EXPERIMENTAL MODELS AND INSTRUMENTAL SURVEYS FOR RISK ASSESSMENT IN HYGIENE AND EPIDEMIOLOGY

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CHANGES IN THE NERVOUS SYSTEM STATE AND PERIPHERAL BLOOD PARAMETERS UNDER BENZENE INTOXICATION DURING AN EXPERIMENT

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Benzene is a widely spread chemical health risk factor. Our research goal was to examine the nervous system state and the blood system state under benzene intoxication during an experiment. An acute experiment was performed on 45 white mice with 5-fold poisoning with benzene; a chronic one was performed on 72 rabbits being under inhalation exposure to benzene during 4 months, its concentrations increasing and fluctuating. We determined the following blood parameters: number of reticulocytes, eosinophils, basocytes, and erythrocytes; erythrocytes sedimentation rate; blood clotting period; blood clot retraction; plasma re-calcification period; plasma tolerance to heparin; prothrombin time; prothrombin index; fibrinogen concentration; blood fibrinolytic activity; acetylcholine and choline esterase contents. We also determined adrenalin, noradrenalin, dopamine, and dihydroxyphenylalanine contents in urine.

Acute experiments results revealed that one-time exposure to benzene exerted a narcotic effect on the central nervous system which had an excitation phase and inhibition phase. Under a repeat exposure to benzene animals' drug intoxication was shorter. And here neutrophils / leucocytes gradient first increased to 139.5 % from its standards value and then when down under consequent intoxications.

We detected relevant changes in morphological picture of animals' peripheral blood and their central and vegetative nervous system under chronic exposure to intermittent and increasing benzene concentrations.

So, our research revealed that effects exerted by benzene in small concentrations led to apparent shifts in white blood and catecholamines (adrenalin, noradrenalin, dopamine, and dihydroxyphenylalanine). We also detected certain signs that catecholamines endogenous reserves (dihydroxyphenylalanine) were depleted and, and also signs of eosinophils-basocytes dissociation; such prognostic signs were considered to be unfavorable as it was exactly at that moment of time (the 4th month of poisoning) when substantial changes (leucopenia, granulopenia, lymphopenia, and monocytopenia) occurred in blood. Fluctuating benzene concentrations exerted more apparent toxic effects in comparison with simply increasing toxicant concentrations.

Key words: *benzene poisoning, intoxication, nervous system, drug intoxication, hemogram, catecholamines, acetylcholine, choline esterase.*

Oil-refinery and petrochemical plants tend to have a lot of potentially hazardous chemicals in working areas air; these chemicals are chemical risk factors both for workers employed at these enterprises and population in general [12, 15].

Petrol, acetone and benzene are the most widely used chemicals among those produced by petrochemical industry. Benzene is the most toxic substance [5, 10]. It exerts effects close to those of drugs on the central nervous system (CNS) when it is in high concentrations; and it can become a poison for blood and blood-making organs when it is introduced chronically in low doses [13, 14, 17]. It is also known, that,

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depending on concentrations penetrating a body, benzene can cause various pathological reactions. And clinical signs can be absent during a long period of time but impacts exerted by benzene on a body are truly dangerous in terms of long-term effects as they can lead to leucosis, genetic disorders, and depletion of overall adaptive and protective responses complex [12, 15].

Nowadays, a correlation between the leukocytic reaction and neurohumoral and endocrine changes in a body is established [9, 14]. Thus, when the sympathetic nervous system is excited, leukocytes, eosinophils, and neutrophils grow in number; and when adaptive adrenal cortex hormones are active, eosinophils number drops by 50% or more [4, 9]. It is considered to be correct to make judgments on mineral corticoids activity as per value of neutrophils to leukocytes ratio [9, 17].

Allowing for this strong correlation between the neuro-endocrine system state and the peripheral blood, we set our research goal: to examine a state of these two systems under an acute and chronic intoxication with benzene

Data and methods. We performed acute and chronic experiments on two groups of laboratory animals (white mice and rabbits).

