EVALUATING THE OCCUPATION-RELATED HARM TO THE HEALTH OF ORE MINING WORKERS USING TARGET PROTEIN ANALYSIS

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Exposure to harmful and dangerous factors at ore mining facilities poses health risks to workers that are associated with prolonged exposure to airborne chemicals in the work area. Realized, these risks undermine physical condition of people doing key ore mining jobs underground. Relying on the target protein analysis, this study aimed to assess the job-related harm to the health of ore mining workers resulting from exposure to metals airborne in the work zone. The participants were involved in copper-nickel ores mining. To evaluate the impact of metals from the working zone air on their health, we conducted chemical, proteomic, statistical, and bioinformatic analyses on the collected samples and data. With the mean per-shift exposure to metals of up to 0.2 mg/m^3 (up to 4 times the MPC), the blood supernatant concentrations of cobalt, chromium, nickel, copper, and manganese increased by 1.4 to 2.6 times in the study group compared to the control group. Comparison of proteomics datasets revealed 33 significantly different protein spots. In 15 of them, the change in intensity was related to the increased concentration of the considered metals in the supernatant. Identification and analysis of proteins from these spots revealed their association with impairments in the functions of the nervous, cardiovascular, and digestive systems. The identified proteins were involved in the development of oxidative stress, metabolic and neurodegenerative disorders. Proteomic analysis improves the prediction and early prevention of occupational adverse outcomes among the ore mining industry workers.

Keywords: mining workers, metals, exposure, proteomic profiling, adverse outcomes, risk prognosis and prevention

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Compliance with ethical standards: the study involved employees of a mining facility (copper-nickel production); it was conducted in accordance with international standards of medical practice (Declaration of Helsinki, revision of 2013, 2024) and approved by the Ethics Committee of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (Minutes No. 4 of February 24, 2022).

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ОЦЕНКА НЕГАТИВНЫХ ЭФФЕКТОВ У РАБОТНИКОВ ПРЕДПРИЯТИЯ ГОРНОРУДНОЙ ПРОМЫШЛЕННОСТИ ПОСРЕДСТВОМ ИССЛЕДОВАНИЯ БЕЛКОВ-МИШЕНЕЙ

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Воздействие вредных и опасных производственных факторов на предприятиях горнорудной промышленности формирует риски для здоровья работников при длительной производственной экспозиции химическими веществами с воздухом рабочей зоны. Реализация риска может формировать ущерб здоровью работников основных профессий подземной добычи руд. Целью работы было оценить негативные эффекты у работников предприятия горнорудной промышленности, подвергающихся воздействию металлов с воздухом рабочей зоны, путем исследования белков-мишеней. Методы химико-аналитического, протеомного, статистического и биоинформационного анализа применены для оценки воздействия металлов с воздухом рабочей зоны на работников подземной добычи медно-никелевых руд. Среднесменная экспозиция металлами на уровне до 0,2 мг/м^3 (до 4 ПДК) обуславливает повышение концентрации кобальта, хрома, никеля, меди и марганца в супернатанте крови работников группы наблюдения в 1,4–2,6 раза относительно аналогичных показателей группы сравнения. Сравнительный анализ протеомных карт выявил 33 значимо различающихся белковых пятна, из которых в 15 имела место связь изменения интенсивности с повышением концентрации изучаемых металлов в супернатанте. Идентификация и анализ белков, обнаруженных в указанных белковых пятнах, показали, что они ассоциированы с нарушением функций нервной и сердечно-сосудистой систем, органов пищеварения. Выявленные белки участвуют в развитии окислительного стресса, метаболических и нейродегенеративных нарушений. Внедрение протеомного исследования повышает эффективность прогнозирования и ранней профилактики производственно-обусловленных неблагоприятных исходов у работников горнорудной промышленности.

Ключевые слова: работники горнорудного производства, металлы, экспозиция, протеомное профилирование, неблагоприятные исходы, прогноз и профилактика риска

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Соблюдение этических стандартов: исследование с участием работников горнорудного предприятия (на примере медно-никелевого производства) проведено в соответствии с представленными международными нормами медицинской деятельности (Хельсинкская декларация Всемирной медицинской ассоциации 2013, 2024 г.) и одобрено комитетом по этике ФБУН «ФНЦ медико-профилактических технологий управления рисками здоровью населения» (протокол № 4 от 24 февраля 2022 г.).

