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Influence of various pollutants to the environment on metabolic status laboratory animals organism

This article presents the results of an experimental study of dust containing a complex microconcentrations metals. Quantitative characteristics carcinogenic risk in inhalation and oral intake of chemical compounds have been established. The results of complex ecological studies on the evaluation unfavorable environmental factors, the carcinogenic risk of cancer development among the population living in the zone a dysfunctional region, specifies the features ecological and hygienic situation caused by chemical contamination of some natural environments (air, water, snow, soil). The role of low metal concentrations in the dust composition on the metabolic processes in the body has been experimentally established, the dose-dependent changes have been determined, that the trigger mechanism toxicity of chemical compounds is the inefficiency the cellular mechanisms in the splitting corpuscular microparticles of dust penetrating into the blood stream, provoking increased oxidative stress in the internal organs.

Keywords: environment, dust, experimental animals, chemical pollutants, toxicity, biological equivalent, maximum permissible concentration, biosphere, the soil, cytomorphological index.

Introduction

A feature of any large industrial city is the concentration of a significant number of objects of environmental and hygienic risk in a relatively small area. The main parameters of the hygienic assessment of the habitat in such conditions are, first of all, the level of contamination of the main depositing media, specifically manifested in specific landscape conditions, the factors of an optimal town-planning character with the required organization of sanitary protection zones of objects of ecological and hygienic risk [1].

The basis of scientific and applied research in modern hygiene, in connection with the study of the impact of urban environmental factors on public health, are approaches aimed at identifying, preventing and assessing the adverse effects of man-made pollution of the urban environment on public health and sanitary and hygienic living conditions [2].

In the urban environment, the majority of researchers consider chemical pollutants entering the depositing media—the air basin, snow, soil, as the main risk factors for the health of the population. Among the priority pollutants are heavy metals, dioxins, polycyclic aromatic hydrocarbons, chemical carcinogens (asbestos, nickel, benzene, arsenic, radon, soot, etc.). And the pollution of atmospheric air often serves as a leading indicator of hygienic trouble in the territory [3].

The need for constant monitoring of the state of the soil cover is explained by the fact that it is the ultimate receiver of most technogenic chemicals involved in the biosphere [4, 5].

The soil is significantly different from other components of the biosphere both in terms of the level of organization and complexity of the structure, and in terms of the functions it performs. In addition, the soil plays a special role of the global geochemical regulator of cyclic mass flows of heavy metals — polluting elements, which have high levels of technogenicity and toxicity at high concentrations [6].

Therefore, in order to substantiate the model of the behavior of heavy metals in the soil, it is necessary to study the forms of existence, transformation and migration of heavy metals in a given object, the interrelationships of these factors, as well as control systems over sources of pollution that have anthropogenic origin (industrial emissions of enterprises, polluting chemicals with atmospheric precipitation and thawed snow). Once in the soil, the metals are distributed among the biota of the soil, interacting with it and leaving the negative effects of such interaction everywhere. The danger is aggravated by the fact that metal ions do not undergo chemical and biological degradation, as is characteristic of organic compounds. Therefore, the metals in the soil should be considered almost as permanently present — they are only transferred or transferred from one form to another within the given system. The form of the existence of metals in the soil is complex and their detailed identification with different types of soils is hardly advisable [7].

Materials and methods of research

The object of the study was the city of Temirtau, Karaganda region, it is located in the Central region of Kazakhstan and is a large industrial region with an area of 10 thousand square meters. and a population of 180 thousand people. In the city of Temirtau there are enterprises of ferrous and non-ferrous metallurgy, energy, construction and food industries. According to the number of emissions from industrial enterprises per city inhabitant, 2.1 tons, including about 1 ton in the form of dust precipitation, while at the national level this value should be within 200 kilograms of various chemical compounds [8,9].

The influence of unfavorable environmental factors included analysis of air pollution, snow, drinking water, surface water, soil cover, food. Hygienic assessment of medical and biological monitoring in the determination of cause-effect relationships in the «environment — health of the population» system was carried out in 4 stages.

At the first stage, chemicals were identified through collection and analysis of data on sources and composition of emissions of polluting chemicals. A list of potentially harmful substances in environmental objects that could affect the population was identified. The main source of information on industrial emissions was the annual statistical reports of the Karaganda territorial environmental protection department «On the state of atmospheric air protection in the Karaganda region» for 2010–2016 [10].