An acute experiment was performed on 45 non-linear white mice grown in the vivarium of the Scientific Research Center at Azerbaijan Medical University. Animals weighed 18-22 grams and were divided into 2 groups: the first one was intact animals, the second underwent inhalation poisoning with benzene with repeating 2-day breaks.

72 rabbits of "chinchilla" species underwent chronic benzene poisoning. The intoxication was done daily during 4 months, 4 hours a day with one day a week which was free from it, and there was 1-month recovery period after the poisoning was over. Average poisoning benzene concentration in chambers was equal to 1240±82 mg/m3. The experimental animals were divided into 3 groups: group 1 was poisoned with growing benzene concentrations, group 2 was exposed to intermittent concentrations, and group 3 was a reference one, without any exposure to benzene.

We applied benzene PFA (pure for analysis) as our poisoning substance (State Standard 5055-75)[1]. Benzene concentrations in poisoning chambers were checked regularly via air analysis. Air in chambers was constantly mixed via ventilation systems.

We introduced 0.25 of threshold benzene concentration each 15 minutes to achieve growing concentrations mode allowing for benzene hazardous effects threshold being equal to 2000 mg/m^3 [13].

To achieve benzene intermittent concentrations, we introduced benzene into chambers each 30 minutes in various quantities, to make concentrations fluctuate significantly. Sometimes a chamber was open for 20 minutes ventilating. And here concentrations sequences changed every day during 12 days (Table 1), and then were repeated in the same order during 4 months.

Blood tests were taken in the morning, before feeding, from an ear border vein (rabbits) and from a caudal vein (mice). Reticulocytes number was calculated in preparations dyed with brilliant cresyl-violet via Pappenheim technique. To make chamber calculation of eosinophils, we diluted blood with a liquid offered by I.S. Piralashvili, basocytes chamber calculation was performed as per M.P. Vilchinskiy's modification [2].

[1]State Standard 5955-75. BENZENE reagents: The USSR State Standard. Available at: http://docs.cntd.ru/document/gost-5955-75 (08.07.2017).

Table 1

Animals poisoning with intermittent benzene concentrations

Poisoning days	Benzene concentrations in a chamber, shares from threshold								
1	0,25	2,0	0	5,25	0,5	0	6,0	0	2,0
2	5,0	0	1,75	0	4,0	0,25	0	4,5	0,5
3	4,5	0,25	0	3,75	0	0,5	0	1,5	5,5
4	2,0	0	4,5	0	2,5	0	5,0	0,25	1,75
5	1,75	0,25	0	4,75	0	1,25	0	6,0	2,0
6	1,25	6,0	0	0,25	0	5,5	0	2,0	1,0
7	0,25	0	2,5	0	4,75	1,5	0	3,75	3,25
8	0,5	1,25	0	5,75	0	2,5	0	1,5	4,5
9	4,75	0	1,25	0	4,5	0	1,5	2,25	1,75
10	2,0	0,25	0	6,75	0	4,0	0	0,5	2,5
11	4,0	0	0,25	0	5,75	0,5	1,5	0	4,0
12	0,25	4,75	1,0	0	3,75	0	1,25	0	5,0

We also examined erythrocytes sedimentation rate and erythrocytes acid resistance. The latter was determined as per I.A. Terskov and I.I.Gitelzon technique, modificaiton by A.I. Vorobiyov [2]; acetylcholine (AC), as per S.Hestrin technique [16], choline esterase (CE), as per E.Sh.Matlin technique [7]. We applied trioxyindole fluorimetric technique for determining adrenaline (A), noradrenalin (NA), dopamine (D), and dihydroxyphenylalanine (DHPA) in a single urine portion [13]. To get insight into the blood coagulating system state, we determined blood coagulation time (BCT), blood clot retraction (BCR), plasma re-calcification time (PRT), plasma tolerance to heparin (PTH), prothrombin time and prothrombin index, fibrinogen concentration, and blood fibrinolytic activity (FA) [1, 6].