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Ore mining industry is one of the leading sectors of the economy of the Russian Federation. According to the Federal State Statistics Service, more than 1 million people are employed in this industry. This supports the importance of efforts aimed at preservation of health of workers engaged in the production of metal ores (underground mining in the first place). The said production involves a complex of interrelated process stages, from the extraction of ore, its enrichment, through metallurgical conversion, further processing, to loading and unloading operations and transportation of raw materials and metal products. The key stage, however, is the extraction of raw materials for subsequent processing, and it determines the basic harmful and dangerous factors peculiar to the production environment and the labor process that affect the health of underground miners [1, 2].

There is a number of specific harmful and hazardous dangerous factors associated with underground mining operations, including dust the chemical and fractional composition of which depends on the type of ore being mined [3, 4]. This dust is composed of particles of various sizes, including ultrafine (0.1–10 microns). Such particles can adversely affect critical organs and systems after transition from the lungs into the systemic circulation [5]. They acquire the transition ability once covered with a layer of absorbed proteins and forming a unique crown that allows them to penetrate the aero-hematic barrier barrier [6]. The bloodstream carries the particles to sensitive receptors in critical organs and systems such as the liver, spleen, heart, kidneys, lymph nodes, and brain; there, they initiate metabolic disorders at the cellular and molecular levels [7, 8].

Postgenomic technologies, including proteomics, are widely used to study the toxic effects of chemicals and early detection of the associated negative effects [9, 10]. The study of changes in the expression of proteins and peptides grants insights into physiological processes and disorders of the balance of homeostasis at the cellular and molecular levels [11]. The identified changes in the protein composition of the biological media (plasma, serum, urine, etc.) allow investigating the reactions of the molecular dynamic equilibrium and how the negative effects occur and develop under the influence of harmful and hazardous factors of the industrial environment, including the chemical factor [12, 13].

Relying on the target protein analysis, this study aimed to assess and determine ways for early detection of job-related harm to the health of ore mining workers resulting from exposure to metals airborne in the work zone.

METHODS

The study was conducted from April 14, 2024 to April 15, 2025, and included 113 employees of a mining facility. For both the control group and the study group, the inclusion criteria were: male gender, age 30–65 years, over a year of employment at the facility, satisfactory hygienic conditions and socio-economic standard of living; specific to the study group: professional activities related to underground mining of copper-nickel ores; specific to the control group: professional activities not involving exposure to harmful factors of the production environment and labor process. Exclusion criteria: acute infectious diseases within 4 weeks before the start of the study, intake of medications that have a pronounced effect on the intended target organs less than 30 days before the start of the study. Under these criteria, the study group was comprised of 39 men mining copper-nickel ores underground and representing the key professions: ore face miner, blaster, borer, pitman, fastener, trolley driver, drilling rig driver. Their work experience at the facility was 18.2 ± 1.0 years, mean age — 47.95 ± 0.87 years. The comparison group included 74 employees of the facility's administrative staff. They were employed by the company for 17.1 ± 1.05 years, and their mean age was 47.1 ± 0.83 years ($p \leq 0.05$ relative to the study group, both figures). Thus, by the above criteria, the groups were comparable, and differed in the fact of exposure to harmful and hazardous factors of the production environment.

The participants' blood was sampled at the Occupational pathology Department of the Regional Center for Occupational Pathology of the Krasnoyarsk Regional Clinical Hospital (head of the department — O.N. Zakharinskaya, occupational therapist — A.V. Galiulina). The samples were analyzed at the on the basis of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

The fact of exposure was confirmed by chemical testing of supernatant isolated from whole blood, conducted to determine the concentration of ions of six metals (cobalt, manganese, copper, nickel, lead, chromium). Fasting blood samples were collected from the ulnar vein using a disposable device. To make supernatant, we destroyed shaped blood elements in a lysing buffer (ratio 3 : 10), put the samples through centrifugation at 14,000 rpm for 10 minutes (twice), and decanted them (separated solid and liquid phases). Metals were quantified by inductively coupled plasma mass spectrometry (ICP-MS) as prescribed in guidelines MUK 4.1.3230-14, MUK 4.1.3161-14. Agilent 7500cx mass spectrometer (Agilent Technologies; USA) was used for this task. The results were compared to those obtained in the control group. The concentration of proteins in the supernatant is higher than in whole blood, which supports the selection of the former as the biosubstrate.

