechanical behavior of their microscopic analogues that can't be applied at the molecular level. It is true in particular when a concept of viscous friction and lubrication is applied. There was recent experimental research that revealed it was important to distinguish between physical properties of water in bulk and levels of nanoscopic interphase water layers that were screening surfaces. Nanoscopic water layers that are linked to hydrophilic surfaces have viscosity properties that are significantly greater than those of water in bulk. Besides, it was experimentally shown that as confinement between hydrophilic surfaces grew, a drastic increase occurred in viscosity of nanoscopic water layers [24].

The suggested model for ATP-synthase motor load [20] shows that its rotation speed, and ATP-production efficiency as well, depends on the state of water. Calculations included into ATP-synthase motor modeling assumed that water viscosity inside mitochondria was constant and corresponded to viscosity of water itself. According to A.P. Sommer et al. [25], the assumption was not true due to two basic reasons:

1) There were data on water inside mitochondria, and interphase water prevailed there, its share being 100%;

2) Laboratory experiments that focused on interphase water properties assumed that its viscosity was higher than viscosity of water in bulk, especially at hydrophilic boundaries.

A.P. Sommer et al. [25] considered a physical-chemical mechanism that gave some insights into viscosity gradients inside mitochondrial waters and sequentially explained two cellular responses: an increase or a decrease in ATP syn-

thase as a response to reactive oxygen forms and non-destructive levels of NIR laser radiation respectively. The mechanism is based on results obtained via a new experimental procedure that included nano-identification and modulation of interphase water layers with laser radiation. Its results that may include determination of light-induced ATP-production are expected to have significant outcomes for all branches of medicine and ophthalmology in particular, especially when it comes to analyzing degradation processes related to eye retina ageing.

Ageing is associated with cellular decrease and weaker functions that are partly mediated with the mitochondrial compromise. However, the age function of mitochondria is adjusted under infra-red light (670 nm) that improves their membrane potential and adenosine phosphate production as well and decreases age-related inflammation. Some data indicate that 670 nm light can significantly improve aged functions of the retina and it can possibly provide additional adenosine triphosphate production for ion pumps in photoreceptors or for decreasing age-related inflammation. It can have some positive outcomes for treating ageing of the retina and such age-related diseases as macular degeneration [26].

A resolution approved by 3th Global Pediatric Congress listed recent significant achievements in ophthalmology for children but stressed there still was a lot of issues to be solved [27, 28].

Thus, there is an overall trend for creating safe illumination with semiconductor light sources and safe radiation from visual units or monitors. It states it is necessary for any light source or visual unit to have a biologically adequate spectrum that will provide harmonic functioning of the visual analyzer and hormonal system. The Congress participants appealed to heads of states and governments to understand it was necessary to provide funding for state programs aimed at developing national standards for visual work; such activities should involve participation of ophthalmologists and experts in occupational hygiene and labor protection.

Experts who participated in a working group on safe use of buildings approved on the Report No. 02/TP dated July 19, 2017[1]; they also recommended «… to take into account domestic and foreign experience in creating semiconductor white light sources with a biologically adequate radiation spectrum when technical and regulatory documents are being developed» [29].

Conclusions

1. All energy-saving artificial light sources have spectra with a dip within 670 nm section and it exerts negative influence on ATP synthesis in cellular mitochondria in the eye.

2. If a spectrum has a hygienically optimal dose of 670 nm red light, it produces positive effects on viscosity of water nano-layers and ATP synthesis.

3. It is necessary to conduct further research on determining influence exerted by a hygienically optimal dose of 670 nm red light on changes in viscosity of water nano-layers and ATP synthesis related to them.

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NEW LEGAL, REGULATORY AND METHODOLOGICAL DOCUMENTS ISSUED IN THE RF IN THE SPHERE OF HEALTH RISK ANALYSIS

11.03–07.06.2019

The Decision by the Eurasian Economic Commission (EEC) Council dated February 22, 2019 No. 8 "On making alterations into Section II of the Unified list of products (goods) that are subject to state sanitary-epidemiologic surveillance (control) at customs borders and customs territory of the Eurasian Economic Union"

According to the alterations made into Section II of the above mentioned list, the following products are to be included into it: repellents which are dermatological means of individual protection against biological factors (insects) and are applied at industrial objects.

