

FEATURES OF THE BIOLOGICAL RESPONSE OF THE ANIMAL ORGANISM AT SIMULTANEOUS FOUR-MONTH AEROGENIC IMPACT OF BI-, TRI-, TETRO-COMPONENT CHEMICAL MIXTURES AND DEVELOPMENT OF THEIR PERMISSIBLE VALUES IN THE ATMOSPHERIC AIR.

MirzakarimovaMalokhat, Doctor of Biological Sciences, Senior Researcher, Head of the Atmospheric Air Hygiene Laboratory, The Scientific Research Institute of Sanitation, Hygiene and Occupational Diseases of the Ministry of Health of Uzbekistan, Uzbekistan, e-mail: mirzakarimova.malokhat@mail.ru.

KamiljanovAbduvahit, Doctor of Medical Sciences, Professor, The Scientific Research Institute of Sanitation, Hygiene and Occupational Diseases of the Ministry of Health of Uzbekistan, Uzbekistan

AdilovUtkir, Doctor of Medical Sciences, Senior Researcher, Research Institute of Sanitation, Hygiene and Occupational Diseases of the Ministry of Health of Uzbekistan, Uzbekistan.

KhashirbaevaDinora, Doctor of Medical Sciences, Associate Professor of the Department of Medical and Biological Disciplines, Tashkent Pharmaceutical Institute, Uzbekistan

Abstract:

Background: Among the many hygiene problems of a large industrial city, one of the most important is the protection of the environment from pollution by emissions from motor vehicles and industrial enterprises. In the case of chemical pollution of the environment, very resistant and capable of bioaccumulating in large quantities chemicals - substances that have carcinogenic or mutagenic properties or have adverse effects, in particular on the reproductive, endocrine, immune and nervous systems - pose a particular threat to public health. The development of various sectors of the national economy (non-ferrous and ferrous metallurgy), chemical industry, production of synthetic fibers, pesticides and mineral fertilizers and thermal power plants and motor transport in cities is associated with the emission into the atmosphere of a bouquet of toxic substances containing gas, steam and aerosol mixtures of 1-4 classes of danger: hydrogen fluoride (Hf), ammonia (Am), formaldehyde (Fo), acetate lead (Al), dust (D), phenol (Ph-ol), sulphur dioxide (SD), nitrogen dioxide (ND), suspended solids

(Ss). Therefore, the establishment of hygienic regulations only in the isolated intake of substances is insufficient.

Objective: To experimentally determine the peculiarities of the biological response of the rat organism under simultaneous four-month permanent aerogenic exposure of bi-, tri-, tetrocomponent chemical mixtures and development of their permissible values in the atmospheric air.

Methods: A chronic experiment was carried out on 375 male rats weighing 100-130 grams, which were divided into 6 series, with 1, 2, 3, 4 and 5 series having 4 groups and 6 series having 5 groups of 15 individuals each. For inhalation action in the experiment, 200-liter chambers were used, proposed by B.A. Kurlyandsky.

Results: With round-the-clock inhalation inoculation of rats, both binary and three-, four-component complex mixtures in relatively high concentrations, the animals showed general toxic and gonadotoxic effects, which were expressed in functional changes of the CNS, liver and kidney function, reducing the non-specific resistance of the animal body, changing the functional state of sperm and the ability of male rats to fertilize. The results of studying the biological effect of binary mixtures on the example of Hf + Am and Al + Ph-ol on the animal body were found that at the total intake of the biological effect of chemicals is characterized by the dependence of "concentration - effect" and there is an "increase in effect." Analysis of changes in biochemical, physiological, hematological, embryo and gonadotoxic indicators arising in the animal body indicates that Hf + Am and Al + Ph-ol at the combined intake in chronic experiments also have a general toxic effect, manifested in violation of the CNS, liver, blood, enzymes and functional state of sperm.

