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ELEMENTAL MARKERS OF EXPOSURE UNDER COMBINED ORAL INTRODUCTION OF CHEMICAL MIXTURES WITH PREVALENT ANTIMONY AND ARSENIC INTO WHITE WISTAR RATS

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The present paper dwells on assessing oral sub-acute exposure of a warm-blooded body to non-organic compounds that contain antimony and arsenic and are introduced with drinking water. Another issue was to assess changes in their concentrations as well as concentrations of other elements in tissues of certain organs. We accomplished elemental analysis of tissues taken from exposed white Wistar rats; the analysis included the following elements: S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr, Mo, As, Hg, Pb, Sb. As per its results we characterized changes in elemental structure of the liver, kidneys, heart, lungs, femoral muscle, thyroid gland, and whole blood caused in white male Wistar rats by sub-acute combined oral exposure to arsenic and antimony. Arsenic was detected in all the examined internal organs after 3 weeks of combined oral exposure to it in a dose equal to 15 μg/kg together with exposure to antimony in a dose equal to 61 μg/kg. It concerned both test and control group as animals in the test group had arsenic in a dose equal to 0.010 ± 0.002 μg/kg in the thyroid gland tissues and up to 0.950 ± 0.155 μg/kg in blood; animals in the control group, from 0.028 ± 0.003 μg/kg in muscular tissues to 1.56 ± 0.03 μg/kg in blood. Antimony was detected in blood only (0.005 ± 0.0021 μg/kg in the control group and 0.021 ± 0.0009 μg/kg in the test one).

We detected a direct correlation between arsenic contents in organ tissues and contents of potassium, iron, and mercury in the control group and contents of iron and mercury in the test one. Strontium and rubidium concentrations in organs of animals in the test group were inversely correlated with arsenic concentrations.

We analyzed a correlation between growing arsenic contents in tissues of animals in the test group against the control and changes in contents of other elements and revealed a statistically authentic correlation with an increase in concentrations of potassium, molybdenum, iron, and lead, as well as an inverse character of this correlation.

We concluded that there were several markers of oral exposure to arsenic and antimony as components in a complex mixture detected in white Wistar rats; they were arsenic in tissues of the liver, kidneys, muscles, thyroid gland, and whole blood; antimony in whole blood; increase in contents of chlorine, potassium, sulfur, calcium, rubidium, zinc, manganese, and chromium in the liver and kidneys; a decrease in concentrations of chromium, manganese, iron, copper, molybdenum, nickel, selenium, and strontium in blood, heart, thyroid gland, and lungs.

Key words: *antimony, arsenic, water, elemental structure of white rats' organ tissues, markers of exposure, chemical mixtures, combined examination, oral exposure.*

Abnormal contents of chemical elements in the atmosphere exert their impacts on regulation of live processes in a body; induce disorders in morphogenesis, metabolic processes and metabolic coherence; make enzymes less active; cause dysfunctions and endemic diseases. A range of chemicals concentrations between their upper and bottom limits in external and internal environment defines how well a body can regulate its chemical homeostasis [1, 2].

Biogeochemical monitoring substantiates medical and preventive support for demographic, social, and industrial development of a society [3]. Regulatory systems of a body change within certain ranges under exposure to abnormal geochemical conditions and it is vital

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to determine these ranges when general and specific medical and prevention activities are implemented [4–6]. A key focus in implementing monitoring programs should be a choice on criteria that describe influence exerted by adverse environmental factors on a human body and responses it gives to such influence.