Beyond experiments, all the animals were kept and fed under the same conditions. All the experiments on the animals were performed in full conformity with "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasburg, March 18, 1986)[2].

We detected statistically significant discrepancies between the compared groups with Student's parametric criterion. Cause-and-effect relations between exposure factors and their effects were revealed via multi-factor correlation analysis and other mathematical statistics techniques.

Results and discussion. Results obtained in acute experiments revealed that a single exposure to benzene exerted a narcotic effect on the central nervous system which was characterized with excitation and inhibition phases. A repeated exposure to benzene involved shorter periods during which animals suffered from narcotic intoxication. For example, after the 5th exposure, intoxication time decreased from 34.60±3.05 minutes to 12.27±3.71 minutes and it can be considered signs of adaption to benzene effects which evolved in the animals [9, 10].

Table 2

Leuko-formula elements picture under single and repeated exposure to benzene (in % of the background taken as 100)

Leukocytes	Exposure mode	
	Single exposure	Repeated exposure
Leukocytes	73,70	86,70
Eosinophils	41,20	60,0
Neutrophils	87,60	98,30
Lymphocytes	62,80	86,30
Neutrophils/Leukocytes (N/L)	139,50	81,10

Table 2 comprises data reflecting changes in a blood picture under various benzene exposure modes; as we can see from it, after narcotic effects pass, an au-

[2]European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasburg, March 18, 1986. 13 p. Available at: http://docs.cntd.ru/document/gost-5955-75 (10.06.2017).

thentic decrease in leukocytes number and their specific forms occurs. A considerable decrease in leukocytes number makes for neutrophils/leukocyte (N/L) gradient growth up to 139.5% of the background level.

We detected changes in blood components after the repeated exposure to the toxicant; these changes were different from the initial parameters before the first poisoning. There was an increase in leukocytes number with simultaneous N/L gradient fall. Absolute number of eosinophils returned to its initial value before the first poisoning (Table 2).

A significant decrease in erythrocytes number (Figure 1) and hemoglobin was detected in the morphological picture of animals (rabbits)' peripheral blood under chronic exposure to intermittent and increasing benzene concentrations. This decrease was more significant during the first month of chronic poisoning with intermittent concentrations.

By the end of the observations period the red blood composition somehow got back to normal as erythrocytes number was close to background values and to the same parameter in animals from the reference group (it as equal to $9.20\pm0.30x10^{12}$/l in the experimental animals; $10,40\pm0,15x10^{12}$/l, in the reference group animals; background level was equal to $11,30\pm0,40x10^{12}$/l).

The animals from this group also had a decrease in overall erythrocytes sedimentation rate which became more apparent by the end of the fourth month under chronic exposure to benzene (Figure 2).

Together with certain quantitative changes, our research revealed a number of qualitative shifts in the red blood picture, their evidence being changes in erythrocytes acid resistance which can be seen in acid erythrograms deviations to the right. These changes appeared at the end of the second month and it proved young and more acid-resistant erythrocytes occurred in the peripheral blood.