In order to take into account the chemicals entering the environment from existing sources, a universal value, the biological equivalent of emissions (BEV), was used to take into account the hazard class of chemical compounds, their toxicological characteristics, the mechanism of action on the organism and assess the state of the city atmosphere. the value of MPC or OBUV and the hazard class of chemicals [10].

In the analysis of pollution of environmental objects, the method of zoning of urban territory into 6 plots was used, based on the geometric scheme of division of the city's territory into equal areas — the «squares method» [11], which allowed:

1. Obtain differentiated quantities of chemical loading in individual territories.
2. To rank the territory of the city, regardless of administrative boundaries and to identify those areas where in-depth medical and biological research should be conducted.

For this purpose, a coordinate grid of the city's territory was composed of 12 squares equal in area, divided into 6 zones. Each zone was a separate unit of observation, carrying in itself an independent characteristic of the chemical load.

Studies included: environmental objects, experimental animals (organs: bone marrow, lungs, liver, kidneys stomach).

The research consisted of the following stages:

- Sanitary and hygienic assessment of Temirtau;
- experimental modeling to prove the mutagenicity of urban dust as the leading factor of pollution in animals (male rats).

Based on the rank evaluation of each chemical element included in the square, a quantitative assessment of the degree of total chemical loading was determined. The squares were grouped by comparison zones, which made it possible to identify the most unfavorable zones by the level of atmospheric pollution.

When using dust, which includes metals, as a marker affecting metabolic processes, inducing mutagenesis, it was planned to observe the following:

- the possibility of detecting the dependence «dose-effect», «dose-time-response»;
- the establishment of a link between the manifestation of intoxication and a specific action.

Under the action of small doses according to the literature, the specific component does not prevail in the general picture of intoxication. At the same time, the specificity of metals was mainly studied with an isolated action.

A study of the possible adverse effects of a complex of metals in the dust on the organism of residents of Temirtau was carried out during a toxicological experiment. Integral and specific indicators were used reflecting the state of the most important vital systems of the organism (body weight, summation — threshold value (SPP), biochemical, cytomorphological and cytogenetic studies). Method of exposure: intratracheal, inhalation, for 3, 70 days and 4 months, and also the recovery period.

In the first stage of the rat, males received dust containing a complex of metal microconcentrations (PSCMM) once intratracheally at a dose of 50 mg/ml. The experiment lasted for 3 and 70 days. The technique of intratracheal administration is technically simple, it provides an accurate dosage of the introduced dust and allows to cause a pathological process in the lungs.

The second step lasted 4 months when rats received inhalation dust in the chamber at a concentration of 0.25 mg/m^3 , the average daily concentration of dust exceeds 5 times (their MPCs — 0.05 mg/m^3) and corresponds to the actual dust load for the residential areas of the city.

The third stage of the experiment included the inhalation administration of SSCMM for 4 months at the MPC level of 0.05 mg/m^3 and a recovery period of 12 days.

The animals of the experimental groups were daily subjected to a 4-hour inhalation primer during the second and third stages of the experiment.

Based on the micronuclear test, a study was made of the mutagenic properties of dust in mammals in vivo, taking into account organ specificity. In the experiment, the cytogenetic effect was evaluated in the body's barrier systems, which are target organs:

The duration of the experiment was determined by the time of cellular renewal of tissues, so that the maximum number of cells of different organs passed the mitotic cycle. The chronic experiment used was due to the time of renewal of the epithelium of the organs studied. The micronuclear test is based on recording the increase in the frequency of cells with micronuclei in the organs of experimental animals.

Biochemical methods of investigation in various organs of experimental animals. Analysis of biochemical indices of endogenous intoxication by generation of nitric oxide, lipid peroxidation and system antioxidant protection.

To identify the shifts that occur in the body of experimental animals subjected to physical stress, integrated research methods were used, the use of which helped to identify the intensity of the physical load and the general characteristics of metabolic processes in the body: body mass dynamics, muscle dynamometry, and SPP. In this connection, during the experiments of 1 and 2 series, an «open field» test was carried out on the first day, in the middle and at the end of it in the 2nd series of the experiment. In the first series of experiments, the «open field» test was carried out at the first and at the end of the experiment.