Proteomic testing implied obtaining peptide samples from the blood supernatant, so it was subjected to 2D electrophoresis in polyacrylamide gel and stained with silver using the PROTEAN I12 IEF System and the PROTEAN II XL vertical electrophoresis cell (Bio-Rad; USA). The intensity of protein spots was determined with the help of GeL Doc XR+ gel documentation system (Bio-Rad; USA). To compare the groups' proteomics datasets by intensity, we used PDQuest (Bio-Rad; USA). Significantly different protein spots were excised and sequentially analyzed in an UltiMate 3000 chromatograph (Thermo Fisher Scientific; USA) and a 4000 QTRAP mass spectrometer (AB Sciex; USA), HPLC and tandem mass spectrometry, respectively. The resulting spectra were processed in the ProteinPilot program (AB Sciex; USA); for identification, we used the UniProt database, sampling by the Homo sapiens (Human) taxon. The assessment of metabolic disorders relied on the publicly available bioinformatic resources: UniProt (http://www.uniprot.org), Comparative Toxicogenomics Database (http://ctdbase.org/) and DisGeNET (https://www.disgenet.org/dbinfo).

The results of the data collection and processing stage were analyzed in Statistica 10 (StatSoft; USA), with the differences evaluated for statistical significance using the Mann–Whitney *U* test, $p \leq 0.05$). To build models illustrating the causal relationship between metal concentration in the supernatant and changes in the intensity of the protein spot, we used regression analysis. The determination factor (R^2) and the Fisher's exact test ($F > 3.96$) allowed assessing the statistical adequacy of the model. The Student's *t*-test ($p \leq 0.05$) was used to verify the statistical significance of the model.

RESULTS

Long-term mean per-shift exposure to airborne metals (cobalt, manganese, copper, nickel, lead, chromium) at the level

Table 1. Comparative analysis of the blood supernatant metal content, samples from workers in the main professions of underground mining of copper-nickel ores

Substance	Mean (M ± *m*), mg/dm³		The share of workers from the study group with indicator values higher than in the control group, %	The order to which the indicator value is greater than in the control group	Significance of differences between groups ($p \leq 0.05$)
	Study group	Control group			
Cobalt	0.0004 ± 0.00005	0.0002 ± 0.00006	66.4	2	0.011
Manganese	0.010 ± 0.001	0.004 ± 0.001	79.5	2.5	0.0001
Copper	0.834 ± 0.034	0.446 ± 0.041	79.1	1.9	0.0001
Nickel	0.0039 ± 0.001	0.0015 ± 0.0003	82.2	2.6	0.0001
Lead	0.113 ± 0.018	0.081 ± 0.010	28	1.3	0.994
Chromium	0.0038 ± 0.001	0.0021 ± 0.0005	95.4	1.8	0.002

of 0.004–0.2 mg/m³ (from 0.1 to 4 times the MPC) raises the concentration of these substances in the blood supernatant by 1.4–2.6 times, study group vs. control group (p = 0.000–0.011; Table 1).

The proportion of workers with elevated blood levels of metals in the study group relative to the comparison group was 66.4–95.4% of the total number of the examined participants.

Quantification of the supernatant proteomics datasets revealed 46 protein spots that exhibited different intensity in the study and control groups. The comparison of intensity of these pots identified significant differences for 33 of them. In 15 protein spots, the synthesis was boosted by 1.1–26.3 times (p = 0.000–0.021), and in 18 spots, it was decreased by 1.2–38.8 times (p = 0.000–0.033). Mass spectrometric identification showed that the detected amino acid sequences match 80 proteins from the ProteinPilot library.

For 15 protein spots out of 33 identified, we obtained the intensity change probability dependence models describing a situation in which the content of all elements (cobalt, chromium, nickel, copper, and manganese) grows in the blood supernatant. This allowed labeling the proteins of these spots as indicator proteins (Table 2).