A significance this or that element can have for a body is determined by a role it plays in vital activities [7]: sulfur is a component in many proteins and hormones, liver cells use it to neutralize toxic metabolic products. Potassium is a constant component in all body cells and tissues; its greatest quantities can be found in cells of the liver, kidneys, skin, muscles, and nervous system as salts such as chlorides, phosphates, carbonates, and sulfates. Ionized potassium or potassium bound to proteins or other organic compounds regulates activity of certain enzymes, notably K+-ATP-ase, acetyl kinase, and pyruvate phosphokinase; the element is regulated by mineral corticosteroids produced by the adrenal cortex [8]. Chlorine is found in a body as anions of salts (sodium, potassium, calcium, manganese and others) in all body fluids [9]. Calcium participates in bone tissue formation, provides contractile function of plain and skeletal muscles, and regulates functions of the heart [10]. Titanium is necessary for erythrocytes creation in the bone marrow; it influences immune system functioning, regulates cholesterol and carbamide (urea) contents in blood, and participates in many metabolic reactions [11]. Chromium is a component in the low-molecular organic complex, tolerance factor to dextrose; it stimulates biosynthesis of glycogen and protein, and normalizes lipid metabolism as well [12]. Manganese is a part of multiple enzymes, such as phosphoglucomutase, enolase, mitochondrial superoxide dismutase, etc.; it activates phosphofructokinase, arginase, dipeptidase, and bone and alkaline phosphatase [13]. Iron is contained in hemoglobin and muscle hemoglobin and is also a component in respiratory enzymes that catalyze breathing processes in cells and tissues [14]. This chemical element is a part of more than 70 various enzymes and it explains significant influence exerted by it on body growth and development, tissue breathing, hemopoiesis, immune genesis, and other physiological processes. Nickel participates in structural organization and functioning of DNA, RNA, and proteins [15]. Copper catalyzes hemoglobin formation in blood and is a part of many key enzymes such as cytochrome-c-oxidase, tyrosinase, ascorbinase, etc. [16]. Zink can be found in more than 80 enzymes, the most significant ones being alcohol dehydrogenase, lactate dehydrogenase, glutamate dehydrogenase, carbonic anhydrase, DNA- and RNA-polymerase, carboxypeptidase, glyceraldehyde-3-phosphate dehydrogenase, and aldolase. Selenium participates in redox processes at the cellular level (dextrose metabolism, the Krebs cycle, potassium-sodium-calcium metabolism etc.) and is involved in activities of more than 100 enzymes that take part in metabolic products detoxication; it is a component in most hormones [17]. Bromine can be found in an adult body in blood, bone, and muscle tissues, and its concentrations are the highest in the kidneys, hypophysis, and thyroid gland [18]. Rubidium can substitute potassium and is its synergist [19]. Rubidium also accumulates in the intracellular fluid and can substitute equivalent amounts of potassium in various processes. Strontium is concentrated in bones and it partially substitutes calcium [20]. Strontium is basically excreted with urine and to a lesser extent with bile. Molybdenum is a component in prosthetic groups of enzymes including xanthine oxidase, aldehyde oxidase, xanthine dehydrogenase, and sulfite oxidase [21]. Arsenic is intensely accumulated when there is shortage of selenium and can make for deficiency of this microelement [22]. Excessive quantities of arsenic are accumulated in the thyroid gland and cause endemic goiter. Arsenic interacts with thiol protein groups, cysteine, glutathione, and lipoic acid. Antimony (Sb) is similar to arsenic in its properties and is known to inhibit enzymes that participate in carbohydrate, fat, and protein metabolism [23, 24].

Therefore, a human body provides homeostasis involving a lot of elements that differ from each other conditionally; sulfur and strontium predominantly have structural roles; chlorine, potassium, rubidium, calcium, zinc, and selenium are predominantly enzyme-dependent; copper, titanium, chromium, manganese, iron, molybdenum, nickel, and bromine have apparent metabolic and antioxidant activities. Multi-

functional properties of certain elements and their metabolic dependence determine a variety of responses to multiple exposures that can give grounds for revealing initial shifts in elemental homeostasis of a body. At the same time, combined exposure to several elements in hazardous concentrations makes it difficult to predict probable effects produced by it.

Our research goal was to experimentally substantiate elemental exposure markers for tissues of internal organs taken from experimental animals after combined oral exposure to arsenic and antimony that were introduced into them as a complex mixture contained in drinking water.

Our research tasks were:

1) to perform hygienic assessment of chemicals concentrations in sewage from a tailings dam of a gold mine;

2) to assess doses in which antimony and arsenic were introduced into experimental animals;

3) to analyze dynamics of antimony and arsenic concentrations in blood of experimental animals under sub-acute oral introduction;

4) to describe changes that occurred in elemental structure of the liver, kidneys, heart, muscles, and thyroid gland under combined subacute oral exposure to antimony and arsenic.

Research objects. In Komsomolskiy settlement located in Kemerovo region specific local conditions could make for penetration of sewage that contained antimony and arsenic from a tailings dam into underground water-bearing layers used for providing drinking water supply to population. Antimony and arsenic produce similar toxic effects on a warm-blooded body as they deactivate thiol protein groups, amino acids, and metals in enzyme systems; taking it into account, we performed an experiment with a model mixture that contained sewage from a gold mine. This mixture contained high concentrations of antimony and arsenic. The experiment was performed to predict changes in a body under combined oral exposure to arsenic and antimony. Animals from the control were given drinking water from centralized water supply systems in Novosibirsk.