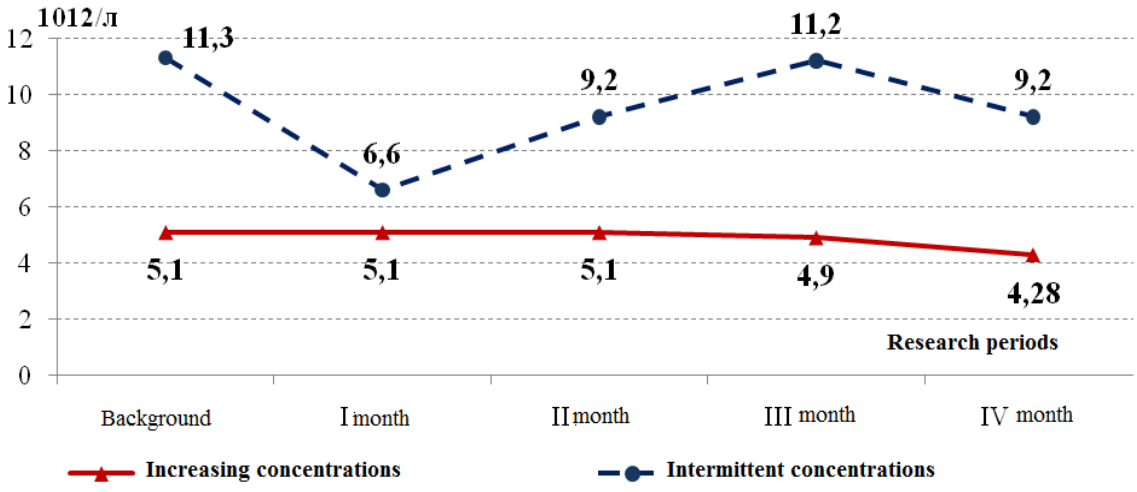

Figure 1. Dynamics of erythrocytes number changes in animals' peripheral blood under various modes of exposure to small benzene concentrations.
(Parameters are authentic against the reference group ($p<0.01$)

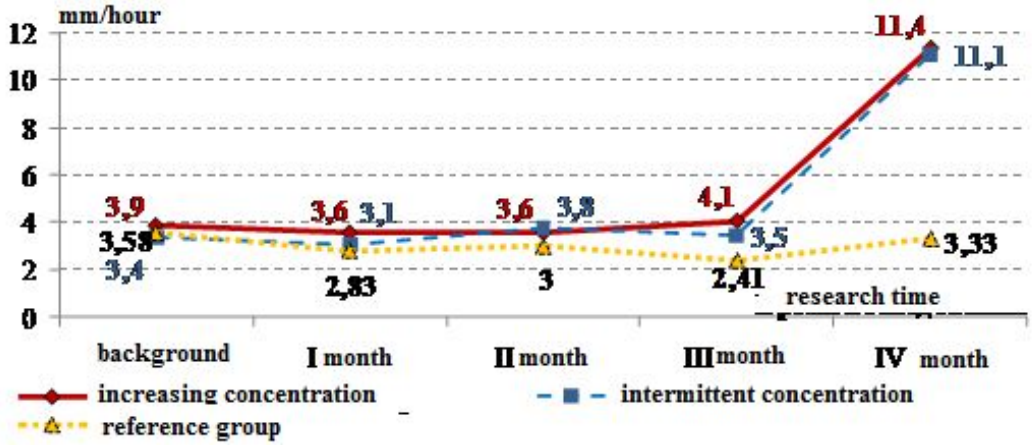

Figure 2. Dynamics of changes in overall erythrocytes sedimentation rate in animals' peripheral blood under chronic inhalation poisoning with benzene
(The parameters are authentic against the reference group ($p<0.05$)

As we can see from Table 3, a month animals' exposure to benzene caused a 61.8% decrease in eosinophils/basocytes gradient due to a drastic growth in basocytes number (187.70%, $p<0.01$), while eosinophils number increased only slightly (107.70%, $p<0.05$).

Table 3

Dynamics in blood coagulation system parameters and eosinophils/basocytes association

Parameters	Research periods and parameters values (in % from the initial level)			
	I month	II month	III month	IV month
Eosinophils/basocyte	61,80	110,20	100,0	112,40
Blood clot retraction	123,1*	128,4*	140,4*	140,0*
Blood coagulation time	112,70	125,50*	144,50*	147,50*
Plasma re-calcification time	109,30	101,20	107,60	106,70
Prothrombin time	04,50	94,0	97,80*	94,0
Prothrombin index	94,10	102,30	99,30	101,20
Plasma tolerance to heparin	115,40	97,60	145,9*	178,8*

Note: * – means these parameters are authentically different from the reference group ($p < 0,02$).