The Decision by the Eurasian Economic Commission (EEC) Council dated March 19, 2019 No. 40 "On making alterations into the Program on developing (making alterations or revising) interstate standards the application of which should provide voluntary compliance with the Customs Union Technical Regulation (CU TR 033-2013) "On safety of milk and milk products", as well as interstate standards that contain rules and procedures for research (tests) and measurement, including rules for sampling, necessary to apply the Customs Union Technical Regulation (CU TR 033-2013) "On safety of milk and milk products" and to provide compliance with the requirements fixed in it as well as to assess conformity of objects which are subject to technical regulation"

The Decision adjusts dates by which certain interstate standards are to be created; several sections are edited; several new sections are added.

The Decision comes into force after 30 days have passed since the date it has been published in open sources.

The Decision by the Eurasian Economic Commission (EEC) Council dated March 19, 2019 No. 41 "On making alterations into certain decisions made by the Eurasian Economic Commission (EEC) Council"

The Decision makes alterations into the Decision by the EEC Council dated April 24, 2013 No. 91 "On Approval of the list of products which should be declared at the customs together with supplying an additional document on assessment of conformity to the CU TR 004/2011 "On safety of low-volt equipment" and the Decision dated January 16, 2014 No.2 "On Approval of the list of products which should be declared at the customs together with supplying an additional document on assessment of conformity to the CU TR 020/2011 "Electromagnetic compatibility of technical appliances". Several positions in the above-mentioned lists are edited and these alterations are fixed in the above-mentioned Decision.

The Letter by the EEC dated March 06, 2019 No. 16–327 "Concerning the technical regulations "On safety of alcohol products" coming into force"

The Eurasian Economic Commission has considered an application on The Customs Union Technical Regulations (CU TR) "On safety of alcohol products" coming into force. It is reported that Section 53 in the Treaty on the Eurasian Economic Union signed on May 29 2014 fixes that all the Union member states are to provide distribution of alcohol products which conform to the requirements fixed by the Union technical regulations on their territory without making any additional demands to such products which are not mentioned in the technical regulations and without any additional procedures aimed at assessing conformity excluding certain positions regulated by transitional provisions. As regards alcohol products that are subject to EAEU TR 047/2018, all transitional provisions are to be determined by a decision made by the EEC Council.

The Decision by the Eurasian Economic Commission (EEC) Board dated April 02, 2019 No. 52 "On the list of the Eurasian Economic Union Technical Regulations (Customs Union Technical Regulations)

The Decision approves the list of Technical Regulations of the Eurasian Economic Union (Customs Union Technical Regulations or CU TR). It is

fixed that the list is to be applied since the date the present Decision comes into force. The stated list is included into resources contained in the EAEU unified regulatory and reference information system. It is obligatory to apply nomenclature codes given in the list.

The Order by the EEC Board dated April 02, 2019 No. 57 "On Introduction of the unified process entitled "Creation and maintenance of unified registers that contain issued or accepted documents on assessing conformity to the requirements fixed in the Eurasian Economic Union Technical Regulations (Customs Union Technical Regulations)" into practice as regards a unified register of issued certificates of conformity and registered declarations of conformity"

The unified process "Creation and maintenance of unified registers that contain issued or accepted documents on assessing conformity to the requirements fixed in CU TR" comes into force on June 01, 2019, as regards a unified register of issued certificates of conformity and registered declarations of conformity. New participants can join the unified process according to the procedure that regulates joining the unified process.

The Order by the EEC Board dated April 16, 2019 No. 60 "On making alterations into the list of standards that contain rules and procedures for research (tests) and measurement, including rules for sampling, necessary to apply the Customs Union Technical Regulation "On safety of package" (CU TR 005/2011) and to assess compliance of objects that are covered by the said Technical Regulations"

The Order specifies dates on which certain standards become valid; these standards are necessary in terms of applying and providing compliance with the requirements fixed in the CU TR 005/2011 "On safety of package". According to the latest alterations, words "is valid till January 01, 2019" are replaced with "is valid till a corresponding interstate standard is included into the list" in Section 5 of the items 25-68 and 259-302 in the list of standards containing rules and procedures for research (tests) and measurement, including rules for sampling, necessary to apply the Customs Union Technical Regulation "On safety of package" (CU TR 005/2011) and to assess compliance of objects that are covered by the said Technical Regulations"; the said list was approved by the Decisions of the Customs Union Commission on August 16, 2011 No. 769.