We examined tissues of internal organs, notably the liver, kidneys, heart, lungs, muscles, and thyroid gland, as well as whole blood of white male Wistar rats with their body weight being equal to 280–340 grams; there were 2 groups, the test and the control, 6 animals in each.

Data applied in the research:

– results obtained via analyzing structure of a model complex mixture in water that contained arsenic and antimony;

– hygienic standards for chemical contents in water;

– results of determining elements concentrations in tissues of internal organs, namely the liver, kidneys, heart, lungs, muscles, thyroid gland, and whole blood of white male Wistar rats from the test and control groups.

Animals in the test group were given model drinking water with increased quantities of antimony and arsenic and we kept daily accounting of water quantities consumed by them. Animals from the control group were given drinking water taken from centralized water supply systems in Novosibirsk. We took samples of organs and tissues after animals had been autopsied. Prior to autopsy all animals were taken out of the experiment via intraperitoneal introduction of Nembutal in a dose equal to 4 mg/100 g of body weight.

We performed atomic absorption analysis with “Kvant-2A” atomic absorption spectrometer (Moscow, KORTEK) equipped with deuterium correction device with non-selective absorption and relevant hollow cathode lamps. Heavy metals in samples were determined in accordance with requirements fixed in conventional procedures. Lead (Pb) was determined in “propane-air” flame. We applied standards samples with IAEA-SOIL-7, IAEA-336 (Lichen), SRM 1572 (CitrusLeaves), SRM 1575 (PineNeedles) structures as reference ones in both analysis procedures.

As a result, we determined elemental structures of internal organs taken from experimental animals; all the obtained data were statistically processed with calculating mean values and standard error of the mean, coefficients of variation, validity of discrepancies in dispersion, and average values of parameters in both groups as per Fischer and Student’s tests. To do that, we used applied Excel software package. Discrepancies with their validity being $p \leq 0.05$ were considered to be statistically significant.

Research results. The model complex mixture contained in water consumed by animals in the test group was orally intro-

duced into them via providing them with free access to standard drinking bowls; animals from the control group consumed drinking water from similar bowls. Therefore, to calculate doses of chemicals consumed by rats, we kept daily accounting of water consumption volumes. We analyzed water consumption during three weeks of the experiment and revealed that there were no statistically authentic discrepancies in water consumption between the test and the control groups taken both in ml per day and recalculated per 1 kg of animals' body weight a day ($p>0.05$). So, having free access to drinking water from automated water bowls, animals from both groups consumed it in similar quantities, 71.9±4.9 ml/kg/day in the test group, and 65.6±4.4 ml/kg/day in the control group, ($p>0.05$) (Table 1).

We took data on actual water consumption by animals from the test and control groups and concentrations of chemicals in consumed water and calculated average daily doses of chemicals introduced into animals from both groups with drinking water.

We revealed that animals from the test group consumed higher quantities of chemicals than animals from the control group; for example, doses of strontium were 1.1 times higher; doses of potassium, calcium, chromium, manganese, iron, copper, zinc, rubidium, molybdenum, and lead, 7.7 times higher; sulfur, 18.3 times higher; nickel, 26.3 times higher (Table 2).

Table 2

Summary table showing water consumption by white male Wistar rats from the test and control group; water was consumed from automated water bowls in cages

Dates of experiment	Test, ml/day	Control, ml/day	*t*	*P*
March 04–10, 2018	122±9.6	113±7.8	0.06	0.9521
March 11–17, 2018	127±8.1	105±5.9	0.22	0.8289
March 18–25, 2018	125±7.8	105±8.1	0.16	0.8786
Total	125±8.1	108±7.2	0.14	0.8936
Average body weight, г	289±14.5	273.5±12.4	0.04	0.9669
Water consumption, ml/kg/day per 1 animal	71.9±4.9	65.6±4.4	0.15	0.8867

Table 3

Concentrations in water (Ct, Cc) and daily doses (ADt, ADc) of chemicals consumed by animals form the test and control groups