The change in body weight of animals is a sensitive integral indicator of the body. The decrease or insufficient weight gain of the experimental groups of animals in comparison with the control was regarded as an unfavorable effect of physical activity, provided that the animals were kept in the same condition. The nervous system reacts most early to all changes in the external and internal environment. To assess the functional state, we used the method of determining the ability of the central nervous system to summarize the subthreshold pulses. The summation-threshold value (SPR) in rats was determined with the help of a pulse electronic stimulator «IES-01».

Toxico-hygienic characteristics of the effect of PMSM in intratracheal administration at a dose of 50 mg/ml.

Dust of atmospheric air in Temirtau was selected on the territory of a kindergarten located in the center of a residential area at a distance of 2 km from the industrial complex «Mittal Steel Temirtau» and 2.5 km from the motorways. The composition of dust included the following metals: lead, zinc, nickel, chromium, copper, arsenic, manganese, beryllium, cobalt, cadmium. The content of metals in dust fluctuated at the level of «small» values from $3 \cdot 10^{-3}$ to 10^{-7} %. Dispersion of dust to 5 microns was 80 %, up to 8 microns — 20 %. The organic part of the dust was a complex composition of humic acids, forming chelate compounds with metals and structured elements of soot.

At the first stage, dust at a dose of 50 mg/ml was administered intratracheally once. The trial period was 3 and 70 days. After 3 days, BAL was examined, reflecting lung function as a target organ for assessing early changes.

The results of the study showed that after 3 days in the BAL fluid of the experimental group, the amount of neutrophils (NF) increased to 7.9 ± 1.3 % compared to the control 4.0 ± 2.1 , which exceeded the control value by 50.6 % (Table 1). A significant accumulation of degenerated NF was detected up to 12 ± 0.6 % ($p < 0.001$). The number of full alveolar macrophages (AM) was significantly decreased 3.3-fold and was 25.3 ± 6.4 %, the amount of degenerated AM was at the level of 51.4 ± 7.8 %, which significantly exceeded the physiological level of the control group of rats in 6,1 times. Analysis of the results of the conducted studies showed that the earliest changes in the respiratory system occur in cells from the side of NF and AM.

**Cytomorphological indices of BAL cells of rats/males after 3 days
with single intratracheal dosing of 50 mg/ml (%)**

Group of animals	NF	NF degener.	AM	AM degener.	lymphocytes (LF)	Ciliated epithelial cells	
						Normal	Degener.
Control n-8	4.0±2.8	0.8±0.4	84.1±4.7	8.4±3.9	1.3±0.01	0.2±0.01	1.2±0.5
Dust n-8	7.9±1.3	12±0.6 ⁺	25.3±6.4 ⁺	51.4±7.8 ⁺	2.1±0.5	0±0	1.3±0.6

Note. Reliability ⁺ <0.001.

The study of cytomorphological indices of thyroid cells in rats/males (Table 2) after 3 days revealed a change in A-cells, which was manifested by the accumulation of its granulated cells to 30.1±5.3 %, 2.4 times higher than the control values, more the expressed changes were from the side of degranulated A-cells, the number of which increased significantly by 3.3 times.

Table 2

**Cytomorphological indices of thyroid cells of rats/males after 3 days
with single intratracheal administration of 50 mg/ml dose (%)**

Group of animals	A-cells		B-cells		C-cells	
	Pellets	Degranul.	Pellets	Degranul.	Pellets	Degranul.
Control n-8	12.5±3.2	3.6±1.05	17.6±4.2	10.7±1.9	48.4±1.9	7.2±0.7
Dust n-8	30.1±5.3 ⁺	12±1.8 ⁺⁺	24.3±4.0	16.5±3.3 ⁺	14.0±2.9 ⁺⁺⁺	3.1±0.6 ⁺⁺

Note. Reliability ⁺ <0.05; ⁺⁺ <0.01; ⁺⁺⁺ <0.001.

From the side of B cells, significant changes in only degranulated cells were revealed, whose level increased 16.5±3.3 (p <0.05). Granulated and degranulated forms of C-thyroid cells in the rats of the experimental group were significantly reduced in 3.4 (p <0.001) and 2.3 times (p <0.01) compared with control values of 48.4±1.9 % and 7.2±0.7 % respectively.

Thus, the results obtained indicate that a dose of 50 mg/ml contributes to a change in the cellular composition of the thyroid gland, which is apparently due to the production of thyroid hormones that stimulate protein synthesis, the immune system, and provide a complex of adaptive responses.