The Decision by the EEC Board dated April 16, 2019 No. 61 "On transitional regulations in the EEC TR 047/2018 "On safety of alcohol products"

Documents that confirm compliance of the products which are subject to regulation by the EAEU TR 047/2018 "On safety of alcohol products" issued before the said EEC TR came into force are valid till their validity expires but not longer than till January 09, 2024. Also, up to January 09, 2024, it is allowed to: manufacture and distribute products on territories of the EAEU member states that were not subject to obligatory assessment of conformity with obligatory requirements fixed in legislative acts issued in the EAEU or in a EAEU member state before the above mentioned EAEU TR came into force, and the said products were allowed to be manufactured and distributed without documents on obligatory assessment of conformity and without a national mark of conformity; manufacture and distribute products on territories of the EAEU member states in conformity with obligatory requirements fixed in previously issued legislative acts of the EAEU or a EAEU member state provided that products are supplied with documents on assessment of conformity to the above mentioned obligatory requirements which were issued or submitted before the EAEU TR 047/2018 came into force.

The Decision by the EEC Board dated April 16, 2019 No. 63 "On making alterations into Section 2.1 in the list of goods for which a license is required in order to import them on the Eurasian Economic Union customs territory and (or) export them from the Eurasian Economic Union customs territory"

The Decision specifies the list of ozone depleting substances for which an import or an export license is required. The word "1,1-difluorinechlorine,2-difluorine,3-dichlorinepropane" is replaced with the word "1,1-difluorinechlorine,2-difluorine,3-chlorinefluorinepropane" in Section 2.1 in the list of goods.

The Decision by the EEC Board dated May 21, 2019 No. 78 "On making alterations into Section 20, Chapter II, of the Unified sanitary-epidemiologic and hygienic requirements to products (goods) which are subject to sanitary-epidemiologic surveillance (control)"

The document actualizes a procedure for distribution of disinfectants on the EAEU territory. Section 20 in the Chapter II of the document has

been edited. The document fixes basic requirements to disinfectants, disinsectants, and deratization means aimed for using in households, medical and prevention institutions, and at other objects in order to provide population health and safety (apart from those applied in veterinary) (EAEU CN FEA code 3808). The latest edition covers disinfectants for professional use and those sold in retail outlets (commodity items 2801-2853, 2901-2942, 3307, 3604, and 3808 in the EAEU CN FEA), excluding substances that are applied in veterinary and agriculture. The document fixes that disinfectants can be distributed on the EAEU territory provided that there is a certificate of state registration issued prior to the present Decision has come into force and distribution is allowed only until validity of the said certificate expires. Requirements to repellent dermatologic means of individual protection from biological factors (insects) applied at industrial objects come into force on a date on which the Decision by the EEC Council on making alterations into CU TR 019/2011 "On safety of individual protection means" as regards requirements to toxicity, safety, and physical-chemical properties of the said means becomes valid.

The Decision by the EEC Board dated May 21, 2019 No. 80 "On making alterations into the Decision by the Customs Union Commission dated September 23, 2011 No. 799"

The latest edition contains the list of standards application of which ensures voluntary conformity with the requirements fixed in the CU TR 008/2011 "On safety of perfume and cosmetic products". The document fixes alterations into the list of standards that contain rules and procedures for research (tests) and measurement, including rules for sampling, necessary to apply the said CU TR and to provide conformity with the requirements fixed in the said CU TR. In particular, the list includes a number of new items; also, a term of validity has been adjusted for several items.

The Decision by the EEC Council dated March 29, 2019 No. 32 "On making alterations into the Customs Union Technical Regulations "On safety of perfume and cosmetic products" (CU TR 009/2011)"

The CU TR 009/2011 "On safety of perfume and cosmetic products" has been improved. Multiple adjustments include the following: new terms and concepts have been introduced, including "perfume and cosmetic products for artificial suntan", "products with low microbiological risks", and some others; a prohibition to apply sucrose and other carbohydrates that are easily fermented as ingredients in hygienic means for the oral cavity; obligatory additional warnings on aerosol products with propellants; obligatory information on products samples that are only for testing properties of a product and are not for sale to consumers; a procedure for providing conformity of perfumes and cosmetics to the requirements fixed in the CU TR and cases in which a declaration on conformity of products to the CU TR requirements can be accepted without any additional or repeated tests (research); a wider list of substances that are prohibited for use in perfume and cosmetic products, or there are certain limitations imposed on their application.

The Decision by the EEC Council dated May 28, 2019 No. 55 "On making alterations into the Customs Union Technical Regulations "On safety of individual protection means" (CU TR 019/2011)"

The document adjusts the CU TR 019/2011 "On safety of individual protection means". In particular, it makes alterations into the safety requirements to means of individual protection from mechanical impacts, chemical factors, lower temperatures, heat and thermal radiation, thermal risks caused by an electric arc, non-ionizing radiation, damage caused by electric current, as well as from impacts caused by static electricity.