Through bioinformatic analysis, we identified the genes encoding the indicator proteins and their associated diseases. It has been shown that changes in the expression of these genes play a certain role in the pathogenesis of negative effects in workers who had a higher amount of the considered metals in their blood supernatant. Primarily such effects were detected in the nervous (genes *MAP3K9, HLA-A, LDLR, LAMP2, AKT2, FNDC3B, FRRS1L, SPTBN4*) cardiovascular (genes *MAP3K9, HLA-A, LDLR, LAMP2, HRH1*) systems, and digestive organs (genes *LDLR, LAMP2, AKT2*). The initiation of these changes determines disorders at the molecular and cellular level, which are signaled by alterations of expression of the identified target proteins (Table 3).

Thus, these results show that the identified target proteins may be involved in the pathogenesis of diseases associated with elevated metal content in the supernatant of blood sampled from workers involved in underground mining of copper-nickel ores. Monitoring the expression of these proteins is necessary to predict the development of negative effects caused by exposure to the considered metals in the air of the working area, as well as to develop measures to prevent such effects.

DISCUSSION

Mining activities have a significant impact on the health of underground miners: the air of the work zone contains metals and their compounds in the form of dust and aerosols [3, 4]. Ultrafine particles can be caught in the alveoli of the lungs, then enter the bloodstream, and ultimately deposit in various organs and tissues [5]. Proteins and other biomolecules absorbed on the particles enable their transportation and penetration into cellular structures [6]. Through proteomic testing, we identified proteins — MAP3K9, HLA-A, LDLR, LAMP2, AKT2, FNDC3B, FRRS1L, SPTBN4, and HRH1 — that contribute to negative effects and related metabolic disorders, which may ultimately increase the incidence of occupational diseases.

Negative effects are associated with the influence of metal ions on protein targets through that translates into increased generation of reactive oxygen species (ROS), which boost or slow down their expression. Damage to proteins impairs activity of the enzymes, which raises the level of endogenous cellular hydrogen peroxides and short-lived ROS, both of which have a significant effect on lipid, protein, and carbohydrate metabolism [14]. The MAP3K9 protein discovered in this study participates in the cascades of cellular responses caused by changes in the environment. In addition, it is involved in the signaling pathway triggered by mitochondrial death (including the release of cytochrome C) and leading to oxidative stress and apoptosis [15]. The biological functions of another protein, LAMP2, are not entirely clear. It is believed to be heavily involved in the work of lysosomes, including maintaining integrity, pH, and catabolism. In addition, one of the functions of LAMP2 is to protect the lysosomal membrane from proteolytic enzymes and methylating mutagens leading to the development of oxidative stress [16].

Metal exposure also increases the risk of developing metabolic syndrome characterized by hypertension, insulin intolerance, central obesity, and dyslipidemia [17]. his effect is considered to be associated with excessive oxidative stress resulting from the said exposure [18]. As part of the insulin signaling pathway, the identified protein AKT2 plays an important role in the control of glycogenesis, gluconeogenesis, and glucose transport [19]. The LDLR protein mediates endocytosis of cholesterol-rich low-density lipoproteins (LDL) and thus maintains their plasma levels [20]. A change in the expression of this protein significantly correlates with growth of the LDL levels, which leads to the development of atherosclerosis, metabolic syndrome, and steatohepatitis [21]. The FNDC3B protein may be a positive regulator of adipogenesis [22]. However, abnormal adipogenesis can trigger pathological conditions such as obesity, insulin resistance, and other metabolic disorders [23].

Diseases of the nervous system are the most common conditions causing temporary disability among ore miners [24]. The discovered FRRS1L protein is involved in the glutamate signaling pathway (the main excitatory neurotransmitter) [22]. Increased expression of this protein can lead to excessive excitability of neurons. Another protein, HRH1, mediates smooth muscle contraction and increases capillary permeability

Table 2. The parameters of the intensity change probability dependence models, simultaneous increase of the content of all elements (cobalt, chromium, nickel, copper, and manganese) in the blood supernatant