Analysis of the results of the experiment for a period of 70 days after seeding the animals (Table 3) revealed a significant decrease in the number of full-fledged AM in the BAL by 2.9 times, which was 28.1±5.5 % (p <0.05). The number of degenerated AM significantly increased to 63.3±5.0 (p <0.05), which was 7.5 times higher than the physiological level of the animals in the control group. A significant accumulation of degenerated ciliated epithelial cells was found up to 8.6±1.2 %, which is 5.6 times higher than the physiological oscillations of the control group 1.3±0.4 %.

Table 3

**Cytomorphological indices of BAL cells of rats/males after 70 days
with single intratracheal administration of 50 mg/ml dose (%)**

Group of animals	NF	NF degener	AM	AM degener.	lymphocytes (LF)	Ciliated epithelial cells	
						Normal.	Degener.
Control n-8	4.1±1.9	0.82±0.32	82.9±3.9	8.3±2.9	1.2±0.01	0.2±0.01	1.3±0.4
70 days(n = 8)	0±0	0±0	28.1±5.5 ⁺	63.3±5.0 ⁺	0±0	0±0.0	8.6±1.2 ⁺

Note. Reliability ⁺ <0.05.

The results of the analysis of the conducted studies on the 70 days of cytomorphological indices of thyroid cells revealed a significant accumulation of granulated A cells 35.8±0.9 %, 2.8 times higher than the reference values of 12.5±3.2 % (Table 4). A similar situation was observed from the side of degranulated B-cells 18.8±1.5 % (p <0.01). The content of C-cells of granular and degranulated form was significantly decreased. The granular form of the C-cells was 6.6±0.6 % (p <0.001) and the non-granulated form the C-cell content was 3.2±0.4 % (p <0.001), respectively.

Table 4

**Cytomorphological indices of thyroid gland cells of rats/males after 70 days
with single intratracheal dosing of 50 mg/ml (%)**

Group of animals	A-cells		B-cells		C-cells	
	Pellets	Degranul.	Pellets	Degranul	Pellets.	Degranul
Control n-8	12.5±3.2	3.6±1.05	17.6±4.2	10.7±1.9	48.4±1.9	7.2±0.7
70 days (n = 8)	35.8±0.9 ⁺⁺	7.9±1.8	27.7±4.1	18.8±1.5 ⁺	6.6±0.6 ⁺⁺	3.2±0.4 ⁺⁺

Note. Reliability ⁺ <0.01; ⁺⁺ <0.001.

The decrease in C-cells that are responsible for the production of calcium can be characterized as a change in the sensitivity of mineral metabolism when exposed to PSCMM.

Analysis of cytomorphological parameters of liver cells (Table 5), as a detoxification organ, revealed the accumulation of degenerated light cells (DSC) to 10.6±2.6 %, a significant increase in the number of neutrophils and Kupffer cells by 2.9 times (p <0.05) and 5.5 times (p <0.05), respectively. Accumulation of neutrophils and Kupffer cells is associated with the processes of reaction of the primary link of liver protection to the damaging effect of dust. It is believed that neutrophils and Kupffer cells have a pronounced cytomorphological activity in relation to various cells, especially transformed cells.

Table 5

**Cytomorphological parameters of liver cells after 70 days
with single intratracheal administration of 50 mg/ml dose (%)**

Group of animals	Hepatocytes			EF	NF	Cells Kupfer
	TK	SK	DSK			
Control n-8	39.1±5.5	47.7±5.7	6.4±1.1	11.2±0.6	4.4±0.8	1.2±0.2
Dust n-8	30.2±3.8	39.6±5.08	10.6±2.6	0±0	13.0±3.06 ⁺	6.6±2.4 ⁺

Note. reliability ⁺ <0.05.

To determine the mutagen load under the effect of PSCMM in the experiment for 3 and 70 days, a micronuclear test (MIC) was performed in polychromatophilic erythrocytes (PCE) of bone marrow in rats / males.

At the end of the 70-day period after intratracheal administration of dust at a dose of 50 mg/ml, the physiological parameters of the experimental animals were studied. Analyzing the weight index at a given dose of dust, it can be noted that in the experimental group there was a decrease in weight after 3 days of the experiment, and a significant decrease in this index after 70 days (p <0.001).