Elements	Hygienic standard 2.1.5.1315-03, mg/dm^3, MPC	Control		Test	
		Cc, mg/dm^3	ADc, mg/kg/day	Ct, mg/dm^3	ADt, mg/kg/day
Ca	130 physiological*	15	0,98	86	6,2
Ti	0.1 overall	0.002	0.0001	0.0053	0.0004
Cr	0.05 c.т.	<0.001	0.00003	0.0012	0.0001
Mn	0.1 organoleptic	0.0068	0.0004	0.02	0.0014
Fe	0.3 organoleptic	0.1	0.006	0.35	0.025
Ni	0.02 sanitary	<0.001	0.00003	0.012	0.0009
Cu	1.0 sanitary	0.01	0.0006	0.07	0.0050
Zn	1.0 overall	0.03	0.0019	0.042	0.0030
Rb	0.1 sanitary	<0.001	0.00003	0.0021	0.0002
Sr	7.0 sanitary	0.3	0.01968	0.31	0.022
Mo	0.07 sanitary	<0.001	0.00003	0.0016	0.0001
As	0.01 sanitary	<0.001	0.00003	**0.21**	**0.015**
Pb	0.01 sanitary	<0.001	0.00003	0.001	0.0001
Sb	0.005 sanitary	<0.008	0.0002	**0.85**	**0.061**

Note: * means as fixed in section 4.7 of the Sanitary-Epidemiologic Rules 2.1.4.1116-02. Drinking water. Hygienic requirement to quality of bottled water. Quality control.

Antimony and arsenic were orally introduced into animals from the test group in doses that were equal to 0.061 mg/kg/day and 0.015 mg/kg/day respectively; deviations from the hygienic standards were the highest for these two metals as their doses exceeded MPC by 605 times for antimony and by 500 times for arsenic.

We analyzed chemicals contents in tissues of organs taken from experimental animals after a three-week experiment and detected arsenic in all internal organs of animals form both groups, its quantities in the control group being from 0.010±0.002 μg/g in thyroid gland tissues to 0.950±0.155 μg/g in blood; from 0.028± ±0.003 μg/g in muscle tissues to 1.56±0.03 μg/g in blood in the test group (Table 3).

Antimony was detected in blood only, 0.005±0.0021 μg/kg in the control group and 0.021±0.0009 μg/kg in the test group.

Although animals from the test group consumed other elements that were included into the complex mixture in concentrations not exceeding MPC but being higher than those consumed by the control group, concentrations of such elements in tissues of their internal organs were still statistically authentically different from those taken out of animals from the control group.

We detected higher concentrations of some elements in blood of animals from the test group against the control group; these higher concentrations were detected for arsenic (on average by 64%, with scaled-down dispersion ($pF<0.05$)); antimony (on average by 320% ($p<0.05$); chlorides (by 110%, $p<0.05$)), calcium (by 52%, $p<0.05$). We also detected lower concentrations of such elements as chromium (by 43%, $p<0.05$); manganese (by 42%, $p<0.05$); molybdenum (by 75%, $p<0.05$); lead (by 36%, $p<0.05$); we also detected scaled-down dispersion for contents of iron, nickel, and copper ($pF<0.05$) with a 30–56% descending trend for their average values.

There was a decrease in dispersion of arsenic contents in liver tissues taken out of animals from the test group against the control group ($pF<0,05$) with a simultaneous trend for a 46% increase in its average value; liver tissues of animals from the test group also contained increased concentrations of chlorides (by 120% higher, $p<0.05$); potassium (by 309%, $p<0.05$); calcium and chromium (by 2 times, $p<0.05$); bromine (by 32%, $p<0.05$); rubidium (by 31%, $p<0.05$); there was also a decrease in dispersion of copper contents ($pF<0.05$), with a trend for decrease in its average value by 20%.

Kidney tissues taken out of animals from the test group contained arsenic in higher concentrations as compared with the control (by 102%, $p<0.05$); they also contained higher concentrations of manganese (by 14%, $p<0.05$) and bromine (by 20%, $p<0.05$), and there was a decrease in dispersion of potassium contents ($pF<0.05$) with a trend for a 364% increase in its average value. We detected an increase in dispersion of sulfur, chlorides, calcium, and zinc contents in the test group against the control one ($pF<0.05$) with a trend for a 47–364% increase in their average values.

Heart tissues taken out of animals from the test group contained arsenic in higher concentrations against the control (by 212%, $pF<0.05$); we also detected lower average molybdenum contents (by 38%, $p<0.05$) and a trend for lower contents of nickel (by 88%, $pF<0.05$) and copper (by 22%, $pF<0.05$) as well as a trend for increased contents of chlorides (by 431%, $pF<0.05$), potassium (by 255%, $pF<0.05$), and selenium (by 50%, $pF<0.05$).

There were no statistically authentic discrepancies in arsenic contents in lung tissues between the test and control groups ($p>0.05$), but still we detected increased potassium contents (by 134%, $p<0.05$), a trend for higher bromine contents with increased dispersion of the parameter (by 28%, $pF<0.05$), a trend for higher lead contents with increased dispersion of the parameter (by 70%, $pF<0.05$) and lower contents of iron (by 33%, $p<0.05$), zinc (by 18%, $p<0.05$), and molybdenum (by 24%, $p<0.05$).