The results of the study of the combined resorptive action of small concentrations of a combination of three and four chemicals on the example of phenol + formaldehyde + lead acetate, as well as combinations of lead acetate + formaldehyde + sulfur dioxide + ammonia indicate that the nature of the combined action of substances of these mixtures is shown by the effect of "total summation." Consequently, in the development of air protection measures, the concentrations of individual substances in a mixture of phenol + formaldehyde + lead acetate should not exceed 0.33 MPC of each ingredient separately and 0.25 MPC - in a mixture of lead acetate + formaldehyde + sulfur dioxide + ammonia.

Conclusions: The nature of the biological response of the body, the combined effects of bi-, tri-, and tetrocomponent chemical mixtures in a constant inhalation of animals depends on the number of components that make up the mixture, the physical and chemical properties of the ingredients, their aggregate state and hazard class. In this case, the nature of the combined action of binary and complex mixtures with 3 and 4 components at inhalation intake into the animal body is manifested by the type of "summation effect" or "partial summation." In the atmospheric air of binary and complex mixtures, each substance at the "summation effect" of 0.25 and 0.33 or incomplete summation should not exceed 0.61, 0.68, 0.61 and 0.46 MPC, respectively - at their solo action.

Keywords: Atmospheric air, experimental research; maximum permissible concentrations; combined action factor; bi-, tri-, tetrocomponent mixtures of chemical compounds; hydrogen fluoride; ammonia; lead acetate; formaldehyde; phenol; sulphur dioxide; nitrogen dioxide; suspended substances; dust.

Introduction:

The development of various sectors of the national economy (non-ferrous and ferrous metallurgy, woodworking industry, chemical industry, production of synthetic fibers, pesticides and mineral fertilizers, thermal power plants and motor transport) in cities is associated with the emission into the atmosphere of a bouquet of toxic substances containing gas, steam and aerosol mixtures. [2 p.197-203; 9 p.15-36; 10 p.9-13; 11 p.53-58; 12 p.72-75] Therefore, the establishment of hygienic regulations only at the isolated inflow of substances is insufficient. [1 p.16-20]. This makes it necessary to study the effects of the combined effects of chemicals in environmental facilities. [3 p.1171-1178, 6 p.75-78; 7 p.27-28; 8 p.11-39]. One of the most common combinations in the atmospheric air of populated areas of the republic: Tashkent, Almalyk, Angren, Bekabad, Samarkand, Fergana, Kokand are complex mixtures of vapor, gas and aerosols of chemicals - formaldehyde (2 class of hazard), ammonia (4 class of hazard), sulfur dioxide (3 class of hazard) and lead (1 class of hazard) [4 p.809-812, 5 p.293-296].

The research objective. To experimentally determine the peculiarities of the biological response of rat organisms at simultaneous four-month permanent aerogenic influence of bi-, tri-, tetrocomponent chemical mixtures and development of their permissible values in the atmospheric air.

Research materials and methods. Toxicological experiments were carried out on organism of experimental animals on combined hygienic rationing in the combination of binary and complex mixtures of harmful substances in atmospheric air, most often occurring in the air basin of industrial cities of the republic: hydrogen fluoride + ammonia; lead acetate + formaldehyde; ammonia + formaldehyde + dust; phenol + formaldehyde + lead acetate; sulfur dioxide + nitrogen dioxide + hydrogen fluoride + suspended matter; lead acetate + formaldehyde + sulfur dioxide + ammonia. The following set of tests was used: animal weight and behavior, PPS, serum lysozyme activity, the activity of cholinesterase and blood catalase, lactic acid content, the content of AST, ALT, SDG, serum alkaline phosphatase, SH-group, the content of white blood cells, erythrocytes and hemoglobin, functional sperm motility on the 3-rd indicators, fertilization ability.