Since the moment of dusting in the experimental group of the three-day experiment, almost all the indicators of the «open field» test have clearly changed. Thus, when analyzing the data of general toxicity when exposed to dust at a dose of 50 mg/ml, it is possible to note a significant decrease in weight and grooming of the animals of the experimental group, in an experiment for a period of 70 days. The changes revealed during the «open field» test can be interpreted as the result of a reaction from the nervous system of experimental animals caused by exposure to dust of complex chemical composition.

Conclusion

Technogenic load metals entering the composition of the dust, exceeded the background values in the tens and hundreds of times, but did not reach the absolute content of the normative values. At the same time, it was revealed that the content of chemical elements in the snow cover of the city of Temirtau is higher in comparison with the background values. The content of heavy metals in the snow cover was revealed ten times higher than the background — nickel, arsenic, mercury. Several times — manganese, lead, zinc, chromium. These metals can be attributed to the priority pollutants of the city's environment according to snow sampling data. In general, there was a high level of air pollution — through dust, dry residue of snowmelt snow.

Analyzing the data of general toxicity when exposed to dust at a dose of 50 mg/ml, a significant decrease in the weight and muscle strength of the animals was observed, at the end of the experiment in both experimental groups. The changes revealed during the «open field» test can be interpreted as a result of in-

toxication of the nervous system of experimental animals caused by exposure to dust of complex chemical composition.

Thus, the mutagenic effect of the dust under study was revealed on the basis of the results of the micronuclear test and taking into account chromosomal aberrations in the cells of the bone marrow of experimental animals. Analysis of cytogenetic studies allowed to come to the conclusion that PMSC induces a mutagenic effect in experimental animals, depending on the time of exposure.

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Қоршаған ортаның әртүрлі ластағыш заттарының зертханалық жануарлар ағзасының метаболикалық күйіне әсері

Мақалада металдардың микроконцентрациясы бар шанды эксперименттік зерттеудің нәтижелері келтірілген. Ингаляцияға және химиялық қосылыстардың ауыз қуысының тұтынуына канцерогенді қауіптің сандық сипаттамалары белгіленді. Қолайсыз экологиялық факторларды бағалау бойынша кешенді экологиялық зерттеулердің нәтижелері, дисфункционалды аймақтың аймағында тұратын халық арасында қатерлі ісік ауруларының канцерогенді даму қауіпі кейбір табиғи орталардың (ауаның, судың, қардың, топырақтың) химиялық ластануынан туындайтын экологиялық және гигиеналық жағдайдың ерекшеліктерін анықтайды. Денедегі метаболикалық процестердегі шаңның құрамында металдардың төмен концентрацияларының рөлі эксперименттік түрде орнатылды, дозаға тәуелді өзгерістер анықталған, химиялық қосылыстардың уыттылық механизмі қан ағымына еніп, ішкі ағзалардағы тотығу стрестерін тудыратын шаңның корпускулалық микробірліктерінің бұзылуында жасушалық механизмдердің тиімсіздігі болып табылады.

Кілт сөздер: қоршаған орта, шаң, эксперименталдық жануарлар, химиялық ластағыштар, уыттылық, биологиялық эквивалент, шекті рұқсат етілген шоғырлану, биосфера, топырақ, цитоморфологиялық индекс.

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Влияние различных поллютантов окружающей среды на метаболический статус организма лабораторных животных

В статье приведены результаты экспериментальных исследований пыли, содержащей комплекс металлов в микроконцентрациях. Установлены количественные характеристики канцерогенного риска при ингаляционном и пероральном поступлении химических соединений. Приведены результаты комплексных экологических исследований по оценке неблагоприятных факторов окружающей среды, канцерогенного риска развития онкологических заболеваний среди населения, проживающего в неблагоприятной зоне. Определены особенности эколого-гигиенической ситуации, обусловленные химическими загрязнениями некоторых природных сред (атмосферный воздух, вода, снег, почва). Экспериментально установлена роль низких концентраций металлов в составе пыли на метаболические процессы в организме, определены дозозависимые изменения. Показано, что пусковым механизмом токсичности химических соединений является неэффективность клеточных механизмов в расщеплении корпускулярных микрочастиц пыли, проникающих в ток крови, провоцируя усиленный окислительный стресс во внутренних органах.

Ключевые слова: окружающая среда, пыль, экспериментальные животные, химические загрязнители, токсичность, биологический эквивалент, предельно допустимая концентрация, биосфера, почва, цитоморфологический индекс.

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