Tissues of thigh muscle taken out of animals from the test group contained some elements in higher concentrations as compared with the control group; these elements were arsenic (by 55%, $p<0.05$), sulfur (by 13%, $p<0.05$), calcium (by 31%, $p<0.05$), strontium (by 125%, $p<0.05$), and lead (by 85%, $p<0.05$). We detected a trend for higher concentrations with simultaneous decrease in dispersion of the parameter for potassium contents (by 400%, $pF<0.05$), and a trend for a decrease in the parameter together with a decrease in its dispersion was detected for nickel (by 53%, $pF<0.05$).

Table 3

Elements concentrations in tissues of internal organs (μg/g) taken out of male white Wistar rats after 3 weeks of daily oral exposure to arsenic in a dose equal to 15 μg/kg/day and antimony in a dose equal to 61 μg/kg/day, both metals were contained in sewage from a tailings dam of Komsomolskiy gold mine

Groups, statistical parameters	Chemical elements/organs																			
	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Se	Br	Rb	Sr	Mo	As	Hg	Pb	Sb
	Blood																			
Control, X	4,808	1,229	7,639	14.7	0.58	1.67	5.26	236.2	0.121	0.392	31.5	0.19	0.96	0.10	0.223	1.32	0.95	0.026	0.115	0.005
±m	141	125	981	1.2	0.07	0.14	0.26	15.7	0.015	0.046	1.2	0.01	0.04	0.01	0.018	0.07	0.155	0.0009	0.0068	0.0021
V%	7%	25%	31%	20%	28%	21%	12%	16%	31%	29%	9%	12%	10%	14%	20%	13%	40%	8%	15%	103%
Test, X	4,283	2,601	6,614	22.5	0.40	0.96	3.07	164.8	0.054	0.293	30.9	0.183	0.92	0.11	0.215	0.34	1.56	0.025	0.074	0.021
±m	206	255	406	0.9	0.05	0.07	0.31	3.5	0.005	0.004	0.96	0.007	0.05	0.01	0.016	0.04	0.03	0.002	0.004	0.0009
V %	12%	24%	15%	10%	29%	18%	25%	5%	23%	4%	8%	9%	15%	15%	18%	26%	5%	15%	14%	11%
P	0.0801	0.0029	0.3715	0.0019	0.0661	0.0040	0.0016	0.0044	0.0059	0.0767	0.6810	0.657	0.544	0.1732	0.7670	0.0000	0.0086	0.5037	0.0022	0.0004
pF	0.2417	0.0990	0.0577	0.2834	0.2739	0.1078	0.3755	0.0068	0.0272	0.0002	0.3451	0.317	0.273	0.3501	0.4063	0.1233	0.0047	0.8460	0.1996	0.0750
	Liver																			
Control, X	2,772	378	1,373	14.73	0.21	0.26	5.59	30.02	0.064	1.3	84.3	0.270	0.317	0.189	0.112	2.565	0.037	0.0007	0.059	
±m	297	96	390	2.26	0.07	0.009	0.27	1.25	0.021	0.091	2.0	0.025	0.033	0.018	0.008	0.030	0.006	0.0001	0.009	
V%	26%	62%	70%	38%	81%	9%	12%	10%	79%	17%	6%	22%	26%	23%	18%	3%	40%	35%	37%	
Test, X	3,312	848	5,688	28.7	1.19	0.53	6.06	29.03	0.087	1.1	86.7	0.249	0.414	0.25	0.295	2.7	0.054	0.0009	0.125	