Several new items have been added into Appendix 4 "Forms of certificates for confirming compliance of the individual protection means" and Appendix 5 "The list of individual protection means that are subject to obligatory confirmation of their compliance when allowed to be distributed on the territory of the Customs Union member states".

The Federal Law issued on March 18, 2019 No 38–FZ "On making alterations into the RF Federal Law "On consumers rights protection" as regards improvements made into the state policy in the sphere of consumer rights protection"

The Law specifies how consumers can make an application concerning protection of their rights. It is fixed that a consumer can make an application in written form, both on paper or electronically, to a state surveillance authority, other authorized federal executive bodies, a regional executive authority, or a local authority. An application can be mailed; submitted via the Internet; made on an official web-site of a surveillance authority, other

authorized federal executive bodies, a regional executive body, or a local authority; made on a unified portal for state and municipal services, or a regional portal for state and municipal services; it can also be submitted by an applicant in person.

Multi-functional centers that render state and municipal services can accept applications made by consumers and consult them on issues related to protection of their rights; this activity is performed basing on the agreements on interaction between multifucntional centers and executive or local authorities.

It is fixed that executive authorities in the RF regions are to develop regional programs aimed at protecting consumer rights and to render assistance to local authorities and public groups of consumers (their associations or unions) when they protect consumer rights. The Rospotrebnadzor is authorized to approve on methodical guidelines on developing and implementing regional and municipal programs aimed at consumer rights protection.

The Federal Law issued on May 01, 2019 No. 93–FZ "On making alterations into the federal law "On protecting children from information that can cause damage to their health and development" and specific legislative acts issued in the Russian Federation"

The Law specifies the procedure for prohibiting distribution of information among children that contains images or descriptions of sexual violence. Anyone who organizes an entertaining event (including movie showing when such services are provided) during which such information is to be shown is obliged to not admit participants that are younger than 18. In order to meet this obligatory requirement, a person who sells entrance tickets or controls admittance to such an event has the right to ask any participant to show a legal document that confirms a holder's identity and age. The list of such documents will be approved upon by a federal executive body authorized to do it by the RF Government. The Law also prohibits admittance to distribution of the said information at a distance shorter than 100 meters away from educational establishments, medical, recreation, and sport facilities for children, culture institutions, establishments for children's rest and health improvement.

The RF President Order issued on March 11, 2019 No. 97 "The Basics of the RF state policy in the sphere of providing chemical and biological safety up to 2025 and beyond"

The Order enlists the following priorities in the RF state policy in the sphere of providing chemical and biological safety: monitoring over chemical and biological risks; further development of legislative regulation and public management; development of resource provision for safety; implementation of measures aimed at neutralizing, preventing, and minimizing chemical and biological risks, enhanced safety of population and the environment from adverse impacts caused by hazardous chemical and biological factors; as well as assessing efficiency of the said measures. The document entitled "The Basics of the RF state policy in the sphere of providing chemical and biological safety up to 2025 and beyond" approved on November 1, 2013 No. Pr-2573 is no longer valid.

"The list of orders issued as per results of the enlarged session of the State Council Presidium"

The RF President approved on the list of orders as per results of the enlarged session of the RF State Council Presidium held on February 12, 2019. Following the session, The RF President also stated a number of issues, including the following: a necessity to consider whether it was advisable to extend "The Unified national system of digital marking and tracking of goods" to basic construction materials as well as other materials that influence safety of capital construction objects.

Information by the RF Presidential Council on civil society development and human rights dated June 11, 2019 on the following subject: "The Ministry of Natural Resources and the Environment of the Russian Federation and Rospotrebnadzor have submitted their proposals on wastes reduction to the Ecological Commission of the European Court of Human Rights"

Within activities performed by the RF Presidential Council on civil society development and human rights, the RF Ministry of Natural Resources and the Environment and Rospotrebnadzor have voiced their opinion on the policy in the sphere of wastes treatment.

Rospotrebnadzor believes it is important to fix the following in the country legislation: stage-by-stage decrease (up to complete prohibition) in production of disposable packets used in retail trade; stimulating production and application of reusable package and polymer materials that are able to self-destruct; stimulating re-use of package; obligatory certification of a sign "biologically degradable" or "ecologically safe disposal"; fixing obligatory standards for multiple use of specific package.

Rospotrebnadzor believes it is necessary to develop legislative acts that prohibit dumping and elimination of food products that are esculent as well as to provide regulatory support for use of food wastes and unclaimed food products as biological energy sources.

The RF Ministry of Natural Resources and the Environment supports the initiative to prohibit sales of disposable products made of plastic in order to reduce negative impacts on the environment. It is stipulated that such a prohibition should be well-grounded and take into account economic consequences that economic entities might bear after its introduction.