The chronic experiment was carried out on 375 male rats weighing 100-130 grams, which were distributed into 6 series, with 1, 2, 3, 4 and 5 series having 4 groups and 6 series having 5 groups of 15 individuals each. The studies were conducted under the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (Strasbourg, 18 March 1986 ETS N 123.). For inhalation, 200-liter chambers proposed by B.A. Kurlandsky were used in the experiment. The actual concentrations of chemical ingredients entering the animal organism in the seed chambers are presented in Table 1.

Table 1

Actual concentrations of binary and complex mixtures of chemical ingredients entering the animal body in seed chambers

Chemical composition of the mixture	№ groups	Chemical ingredient name, in mg/m ³										Σ
		suspended solids	hydrogen fluoride	sulphur dioxide	nitrogen dioxide	Phenol	Formaldehyde	lead acetate	Ammonia	Dust	Fresh air	
Hydrogen fluoride ammonia	I II III IV		0,1 0,02 0,0015 Contr.						0,29 0,13 0,025		+	47,25 11,2 1,22
Formaldehyde lead acetate	I II III IV						0,1 0,012 0,002 Contr.	0,015 0,001 0,0002			+	83,3 7,33 1,33
Formaldehyde Ammonia Dust	I II III IV						0,036 0,010 0,002 Contr.		0,5 0,1 0,02	0,75 0,30 0,10	+	29,5 7,83 1,82
Phenol Formaldehyde Lead acetate	I II III IV					0,1 0,003 0,001 Contr.	0,1 0,003 0,001	0,012 0,0003 0,0001			+	10,6 3,0 1,0
Weighing things up Hydrogen fluoride Sulfur dioxide Nitrogen dioxide	I II III IV	1,61 038 0,02 Contr.	0,046 0,008 0,0014	0,58 0,25 0,03	0,38 0,12 0,012						+	71,7 18,80 1,86

Sulfur dioxide	I			1,49			0,62	0,042	1,40		+	411,4
	II			0,62			0,30	0,0020	0,60			134,0
Formaldehyde	III			0,05			0,0035	0,0005	0,040			5,3
	IV			0,013			0,0008	0,00008	0,010			1,0
Lead acetate	V			Contr.								
Ammonia												

The determination of chemical content in the air of seed chambers was carried out daily colorimetric method. Physiological, biochemical, hematological, gonadotoxic indicators were studied in a 4-month chronic experiment before the beginning of studies (background) on 15, 30, 45, 60, 75, 90, 105, 120 days and in the recovery period in laboratory animals. Data on the resorptive action of the studied substances in the chronic experiment were statistically processed, with the calculation of Student's criterion (t) and the probability of error (P). The difference in mean values was considered reliable at the level of significance $P < 0.05$.

Results. When studying the biological effect of binary mixtures on the example of Hf + Am and Al + Ph-ol on the animal body, it was found that the total biological effect of chemicals is characterized by the dependence of "concentration - effect" and there is an "increase in effect." Analysis of changes in biochemical, physiological, hematological, embryo and gonadotoxic indicators arising in the animal body indicates that Hf + Am and Al + Ph-olat the combined intake in chronic experiments also have a general toxic effect, manifested in violation of the CNS, liver, blood, enzymes and functional state of sperm.

The results of the study of the action of a complex mixture of 3 and 4 ingredients in the long run in the body indicate a direct relationship between the response of experimental animals on the level and duration of exposure to the studied ingredients. Thus, on the example of experiments on the action of complex mixtures containing Am (0.5 mg/m^3) + Fo (0.036 mg/m^3) + P (0.75 mg/m^3), it was found that chronic inhalation of the studied mixture after 60 days in animals of the 1st group caused a change in the activity of cholinesterase in the blood ($331.65 \text{ } \mu\text{g. ml/min}$ vs. $386.8 \text{ } \mu\text{g. ml/min}$ in control). AST ($1.85 \text{ } \mu\text{. mole/ml}$ vs. $1.91 \text{ } \mu\text{. mole/ml}$ in control), catalase number (7.92 vs. $6.91 \text{ } \mu\text{.mole/ml}$ in control), which later after 90 days were more expressed and were statistically reliable ($P < 0.05-0.01$).