±m	115	102.1	421	3.6	0.57	0.09	0.42	2.05	0.028	0.043	2.4	0.018	0.018	0.012	0.040	0.1	0.003	0.0002	0.030	
V%	9%	30%	18%	31%	118%	45%	17%	17%	81%	10%	7%	17%	11%	12%	33%	9%	12%	54%	56%	
P	0.1415	0.0151	0.0002	0.0169	0.1402	0.0317	0.38	0.69	0.5426	0.0508	0.4763	0.521	0.044	0.0333	0.0042	0.1465	0.0410	0.4055	0.0634	
pF	0.018	0.44	0.4294	0.14	0.0001	9E-06	0.153	0.129	0.2226	0.0473	0.3512	0.222	0.086	0.2044	0.0006	0.0040	0.0383	0.0579	0.005	
	Kidneys																			
Control, X	3,098	872	1,181	26.4	0.26	0.49	3.14	18.88	0.0783	2.1	73.5	0.58	0.80	0.14	0.235	1.94	0.017	5E-05	0.101	
±m	33	63	145	0.91	0.04	0.06	0.13	0.62	0.0059	0.080	0.194	0.031	0.017	0.003	0.022	0.026	0.005	0.00001	0.003	
V%	3%	18%	30%	8%	38%	30%	10%	8%	18%	9%	1%	13%	5%	5%	23%	3%	72%	72%	9%	
Test, X	4,565	2,134	5,487	42.0	0.34	0.42	3.57	19.15	0.093	2.07	83.0	0.657	0.96	0.18	0.3425	1.8825	0.036	0.00008	0.179	
±m	249	253	317	4.5	0.10	0.05	0.11	0.92	0.03	0.23	3.41	0.07	0.03	0.01	0.04	0.19	0.004	0.00002	0.04	
V%	13%	29%	14%	26%	71%	30%	8%	12%	75%	27%	10%	27%	7%	11%	30%	25%	27%	64%	59%	
P	0.0011	0.0028	2E-05	0.0151	0.4787	0.4272	0.0485	0.8177	0.64	0.83	0.03	0.35	0.00	0.00	0.06	0.78	0.02	0.26	0.12	
pF	0.0001	0.0019	0.0394	0.0006	0.0245	0.3483	0.3926	0.1778	0.0007	0.0103	0.0001	0.031	0.123	0.0113	0.0632	0.0001	0.3089	0.1868	0.0001	
	Heart																			
Control, X	5,171.6	280	2,120.4	33.2	0.4674	0.78	2.33	35.6	0.086	1.842	59.54	0.125	0.41	0.104	0.228	0.688	0.079	0.0002	0.079	
±m	182	39	276	0.60	0.042	0.029	0.067	1.2	0.006	0.057	0.352	0.001	0.029	0.007	0.004	0.034	0.009	0.0001	0.002	
V%	9%	34%	32%	4%	22%	9%	7%	8%	17%	8%	1%	3%	18%	17%	4%	12%	28%	83%	6%	
Test, X	5,859	1,488	7,529	31.4	0.31	0.36	1.99	37.65	0.066	1.41	64	0.185	0.78	0.173	0.273	0.435	0.249	0.001	0.073	
±m	426	113	1034	3.9	0.07	0.09	0.2	6.0	0.010	0.17	2.261	0.020	0.16	0.016	0.020	0.040	0.073	0.0002	0.005	
V%	18%	19%	34%	31%	57%	58%	25%	39%	37%	29%	9%	27%	51%	22%	18%	22%	72%	84%	19%	
P	0.1888	0.0001	0.0023	0.6572	0.1032	0.0035	0.1583	0.7555	0.1399	0.0496	0.0992	0.026	0.062	0.0080	0.0682	0.0028	0.0610	0.1560	0.3458	
pF	0.0289	0.0101	0.0027	0.0001	0.1072	0.0092	0.0081	0.0005	0.128	0.0093	0.0001	0.000	0.001	0.0425	0.0003	0.3483	0.0000	0.0211	0.0070	
	Lungs																			
Control, X	4,143	1,067	2,553	41.4	0.44	1.6	2.4	44.6	0.1	1.47	89	0.18	1.07	0.14	0.39	0.78	0.11	0.001	0.098	
±m	87	140	510	1.3	0.07	0.0	0.1	3.6	0.008	0.081	3.9	0.006	0.061	0.012	0.014	0.038	0.023	0.0005	0.006	