The above-mentioned changes were mostly accompanied with blood clot retraction growth (123.1%, $p<0.02$). We also observed a trend for longer blood coagulation time (112.7%, $p<0.02$) and plasma re-calcification time (109.3%, $p<0.02$).During the subsequent experimental months, there was a gradual growth in blood coagulation time (125.50-147.50%, $p<0.001$) and blood clot retraction (128.4-140.0%, $p<0.01$), but plasma tolerance to heparin increased only after 3 months (145.9%-178.8%, $p<0.02$) (Table 3). These data prove that blood anticoagulation properties grow steadily. But still, we didn't detect any bleeding which was specific for benzene, given all the detected changes in the blood coagulation system; absence of eosinophils-basocytes dissociation fully corresponds to the situation. The latter can be explained by a growth in eosinophils number.

The biggest deviations in leukocytes number from the initial value occurred during the second month of exposure to increased benzene concentrations; the number grew from 9.95 to 13.08 $x10^3$ µl (Figure 3). By the end of the 4th month of poisoning leukocytes number decreased to 5.95±0.49 $x10^3$ µl.

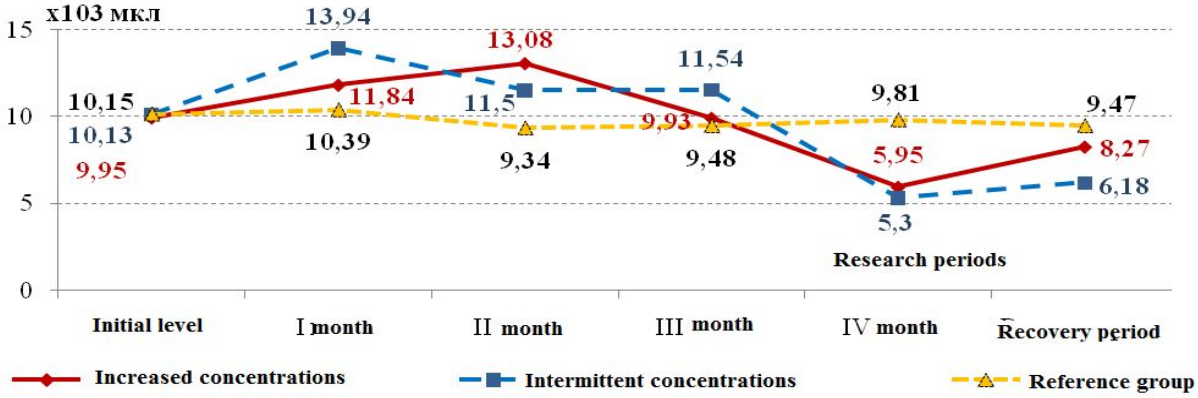

Figure 3. Dynamics of changes in animals' leukocytes in peripheral blood exposed to chronic benzene poisoning (The parameters are authentic against the reference group ($p<0.01$)

The parameters recovered up to their initial level during a month after poisoning stopped; it proves that all the occurred changes are reversible.

Shifts in leukocytic formula of the animals from the second group were more apparent. Thus, wave-like changes in leukocytes and their specific elements content under exposure to benzene in intermittent concentrations were accompanied with a growth in leukocytes number during the first month of poisoning (from 10.13 to 13.94 $x10^3$ µl). By the end of the observations we detected a significant decrease in leukocytes number in the experimental rabbits (5.30 $x10^3$ µl). We should note that statistically authentic low leukocytes level (against the reference one) at the end of a monthly recovery period (initial level was 10.13±0.60 $x10^3$ µl, level during a recovery period was 6.18±0.73$x10^3$ µl) can be an evidence that animals body reactivity decreased considerably under exposure to intermittent benzene concentrations.