The RF Government regulation dated February 28, 2019 No. 222 "On making alterations and confirming validity expiration of certain legislative acts issued by the RF Government"

The document adjusts the rules for sanitary and quarantine control at admission points at the RF state border. In particular, it is fixed that should any products that are subject to assessment (inspection or examination) be detected at any admission point fixed in the RF Government regulations, a customs official makes a decision regarding sending such goods to be assessed (inspected or examined) by Rospotrebnadzor officials. As per results of assessment (inspection or examination) by Rospotrebnadzor officials, a decision should be made whether these goods can be admitted on the EAEU customs territory or their imports should be prohibited.

The RF Government regulation dated March 09, 2019 No. 250 "On making alterations into the Rules for determining a technology as the best available one as well as development, actualization, and publication of information and technical references on the best available technologies"

The document fixes the procedure for determining a technology as being the best available one and for publishing information and technical references on such technologies. Technological processes, equipment, technical procedures and methods that allow to consider a technology the best available one should be examined in terms of their conformity to the following criteria: the lowest negative impacts exerted on the environment calculated as per a time unit or a volume of manufactured goods, accomplished works, or rendered services; high economic efficiency of implementation and operation; application of resource- and energy-saving techniques; the shortest implementation period required; a technology already implemented in industry at two or more industrial objects in the RF.

The RF Government regulation dated March 19, 2019 No. 284 "On making alterations into the Regulations on the Governmental Commission on biological and chemical safety in the Russian Federation"

The document specifies powers granted to the Governmental Commission on biological and chemical safety. The Commission is to provide interaction between federal executive authorities in the sphere of biological and chemical safety, including issues related to creation of a system of monitoring and control over safety in the RF, taking into account risk factors that can lead to emergencies including epidemics and pandemics, increase in infections incidence that is hazardous for population, and a rise in number of intoxications.

The RF Government regulation dated March 13, 2019 No. 262 "On approval of the Rules for creation and operation of the system for automated control over emissions of and (or) discharges of pollutants"

The system for automated control is to be created in order to provide automated measuring and accounting of emissions and (or) discharges, as well as fixating and transferring data on them into the state register of objects that exert adverse impacts on the environment; the activities are to be performed primarily at objects assigned into the 1st rank hazard category in accordance with the legislation on environmental protection.

The above-mentioned Rules fix the following: tasks and stages in creating a system for automated control; requirements to development of a program for creating this system; requirements to sources that exert negative impacts on the environment and are included into the program; requirements to the design of the said system for automated control.

The RF Government regulation dated March 13, 2019 No. 263 "On the requirements to automated measuring devices and means that keep records of emissions and (or) discharges of pollutants, to technical means that fix and transfer data on emissions and (or) discharges of pollutants to the state register of objects that exert negative impacts on the environment"

Automated measuring devices and means that keep record of pollutants emissions are to comply

with the requirements fixed in the RF legislation on providing unanimity of measurements and ensure transfer of data on emissions and (or) discharges of pollutants via information-telecommunication networks into the state register of objects that exert negative influence on the environment. The document fixes types of automated measuring devices; maximum permissible measurement error for such devices; requirements to frequency of transferring data on measurements results; requirements to software applied in technical devices for data fixation and transfer; requirements to a period during which data received from automated measuring devices are to be stored in the register.

The RF Government regulation dated March 21, 2019 No. 289 "On making alterations into specific acts issued by the RF Government"

The said list includes 7 types of the regional state surveillance: ecological, construction, housing, surveillance in the sphere of protecting population and territories from natural and technogenic emergencies, surveillance over providing safety of regional and municipal motorways, surveillance in the sphere of state-regulated prices (tariffs), and veterinary surveillance.

The RF Government regulation dated April 26, 2019 No. 515 "On the system for marking goods with means for identification and tracking of goods"

All stages in distribution of marked goods, starting from their manufacturing and up to retail sales, will be tracked within the system for state information monitoring.

In particular, the RF Government fixed the following: a procedure for marking goods that are subject to obligatory marking with identification means, a procedure for submitting information by those who participate in distribution of products to include it in to the information system; a procedure for creation and operation of the state information monitoring system (SIMS); a procedure for SIMS interaction with other information systems; the requirements to data collection and their protection.

The RF Government regulation dated April 22, 2019 No. 479 "On approval of the federal scientific and technical program for genetic technologies development for 2019-2027"

The Federal scientific and technical program for genetic technologies development is aimed at securing a leading role played by the RF in genetic engineering. Its primary goals are: to provide favorable conditions for development of scientific and technical activities, to create genetic technologies including technologies for genetic editing; scientific staff development and development of professional competencies in the sphere of genetic technologies; a decrease in dependence of the Russian science on foreign databases on genetics and biology, specialized foreign software and devices.