Statistically significant increase in ALT, SDH activity and decrease of sulfhydrylic groups in animals of this group was registered at the end of the 3rd month of inhalation with the mixture, and by the end of the 4th month, the revealed changes of the studied indicators in animals of the 1st group deepened and acquired a higher degree of reliability. The study of urea content and peripheral blood pattern showed statistically reliable changes only at the end of the 4th month of the experiment.

When analyzing the data of the functional state of spermatozoa, it was established a reliable reduction of their mobility time (230 ± 16.74 vs. 283.3 ± 7.45 vs. control, $P < 0.05$) and osmotic resistance of spermatozoa (2.125 ± 0.125 vs. 2.5 ± 0.06 , $P < 0.05$) in the animals of the 1st group by the end of their vaccination. The results of the study of male

fertilization ability allowed to reveal a reliable increase in the total embryonic mortality of fetuses in females paired with males of the 1st group.

The action of a mixture of ammonia, formaldehyde and dust in concentrations of 0.1, 0.01, and 0.3 mg/m³ also manifested itself in violation of some studied parameters (cholinesterase, AST, erythrocytes, leukocytes, hemoglobin, SDH). However, the marked changes occurred at a later date, were unstable and had a lower degree of reliability of differences between the results of the experimental and control groups.

In the animals of the 3rd group exposed to a mixture of ammonia, formaldehyde and dust in concentrations of 0.02, 0.002 and 0.1 mg/m³, no deviations of the researched indicators from the control data were found. Therefore, the changes noted in rats in a chronic 4-month experiment on the part of biochemical, physiological, hematological indicators indicate that under the influence of a mixture of ammonia, formaldehyde and dust in concentrations exceeding the average annual MPC in 32.9 times, there are reversible changes in the function of the CNS and enzymes. Concentrations of ammonia, formaldehyde and dust mixture in concentrations of 0.1, 0.01 and 0.03 mg/m³ were threshold, while 0.02, 0.002 and 0.1 mg/m³ were inactive.

The results of experimental studies on the resorptive action of vapor, gas and aerosol complex mixtures of I and II hazard classes show that the joint action of the mixture As, F and F-ol at the level of 0.012, 0.1 and 0.1 mg/m³, respectively, at 120 day chronic round-the-clock inoculation caused a statistically significant reduction of PPS, SH-groups, increase of ALT, AST, throughout the whole period of the experiment, starting from 60 and 75 days of inoculation.

At a decrease in the concentration of substances in the mixture of Al (40 times), FA (33 times), Ph-ol (33 times), statistically reliable shifts of the studied indicators in animals of group II were not observed, except for SH-groups and PSC. It should be noted, however, that the shifts of SH-group and PSC were less pronounced and short-lived, and came much later, only once at the end of the 120th day of the experiment. On the 120th day of the experiment, an increase of PSC was observed (8.33±0.35 vs. 6.6±0.52 imp/s - in control (P<0.05) and a decrease of SH-group in the blood (62.8±1.32 at 72.17±1.65 mg% - in control, P<0.05). Inhalation of the mixture Al (0,0001 mg/m³), Fo (0,001 mg/m³) and F-ol (0,001 mg/m³) did not cause any deviations in the organism of the rats of the III group in comparison with the animals of the control group, i.e. these concentrations were inactive. Calculated based on the sum of personal values obtained by dividing the experimentally determined inactive concentrations of Al, FA and F-f-ol by the annual average MPC of these substances in the atmospheric air, it was found that Kd of mixture

Al F and Ph-olat 4-month chronic experiment was equal to 1 and was estimated as "summation."