Changes in leukocytic formula parameters occurred at the end of the first month of poisoning. These changes were an increase in neutrophils number (neutrophilic leukocytosis with a shift to the left), as well as lymphocytosis and monocytosis. By the end of the poisoning period shifts in leukocytic formula of experimental animals were quite the opposite: we detected absolute neutropenia, lymphocytopenia, and monocytopenia. 1 months after, when the animals no longer contacted benzene, we didn't detect full recovery in the parameters (they didn't return to their initial levels) and it could be an evidence that a recovery period was too short, or body reactivity decreased too significantly.

So, we examined leukocytic reaction which occurred in the experimental animals as a response to benzene impacts under various intoxications modes; our examination revealed that intermittent benzene concentrations exerted more apparent impacts in comparison with growing toxicant concentrations mode. Thus, authentic decrease in leukocytes number which corresponds to the 3rd and 4th months of poisoning under both intoxication modes didn't disappear in those animals which were exposed to intermittent benzene concentrations. Consequently, concentrations which fluctuated during poisoning caused more considerable changes in animals' bodies and it resulted in adaptation mechanisms depletion.

The results we obtained during our experimental research were analyzed as per catecholamines metabolism and the acetylcholine - choline esterase system. We de-

tected that reactive changes in the sympathoadrenal system are characterized with a certain activity of catecholamines biosynthesis, secretion, elimination, and metabolism, which varies in different periods of exposure to intermittent benzene concentrations. The first stage in the reaction occurred during the 1st experimental month and was characterized with a synchronous sympathoadrenal system (SAS) activation when adrenaline, noradrenalin, dopamine, and DHPA excretion with urine grew (Figure 4). Sympathetic nervous system activity in experimental rabbits grew more substantially at this stage as noradrenalin quantity (noradrenalin being a basic sympathetic nervous system mediator) grew faster than that of adrenaline and it led to a relative decrease in A/NA ratio.

By the end of the exposure period we detected the second stage in the reaction which could be determined as secretory-sympathetic activity dissociation phase. Here we detected a further growth in NA concentration, certain relative decrease in adrenalin, dopamine, and DHPA level in urine.

SAS reserves and precursors level in urine increased initially at the first stage but them at the second one we observed signs of DHPA (endogenous catecholamine reserve) depletion (Figure 4).

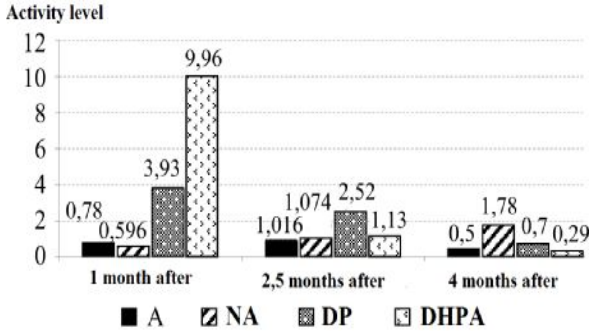

Figure 4. Contents of catecholamines and their precursors in daily rabbits' urine under chronic exposure to intermittent benzene concentrations (A is adrenaline, NA is noradrenalin, DP is dopamine, DHPA is dihydroxyphenylalanine)

Table 4

Acetylcholine content and choline esterase activity in rabbits exposed to small benzene concentrations