The program is to be implemented in 2019-2027 and includes the following: biological safety and technological independence; genetic technologies for agricultural development; medical genetic technologies; genetic technologies for industrial microbiology.

The RF Government regulation dated May 31, 2019 No. 691 "On approval of the rules for placing centralized water discharge systems (sewage systems) among centralized municipal water discharge systems or city district water discharge systems and on making alterations into the RF Government Order dated September 05, 2013 No. 782"

The document fixes the list of grounds that can be given to place a centralized water discharge system (a sewage system) among centralized municipal or city district water discharge systems; the list of grounds to place sewage discharged into a centralized water discharge system (a sewage system) among sewage that is accounted in order to place a centralized water discharge system (a sewage system) among centralized municipal or city district water discharge systems (sewage systems); the procedure for determining volumes of sewage that can be discharged into a centralized water discharge system (a sewage system).

The RF Government regulation dated May 10, 2019 No. 914–r "On making alterations into the list of pollutants that are subject to state regulation in the sphere of environmental protection fixed by the RF Government Order dated July 08, 2015 No. 1316-r"

The document adjusts the list of pollutants that are subject to state regulation in the sphere of environmental protection. A new pollutant, namely "Black coal dust", is introduced; ethenylbenzene (styrene) is now considered a separate pollutant; terephthalic acid is excluded from the list.

The RF Government regulation dated May 29, 2019 No. 1124–r "On approval of the set of activities aimed at implementing the Ecological Safety Strategy in the RF up to 2025"

The document fixes the list of specific activities that are to be performed within implementation of the RF Ecological Safety Strategy up to 2025 fixed by the RF President Order issued on April 19, 2017 No. 176. The list includes the following: making alterations into the RF legislation in order to assign specific legal status to wastes that are applied as recycled resources; developing proposals on how to stimulate companies that use wastes as recycled resources to manufacture goods or render services; making alterations on creating a system for ecological audit; approval on the procedure for determining emissions of hazardous substances (pollutants) from mobile sources (including vehicles that use natural gas as fuel); monitoring over activities in the sphere of solid communal wastes disposal, development of documents that are to provide efficient functioning of regional operators dealing with solid communal wastes disposal; creation and development of the state fund that contains state ecological monitoring data with regional and functional sub-systems and can serve as a source of authentic ecological data for executive bodies at each level, concerned economic entities, and industrial companies; construction, reconstruction, and upgrading of treatment facilities in settlements and city districts; working out proposals on stimulating ecologically safe transport (including vehicles fuelled with natural gas); determination of boundaries for natural protected areas and their protected zones, water protection zones, and protected coastlands, and making an entry on each such object and its boundaries into the Unified state register of real estate objects; determination of optimal values for ecological safety indexes; working out amendments into two-side and multi-side international agreements on cross-border influence on the environment in order to protect national interests of the Russian Federation.

The Order by Rospotrebnadzor No. 42, RF Federal Biomedical Agency No. 13 dated 29, 2019 "On approval of the procedure for submitting documents confirming there are certain circumstances that give grounds for making a decision (suspending or cancelling the previously made decision) on undesirability of a foreign citizen or a person without citizenship staying (residing) in the RF should such a decision be made due to certain circumstances that impose a real threat to population health" (Registered in the RF Ministry of Justice on April 15, 2019 No. 54379)

The Order fixes the procedure for making a decision on undesirability of a foreign citizen staying in the RF due to certain circumstances that impose a threat to population health. Such decisions are to be made by authorized officials of Rospotrebnadzor and RF Federal Biomedical Agency or their regional offices as well as decisions on suspending or cancelling of the previously made ones. Should any circumstances be revealed that are fixed in the RF legislation, an authorized federal body or its regional office are to provide the following: control over timely organization and implementation of a complete set of anti-epidemic (preventive) activities; control over employers implementing anti-epidemic (preventive) activities should any infectious diseases be detected among their employees; submit all the documents that confirm undesirability of a foreign citizen staying in the country. A decision should be cancelled in case there are medical documents confirming a foreign citizen has recovered from an infectious disease that is hazardous for people. The Order fixes all the forms of documents that are to be applied when the procedure is accomplished.