Spot number	Protein name	Direction of change in protein expression compared to the control group	The gene encoding the protein	Parameters of the model illustrating the causal relationship between metal concentration in the supernatant and changes in the intensity of the protein spot		Determination factor	Indicators of adequacy and statistical significance of the obtained models	
				b_0	b_1	R^2	$F > 3.96$	$p \leq 0.05$
3	Protein 2 associated with cerebellar degeneration	↓ 38.8 times	*CDR2*	8507.3 ≤ 12250.4	−3072176.2 ≤ −67257.0	0.43–0.96	5.95 ≤ 188.43	0.001–0.041
	Ceramide synthase 4		*CERS4*					
	Eukaryotic peptide chain release factor, GTP-binding subunit ERF3B		*GSPT2*					
10	Peptidyl-prolyl cis-trans isomerase-like 3	↓ 3.2 times	*PPIL3*	11449.7 ≤ 16213.4	−3194317.1 ≤ −74074.2	0.46–0.90	6.77 ≤ 75.54	0.001–0.031
	Mitogen-activated protein kinase kinase kinase 9		*MAP3K9*					
	Zinc finger protein 316		*ZNF316*					
	Transcription factor LBX2		*LBX2*					
	HLA Class I histocompatibility antigen, alpha A chain		*HLA-A*					
16	Low-density lipoprotein receptor	↑ 23.3 times	*LDLR*	−6354.7≤ −2828.8	57030.1 ≤ 2593427.1	0.47–0.95	7.16 ≤ 145.11	0.001–0.028
17	DnaJ homologue of subfamily C, member 28	↑ 13.4 times	*DNAJC28*	−7376.8≤ −3195.7	68144.0 ≤ 3020105.4	0.47–0.94	6.96 ≤ 129.64	0.001–0.030
20	Lysosomal-associated membrane glycoprotein 2	↓ 5.9 times	*LAMP2*	929.7 ≤ 1286.2	−307021.0 ≤ −6588.4	0.12–0.96	5.67 ≤ 217.35	0.001–0.044
	Zinc finger protein 862		*ZNF862*					
25	RAC-beta-serine/Threonine protein kinase	↓ 7.4 times	*AKT2*	1864.8 ≤ 2551.4	−611457.7 ≤ −13530.8	0.44–0.96	6.16 ≤ 215.24	0.001–0.038
	Gasdermin A		*GSDMA*					
28	Cofilin 1	↑ 11.2 times	*CFL1*	−2559.6≤ −1347.7	25239.5 ≤ 1174851.9	0.41–0.97	5.68 ≤ 221.62	0.001–0.011
	Uncharacterized protein C10orf62		*C10orf62*					
29	Unconventional myosin-Vc	↑ 2.1 times	*MYO5C*	−622.7 ≤ 135.5	10269.5 ≤ 451780.1	0.46–0.73	6.42 ≤ 21.07	0.002–0.035
	Member of the BCLAF1 and THRAP3 3 family		*BCLAF3*					
32	Histamine H1 receptor	↑ 2.9 times	*HRH1*	−412.3≤ −69.3	4725.8 ≤ 197127.0	0.50–0.87	8.14 ≤ 51.47	0.001–0.021
33	tRNA (adenine(58)-N(1))-methyltransferase non-catalytic subunit of TRM6	↑ 1.3 times	*TRMT6*	2510.9 ≤ 4491.1	26816.7 ≤ 1362921.1	0.48–0.93	7.21 ≤ 102.48	0.001–0.028
	Factor 4 associated with DDB1 and CUL4		*DCAF4*					
35	Protein 3B containing the fibronectin type III domain	↑ 1.8 times	*FNDC3B*	−701.0 ≤ 497.14	16066.4 ≤ 721429.5	0.54–0.87	9.48 ≤ 54.18	0.001–0.015
	Peregrine		*BRPF1*					
	Jupiter microtubules associated with homologue 2		*JPT2*					
39	Transforming growth factor beta-1-induced transcript 1 protein	↑ 18.7 times	*TGFB1I1*	−2545.3 ≤ −1344.2	27486.8 ≤ 1244407.3	0.47–0.91	7.22 ≤ 80.58	0.001–0.028
40	Beta protein phospharnesyltransferase subunit	↑ 11.9 times	*FNTB*	−3789.4 ≤ −1756.6	307497 ≤ 1648931.6	0.43–0.97	6.12 ≤ 219.41	0.001–0.038
42	FRRS1L protein containing the DOMON domain	↑ 3.0 times	*FRRS1L*	−4495.1 ≤ −1212.0	62630.9 ≤ 2912018.4	0.41–0.96	5.48 ≤ 214.80	0.001–0.047
	BRISC and BRCA1-A complex member 2		*BABAM2*					
45	Spectrin beta chain, non-erythrocyte 4	↓ 19.4 times	*SPTBN4*	4036.9 ≤ 5811.8	−1414900.1 ≤ −31150.5	0.43–0.96	6.02 ≤ 169.26	0.001–0.040
	Protein 11, containing the domain of disintegrin and metalloproteinase		*ADAM11*					