Animals groups and poisoning modes	Observation periods, months	Examined parameters and their values		
		AC, μg%	IC, μg/min	LC, μg/min
I. Increased concentrations	Initial level (background)	0,20 ± 0,02	0,89 ± 0,05	0,29 ± 0,05
	1 month after	0,33 ± 0,04	0,66 ± 0,06	0,38 ± 0,10
	2 months after	0,46 ± 0,03	0,92 ± 0,03	0,32 ± 0,02
	3 months after	0,49 ± 0,03	0,98 ± 0,04	0,20 ± 0,01
	4 months after	0,40 ± 0,02	0,99 ± 0,08	0,19 ± 0,01
II. Intermittent concentrations	Initial level (background)	0,22 ± 0,03	0,94 ± 0,14	0,28 ± 0,04
	1 month after	0,34 ± 0,05	0,69 ± 0,09	0,44 ± 0,12
	2 months after	0,50 ± 0,06	1,14 ± 0,04	0,32 ± 0,02
	3 months after	0,51 ± 0,04	1,26 ± 0,08	0,21 ± 0,01
	4 months after	0,46 ± 0,04	1,08 ± 0,12	0,18 ± 0,01
Reference	Initial level (background)	0,21 ± 0,01	0,87 ± 0,07	0,27 ± 0,03
	1 month after	0,22 ± 0,02	0,89 ± 0,08	0,26 ± 0,03
	2 months after	0,20 ± 0,02	0,93 ± 0,03	0,27 ± 0,03
	3 months after	0,22 ± 0,02	0,90 ± 0,04	0,26 ± 0,04
	4 months after	0,21 ± 0,02	0,87 ± 0,04	0,25 ± 0,03

We determined parasympathetic nervous system activity simultaneously with sympathetic nervous system activity as these two systems are interconnected. Parasympathetic nervous system activity became apparent through increase in acetylcholine concentration in blood, higher acetylcholine esterase and lower butyrylcholine esterase. By the end of the observation period butyrylcholine esterase activity fell but acetylcholine (AC) level still remained high (Table 4).

Our research results and other authors' works [8,12,13,17] reveal that a primary body reaction to toxic effects exerted by benzene appears in the peripheral blood system which, in its turn, gives information on the sympathetic nervous system state. So, a primary body reaction to endogenous irritation can be seen in the sympathetic nervous system excitation with a corresponding increase in blood sympathetic activity. This primary increase in blood sympathetic activity gives an impulse to a compensatory reaction of the parasympathetic nervous system and other corrective systems [9,15].

So, impacts exerted by benzene in small concentrations caused changes in some regulatory systems of experimental animals' bodies. The most apparent and earliest shifts were detected in the white blood and catecholamines (adrenaline, noradrenalin, dopamine, and DHPA). The detected signs of catecholamines and DHPA endogenous reserves depletion as well as eosinophils-basocyte dissociation are unfavorable as it is exactly at this period (the 4th poisoning month) when significant changes in blood occur, namely leucopenia, neutropenia, lymph- and monocytopenia. And here intermittent benzene concentrations exert more apparent toxic effects in comparison with growing toxicant concentrations. Leukocytes quantity which decreased during the 3rd and 4th poisoning months didn't recover to its initial levels during a recovery period in animals who were exposed to intermittent benzene concentrations.

References

1. Balandina A.N., Panteleev M.A., Ataullakhanov F.I. Sistema svertyvaniya krovi I ee regulyatsiya [Blood clotting system and its regulation]. *Priroda*, 2011, no. 3, pp. 32–38 (in Russian).

2. Vil'chinskii M.P. Uproshchennaya metodika izbiratel'noi okraski bazofil'nykh granulotsitov krovi [Simplified methodology for selective stain of blood basocyte granulocytes]. *Laboratornoe delo*, 1972, no. 1, pp. 58–59 (in Russian).

3. Vorob'ev A.I. Izuchenie kislotoustoichivosti eritrotsitov v gematologicheskoi klinike [Examination of erythrocytes resistance to acid in a hematological clinic]. *Aktual'nye voprosy gematologii*, 1960, no. 2, pp. 314–316 (in Russian).

4. Zyubina L.Yu., Shpagina L.A., Panacheva L.A. Professional'no obuslovlennye gemopatii i professional'nye zabolevaniya krovi [Industrially mediated hemopathies and occupational diseases of blood]. *Meditsinatruda i promyshlennayaekologiya*, 2008, no. 11, pp. 15–20 (in Russian).