The Order by the Rospotrebnadzor dated February 25, 2019 No. 95 "On approval of standard documents on a test purchase of goods (works or services)" (Registered in the RF Ministry of Justice on May 25, 2019 No. 54688)

The Order fixes standard documents on accomplishing a test purchase of goods (works or services). Among other things, such documents should contain data on the following: data on goods (works or services) that have been purchased; data on goods that have been submitted to be tested (examined) by experts and (or) expert organizations; data on officials who have accomplished a test purchase; data on photos taken or video-files made during a test purchase; data on other ways applied to record a test purchase; data on how goods (works or services) have been purchased; data on how good (works or services) have been paid for; data on detected violations of any obligatory requirements; the list of documents attached to the report on a test purchase; when and how a copy of the report on a test purchase was sent to an economic entity that was an object of an inspection accomplished via a test purchase.

The Order by the RF Chief Sanitary Inspector dated March 06, 2019 No. 2 "On accomplishing cleaning-up immunization against measles on the RF territory" (Registered in the RF Ministry of Justice on March 11, 2019 No. 54004)

By the end of 2019, all the employers are to provide immunization against measles among foreign citizens employed by them who haven't ever been ill with measles and haven't been immunized against the disease or don't have any documents confirming the immunization. The activity is accomplished within overall cleaning-up immunization that is to take place from April 01 to October 01, 2019, in all the RF regions and is to cover RF population as well as labor migrants who haven't been immunized against the disease.

Regional authorities are to reveal children and adults (including those without any permanent place of living, refugees, or migrants) who haven't been ill with measles, haven't been immunized against it, have been immunized only once, don't have any documents confirming immunization against the disease, and who are not included into the preventive immunization schedules approved for 2019.

The Order by the RF Chief Sanitary Inspector dated March 25, 2019 No. 6 "On making alterations into the Order by the RF Chief Sanitary Inspector dated July 23, 2008 No. 45 "On approval of the sanitary-epidemiologic requirements 2.4.5.2409–08" (Registered in the RF Ministry of Justice on April 08, 2019 No. 54310)

Iodized salt should become an obligatory ingredient in nutrition provided for schoolchildren at educational establishments. The relevant alterations have been made into the Sanitary-epidemiologic requirements 2.4.5.2409–08 "Sanitary-epidemiologic requirements to organization of nutrition provided for schoolchildren at educational establishments for primary and secondary school education as well as vocational training" approved by the Order by the RF Chief Sanitary Inspector dated July 23, 2008 No. 45. The said alterations come into force on January 01, 2020. Validity of the Sanitary-epidemiologic requirements 2.4.5.2409–08 "Sanitary-epidemiologic requirements to organization of nutrition provided for schoolchildren at educational establishments for primary and secondary school education as well as vocational training" is prolonged up to October 01, 2023.

The Order by the RF Chief Sanitary Inspector dated May 22, 2019 No. 8 "On making alterations into the sanitary-epidemiologic rules and standards SER 2.4.2.2821–10 "Sanitary-epidemiologic requirements to organization of educational processes at educational establishments" (Registered in the RF Ministry of Justice on May 28, 2019 No. 54764)

The Order specifies requirements to orientation of windows in classrooms as well as the list of rooms inside a school where insolation can be absent.

The Order cancels the following requirements: windows in classrooms should look only south, south-east or east; windows in classrooms aimed for lessons in drawing or painting can look north; kitchen windows can also look north; windows in classes for lessons in computer technologies should look north or north-east.

The list of rooms in a school where insolation can be absent not includes school canteen and recreation zones. Classes for lessons in physics, chemistry, painting and drawing are excluded from the said list.

The letter by Rospotrebnadzor dated May 29, 2019 No. 02/7646–2019–27 "On sending explanations"

Economic entities that deal with catering and work with individual orders are now allowed to cook dishes with properties or a degree of readiness stated by a customer. It also concerns dishes cooked out of non-treated raw materials (tartars made of raw meat and fish, sushi, stroganina, etc.), meat dishes with various degree of roasting (steaks), excluding certain dishes that are epidemiologically significant. The above said doesn't prohibit cooking dishes out of food raw materials or products with different thermal treatment (fish or meat) at catering facilities including catering networks.

The letter by Rospotrebnadzor dated April 29, 2019 No. 02/6111–2019–27 "On peculiarities of legal regulation over relationships in the sphere of industrial and consumer wastes distribution"

When sanitary-epidemiologic assessment is accomplished at places where solid communal wastes are accumulated, it is necessary to take into account wastes groups that are collected separately as well as factors that influence epidemiologic and hygienic risks caused by a relevant group of wastes. Accordingly, frequency of disposal for

separately collected wastes can be fixed individually for each wastes group. Overall, practices related to separate collection of wastes in the RF regions prove it is advisable to dispose of them as frequently as their big quantities have been accumulated but not rarer than once a week, excluding wastes that contain food products.