V%	5%	32%	49%	8%	39%	5%	11%	20%	19%	13%	11%	8%	14%	21%	9%	12%	49%	122%	16%	
Test, X	4,606	2,604	5,979	49.7	0.60	1.15	2.05	31.7	0.092	0.928	73.75	0.176	1.081	0.1765	0.3575	0.60	0.18375	0.0004	0.170	
±m	300	739	576	5.8	0.07	0.31	0.18	3.6	0.025	0.245	2.686	0.002	0.174	0.010	0.054	0.027	0.026	0.00027	0.023	
V %	16%	69%	24%	29%	31%	67%	22%	27%	67%	65%	9%	3%	39%	14%	37%	11%	35%	163%	34%	
P	0.189	0.0868	0.0043	0.2114	0.9824	0.2176	0.1174	0.0446	0.7566	0.0794	0.0184	0.250	0.947	0.05	0.5427	0.0098	0.0931	0.3304	0.0252	
pF	0.0175	0.0034	0.4093	0.0063	0.4469	0.0004	0.1256	0.514	0.005	0.008	0.190	0.043	0.033	0.31	0.002	0.226	0.376	0.0757	0.0032	
	Muscle																			
Control, X	3,980	134	1,822	25	0.28	2.13	3.37	19.8	0.145	0.413	32.5	0.063	0.182	0.1675	0.16	0.26	0.018	0.0002	0.045	
±m	106	15	211	1.7	0.04	0.12	0.35	0.96	0.016	0.025	6.3	0.001	0.021	0.012	0.060	0.016	0.003	0.00003	0.003	
V%	7%	27%	28%	17%	37%	14%	26%	12%	27%	15%	48%	2%	29%	17%	92%	15%	34%	31%	19%	
Test, X	4,508	841	9,024	32.6	0.37	0.61	0.92	6.42	0.069	0.36	48.9	0.07	0.35	0.19	0.36	0.22	0.028	0.0003	0.083	
±m	112	141	708	1.21	0.03	0.11	0.21	0.456	0.004	0.016	14.3	0.009	0.075	0.021	0.042	0.011	0.003	0.00004	0.004	
V%	6%	41%	19%	9%	20%	46%	56%	17%	14%	11%	71%	31%	53%	27%	29%	12%	26%	29%	12%	
P	0.0139	0.0024	7E-05	0.0109	0.1298	0.0000	0.0010	0.0001	0.0035	0.1380	0.3343	0.463	0.081	0.3831	0.0346	0.1043	0.0429	0.1660	0.0004	
pF	0.4598	0.0003	0.0189	0.2596	0.2491	0.4631	0.1728	0.0896	0.0100	0.1981	0.0731	0.001	0.016	0.1427	0.2593	0.2614	0.3662	0.3312	0.3699	
	Thyroid gland																			
Control, X	5,346	314	3,718	50.3	0.9	1.0	2.1	13	0.30	0.71	50.28	0.084	0.34	0.14	0.428	0.522	0.010	0.0015	0.097	
±m	263	21	363	4.1	0.1	0.1	0.2	2.2	0.023	0.074	1.6	0.005	0.036	0.011	0.020	0.073	0.002	0.0005	0.003	
V%	12%	16%	24%	20%	34%	29%	26%	41%	19%	26%	8%	14%	26%	19%	12%	34%	39%	82%	7%	
Test, X	3,260	1,880	9,424	41.1	0.5	0.5	1.5	13.7	0.128	0.493	67.1	0.111	0.65	0.204	0.34	0.46	0.060	0.0018	0.16	
±m	106	175	355	4.9	0.1	0.04	0.2	0.72	0.024	0.026	3.1	0.006	0.04	0.007	0.03	0.02	0.008	0.0006	0.030	
V %	8%	23%	9%	29%	39%	20%	28%	13%	47%	13%	11%	13%	16%	8%	22%	12%	35%	82%	46%	
P	0.0003	0.0001	3E-05	0.1962	0.0811	0.0045	0.0675	0.7699	0.0024	0.0305	0.0030	0.014	0.001	0.0027	0.0446	0.4113	0.0015	0.7141	0.0803	
pF	0.0525	0.0005	0.5169	0.3637	0.2756	0.0219	0.3013	0.0274	0.4550	0.0339	0.1268	0.331	0.395	0.2120	0.2298	0.0195	0.0055	0.3662	0.0002	