5. Kutsenko S.A. Osnovy toksikologii [Basics of toxicology]. St. Petersburg, 2004, 570 p. (in Russian).

6. Bokarev I.N., Doronina A.M., Kozlova T.V. [et al.]. Laboratornye metody issledovaniya sistemy svertyvaniya krovi: metodicheskie rekomendatsii [Laboratory techniques for blood clot-

ting system examination: methodical guidelines]. Moscow, ATGPSS, Publ., 2011, 15 p. (in Russian).

7. Matlina E.Sh., Prikhozhan V.M. K metodike opredeleniya kholinesterazy v krovi [On methodology for choline esterase detection in blood]. *Laboratornoe delo*, 1961, no. 6, pp. 10–12 (in Russian).

8. Mikhailova I.V. Otsenka vozdeistviya benzola i khroma na faktory estestvennoi rezistentnosti v eksperimente [Impact assessment of benzene and chrome on the natural resistance factors in the experiment]. *Rossiiskii immunologicheskii zhurnal*, 2014, vol. 8, no. 3 (17), pp. 454–457 (in Russian).

9. Orujov R.A. Nekotorye pokazateli adaptatsionnykh sdvigov organizma pri povtornykh ostrykh intoksikatsiyakh uglevodorodami [Specific parameters of adaptation shifts in a body under repeat acute intoxications with hydrocarbons]. *Zdorov'e*, 2014, no. 3, pp.112–117 (in Russian).

10. Orujov R.A. Razlichie v deistvii promyshlennykh narkotikov na primere benzola, benzina i atsetona [Differences in effects exerted by industrial drugs on the examples of benzene, petrol, and acetone]. *Azmedzhurnal*, 2014, no. 2, pp. 95–98 (in Russian).

11. Piralashvili I.S. K metodike podscheta eozinofilov v perifericheskoi krovi [On methodology for calculating eosinophils in peripheral blood]. *Laboratornoe delo*, 1962, no. 3, pp. 20–22 (in Russian).

12. Yang J., Bai W.n, Niu P., Tian L., Gao A. Aberrant hypomethylated STAT3 was identified as a biomarker of chronic benzene poisoning through integrating DNA methylation and mRNA expression data. *Experimental and Molecular Pathology*, 2014, vol. 96, no. 3, pp. 346–353.

13. Wen H., Yuan L., Wei C., Zhao Y., Qian Y., Ma P., Ding S., Yang X., Wang X. Effects of combined exposure to formaldehyde and benzene on immune cells in the blood and spleen in Balb/c mice. *Environmental Toxicology and Pharmacology*, 2016, vol. 45, pp. 265–273.

14. Hestrin S. The relation acetylcholine and other carbocholine acidi derivatwes with hydroxylamine and its analytical application. *J. biol. Chem*, 1949, vol.180, no.1, pp. 249–251.

15. Kerzic P.J. [et al.]. Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers. *Chemico-Biological Interactions*, 2010, vol. 184, pp. 182–188.

16. Geering B., Stoeckle Ch.., Conus S., Hans-Uwe S. Living and dying for inflammation: neutrophils, eosinophils, basophils. *Trends in Immunology*, 2013, vol. 34, no. 8, pp. 398–409.

17. Rizzo M. [et al.]. Cortisol levels and leukocyte population values in transported and exercised horses after acupuncture needle stimulation. J*ournal of Veterinary Behavior: Clinical Applications and Research*, 2017, vol. 18, pp. 56–61.

Orujov R.A., Dzhafarova R.E. Changes in the nervous system state and peripheral blood parameters under benzene intoxication during an experiment. Health Risk Analysis, 2017, no. 4, pp. 108–116. DOI: 10.21668/health.risk/2017.4.12.eng

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