The information latter "Rospotrebnadzor explains that a regional operator is not entitled to refuse a conclusion of a contract on solid communal wastes disposal with a consumer"

Rospotrebnadzor informs that, according to Clause 426 of the RF Civil Code, an economic entity or a private entrepreneur who accomplishes entrepreneurial or any other income-accumulating activities is not allowed to refuse to conclude a public contract if this economic entity or a private entrepreneur is able to sell relevant goods to a customer, render services, or accomplish relevant works. In all the cases when a regional operator eludes concluding a contract, another party (a consumer) has the right to apply to court and to demand conclusion of a relevant contract. A consumer, who owns a house or an apartment as a part of a house, in his or her turn, is obliged to provide solid communal wastes disposal via concluding a contract with a regional operator as it is fixed in Clause 30 of the RF Housing Code and Clause 24.7 of the Federal Law issued on June 24, 1998 No. 98-FZ "On industrial and consumer wastes"

The information letter by Rospotrebnadzor dated May 08, 2019 "On marking tobacco goods with identification signs"

By June 30, 2019, all manufacturers and importers of tobacco goods are to register themselves within GIS for monitoring over distribution of goods that are subject to obligatory marking with identification signs. This obligation is fixed in the RF Government Order dated February 28, 2019 No. 224.

Since July 01, 2019, it is not allowed to manufacture and distribute cigarettes (All-Russian products Classifier code 12.00.11.130, EAEU CN FEA code 2402 20) and cigarettes without a filter (All-Russian products Classifier code 12.00.11.140, EAEU CN FEA code 2402 20 900 0) without any identifications signs made on them and data on their marking and their first sale being entered into the said GIS monitoring system. Retail sales of the said tobacco goods manufactured (imported) after July 01, 2019, is allowed only provided that data on their sale are entered into the said GIS monitoring system. Distribution of tobacco goods that correspond to the above-mentioned codes and are not marked with identification signs is allowed only till July 01, 2019; other types of tobacco goods, till July 01, 2021.

Methodical guidelines 2.1.4.0143–19. 2.1.4. "Drinking water and water supply in settlements. A procedure for assessing an increase in quality of drinking water supplied from centralized water supply systems. Methodical guidelines" (approved by the RF Chief Sanitary Inspector on March 27, 2019).

The RF Chief Sanitary inspector has approved a new procedure for assessing an increase in quality of drinking water supplied from centralized drinking water supply systems, taking into account implementation of the Federal project "Pure water". The procedure can be applied by authorities responsible for sanitary-epidemiologic surveillance over centralized drinking and communal water supply, executive bodies and local authorities, as well as by juridical persons or private entrepreneurs who deal with everyday water treatment and water supply to consumers. Assessment results can be applied to determine efficiency of implemented regional programs and activity plans aimed at managing quality of drinking water; they can also give grounds for informing population and authorities about provision of population with qualitative drinking water.

The Order by the RF Public Healthcare Ministry dated January 31, 2019 No. 36n "On fixing the procedure for accomplishing an examination on a possible correlation between a disease and an occupation and a medical report on occurrence or absence of an occupational pathology" (Registered in the RF Ministry of Justice on March 19, 2019 No. 54085)

The Order fixes the procedure for accomplishing an examination aimed at determining any cause-and-effect correlation between a disease and an occupation. Any examination of a possible correlation between a disease and an occupation is to be accomplished by a specialized medical organization or a specialized structural division of a medical or any other organization with a license to perform medical activities as regards works (services) related to "occupational pathology" and "examinations of possible correlations between diseases and occupations" (a center for occupational pathology). To accomplish an examination, a permanent medical board is to be formed; a re-

port issued by such a board gives the right to a medical expert who is authorized to accomplish the task by a head of a center for occupational pathology to issue a medical conclusion on occurrence or an absence of an occupational disease; the said conclusion is to be issued within 1 working day since the date a decision was made by a medical board.

The Order by the RF Ministry of Labor No. 52n, The RF Public Healthcare Ministry No. 35n dated January 31, 2019 "On approval of the list of medical examinations which are required to obtain clinical and functional data depending on a specific disease in order to accomplish a social and medical examination" (Registered in the RF Ministry of Justice on March 15, 2019 No. 54059)

The Order fixes the list of medical examinations that are necessary to obtain clinical and functional data depending on a disease in order to accomplish a social and medical examination. The list contains medical examinations (basic and auxiliary ones) for adults and children including limitation periods that should not be exceeded.