by contracting terminal venules. In addition, it promotes neurotransmission in the central nervous system (CNS) and thereby regulates circadian rhythms, emotional and locomotor activity, and cognitive functions [25]. The SPTBN4 protein belongs to spectrins, scaffold proteins that bind the plasma membrane to the actin cytoskeleton. They play a crucial role in determining the shape of a cell, the location of transmembrane proteins, and the organization of organelles. A change in the synthesis of SPTBN4 disrupts ion channels in the tissues of the nervous system by impairing the cytoskeletal system [26]. The HLA complex, which includes the HLA-A protein, serves as the only link between the immune system and the intracellular

Table 3. Prediction of negative effects (adverse outcomes) associated with changes in the expression of target proteins induced by high content of metals in the supernatant

Gene-associated negative effects (adverse outcomes)	Protein-encoding genes
Diseases of the immune system	*HLA-A*
Blood diseases	*HLA-A, FNDC3B*
Diseases of the urinary system	*SPTBN4*
Diseases of the nervous system	*MAP3K9, HLA-A, LDLR, LAMP2, AKT2, FNDC3B, FRRS1L, SPTBN4*
Respiratory diseases	*HRH1*
Diseases of the digestive system	*LDLR, LAMP2, AKT2*
Skin diseases	*AKT2*
Diseases of the cardiovascular system	*MAP3K9, HLA-A, LDLR, LAMP2, HRH1*
Diseases of the endocrine system	*SPTBN4*
Cognitive impairment	*HRH1*

state. The expression of this complex causes neuronal effects in the thalamus and hippocampus, which lead to functional disorders in the brain [27, 28].

The results of this study underpin the inclusion of proteomic testing into chemical exposure assessment with the aim to identify the key molecular points and mechanisms leading to adverse outcomes, with the ultimate goal of this inclusion being development of the early prevention measures for occupational diseases.

CONCLUSIONS

1. Long-term, per-shift exposure to metals (cobalt, chromium, nickel, copper, and manganese) dispersed in the air of the working area at concentrations ranging from 0.004 to 0.2 mg/m^3 (up to 4 times the MPC) causes up to a 2.6-fold increase in the concentration of these metals in the blood supernatant of workers in the primary occupations involved in underground mining of copper-nickel ores.

2. The revealed transformation of the proteomic profile in the workers' blood supernatant—characterized by up to a 26.3-fold increase in expression in 15 protein spots and up to a 38.8-fold decrease in 18 spots is the result of increased concentrations of these metals in the body.

3. The expression of the proteins considered is associated with the development of a number of negative effects, primarily from the nervous (*MAP3K9, HLA-A, LDLR, LAMP2, AKT2, FNDC3B, FRRS1L, SPTBN4* genes) and cardiovascular (*MAP3K9, HLA-A, LDLR, LAMP2, HRH1* genes) systems, and digestive organs (*LDLR, LAMP2,* and *AKT2* genes), which confirms the development of disorders at the molecular and cellular level.

4. The identified target proteins are involved in the pathogenesis of oxidative stress, metabolic, and neurodegenerative disorders, which may increase the prevalence of occupation-related diseases associated with exposure to metals in the workplace air among copper-nickel miners.

5. The results of this study emphasize the importance of integrating proteomic testing into the assessment of the impact of metals on copper-nickel miners for early detection of changes in the key molecular points and assessment of the mechanisms of development of adverse outcomes, with the ultimate goal of this integration being the development of early prevention measures for occupation-related diseases.

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