Table 2

Comprehensive assessment of the impact of gas, gas-aerosol and gas-vapor-aerosol mixtures on the body in case of their prolonged inhalation supply

№ Mixtures	Hazardous Substances Name	Hazard class	Aggregate condition	Combined action		Permissible share in the mixture of each substance	Normative document (SanPiN, methodological guidelines)
				coefficient	feature		
1	Hydrogen fluoride Ammonia	2 4	gaz gaz	1,22	Incomplete sum	0,61 MPC	SanPiN№0293-11 (2011.)
2	Lead acetate Formaldehyde	1 2	mist gaz	1,36	Incomplete sum	0,68 MPC	
3	Ammonia Formaldehyde Dust	4 2 3	gaz gaz mist	1,82	Incomplete sum	0,6 MPC	
4	Phenol Formaldehyde Lead acetate	2 2 1	vapour gaz mist	1,0	Complete sum	0,33 MPC	№012-3/0287 (2016.)
5	Sulfur dioxide Nitrogen dioxide Hydrogen fluoride Weighted substances	3 2 2 3	gaz gaz gaz mist	1,86	Incomplete sum	0,46 MPC	SanPiN№0293-11 (2011.)
6	Lead acetate Formaldehyde Sulfur dioxide Ammonia	1 2 3 4	mist gaz gaz gaz	1,0	Complete sum	0,25 MPC	№012-3/0287 (2016.)

Thus, aerogenic 120-day exposure to Al (0.012 mg/m³), Fa (0.1 mg/m³) and F-ol (0.01 mg/m³) has a negative impact on the rat body, causing statistically reliable changes in the functional indicators of the CNS, blood liver and the functional status of sperm. The coefficient of combined action on the results of comparative analysis of the obtained data 1, 2, 3, 4, 5 and 6 series of experience showed that the coefficient of combined action at

the level of small concentrations coincides and is estimated as "summation" (Ccd-1) or by the type of effect "partial summation" (Ccd 1,22-1,86) (Table 2). This is probably explained by the fact that in the mechanism of toxic action of studied substances on the body, there is a general similarity (oppression of metabolic processes underlying the vital functions of cellular structures of the body, blocking the SH-group, etc.) - unidirectional action.

Conclusion

1. Gas-and-gas, binary mixture of hydrogen fluoride (class 2) + ammonia (2) and aerosol-gas mixture of two substances lead acetate (class 1) + formaldehyde (class 2) with simultaneous prolonged 4-month inhalation of the nature of the combined action of the studied mixture of substances is shown by the type of incomplete "summation".

2. 3-component gas-gas-aerosol mixture - ammonia (4 class) + formaldehyde (2 class) + dust (3 class) at simultaneous 4-month constant inhalation input the character of the combined action can be estimated as "partial summation". And under the influence of similar aggregate mixture - phenol (class 2) + formaldehyde (class 2) + lead acetate (class 1) as "summation".

3. Tetrocomponent mixture: sulfur dioxide (class 3) + nitrogen dioxide (class 2) + hydrogen fluoride (class 2) + suspended solids (class 3) gas-gas-aerosol, with simultaneous 4-month inhalation, the nature of the combined action can be estimated as an incomplete summation, and with exposure to lead acetate (class 1) + formaldehyde (class 2) + sulfur dioxide (class 3) + ammonia (class 4) as a summation.

4. the nature of the biological response of the combined impact of bi-, tri-, tetrocomponent chemical mixtures under constant inhalation of animals depends on the number of components in the mixture, the physical and chemical properties of the ingredients, their aggregate state and class of hazard. In this case, the nature of the combined action of binary and complex mixtures with 3 and 4 components at inhalation intake into the animal body is manifested by the type of "summation effect" or "partial summation." In the atmospheric air of binary and complex mixtures, each substance at the "summation effect" of 0.25 and 0.33 or incomplete summation should not exceed 0.61, 0.68, 0.61 and 0.46 MPC, respectively - at their solo action.

Conflict of interest. All authors state that there is no potential conflict of interest that should be disclosed in this article.

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