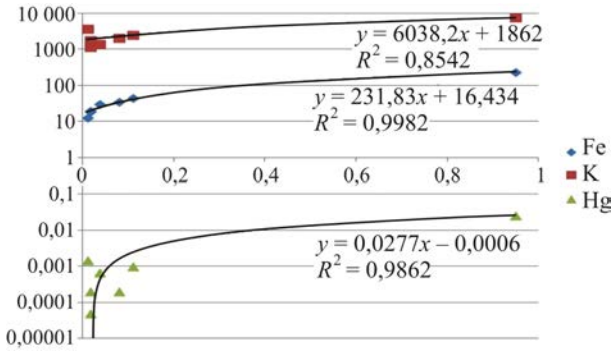

Figure 1. A relationship between arsenic concentrations and contents of various elements in tissue of internal organs taken out of white male Wistar rats from the control group (X axis shows arsenic concentration, μg/g; Y axis shows concentrations of elements, μg/g)

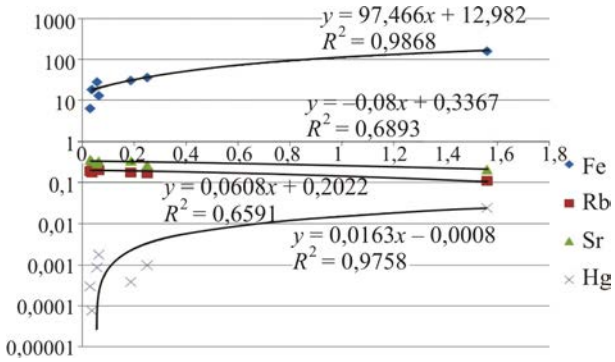

Figure 2. A relationship between arsenic concentrations and contents of various elements in tissue of internal organs taken out of white male Wistar rats from the test group (X axis shows arsenic concentration, μg/g; Y axis shows concentrations of elements, μg/g)

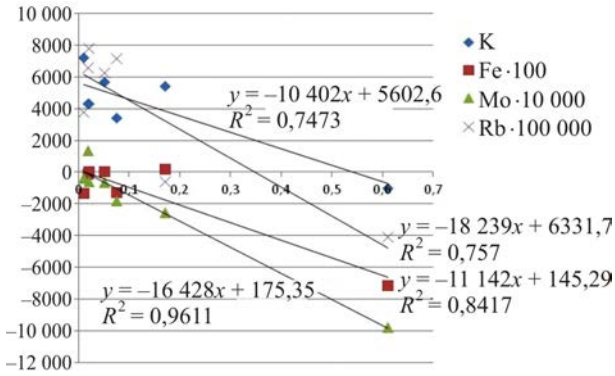

Figure 3. A correlation between changes in arsenic concentration and concentrations of other elements in tissues of organs taken out of white male Wistar rats under sub-acute oral combined exposure to arsenic and antimony in doses equal to 0.015 mg/kg/day and 0.061 mg/kg/day respectively (X axis shows a difference between arsenic concentration in the test and control groups, μg/g; Y axis shows a difference between concentrations of elements in the test and control groups, μg/g; to bring all concentrations to the same dimension, we multiplied iron concentration by 100; molybdenum, by 10,000; lead, by 100,000)

We detected lower contents of chromium (by 72%, $p<0.05$), manganese (by 83%, $p<0.05$), iron (by 68%, $p<0.05$), and nickel (by 53%, $p<0.05$).

We examined tissues of thyroid gland taken out of animals from the test group and detected an apparent trend for increased average arsenic contents and an increase in dispersion of the parameter (by 500%, $pF<0.05$); there were also higher concentrations of potassium (by 153%, $p<0.05$), zinc (by 34%, $p<0.05$), selenium (by 37%, $p<0.05$), and bromine (by 91%, $p<0.05$). We also detected a trend for higher chlorides concentrations together with an increase in dispersion of the parameter (by 498%, $pF<0.05$). A trend for lower concentrations was detected for chromium (by 50%, $pF<0.05$), copper (by 31%, $pF<0.05$); there were statistically authentically lower concentrations of nickel (by 58%, $p<0.05$) and strontium (by 21%, $p<0.05$).

We detected a direct relationship between arsenic concentration and contents of potassium, iron, and mercury in the control group (Figure 1); arsenic concentration and contents of iron and mercury in the test group (Figure 2).

There was an inverse relationship between arsenic concentration and contents of strontium and rubidium in organs of animals from the test group (Figure 2).

We analyzed a correlation between higher arsenic concentrations in tissues of organs taken out of animals from the test group against the control and changes in contents of other elements; our analysis revealed a statistically authentic inverse correlation between arsenic concentration and an increase in concentrations of potassium, molybdenum, iron, and lead (Figure 3).

Results and discussion. Our experiment allowed us to obtain data on changes in elemental structure of internal organs taken from white male Wistar rats under combined sub-acute oral exposure to arsenic and antimony in doses equal to 0.015 mg/kg/day and 0.061 mg/kg/day. In general, all the data are well in line with common toxic effects produced by these semimetals known as “endocrine destroyers” and “thiol poisons”. It becomes apparent via prevailing arsenic accumulation (1.56 μg/g) and antimony accumulation

(0.021 μg/g) in blood; arsenic was also accumulated in the heart (0.249 μg/g), lung tissues (0.183 μg/g), and thyroid gland (0.060 μg/g). Antimony concentration was lower than detection limit in other internal organs and it is coherent with data on antimony compounds being scarcely capable to be absorbed into a body out of the digestive tracts [7, 24].

Although antimony and arsenic were the only elements consumed by animals from the test group in substantially higher quantities, tissues of all the examined organs contained other elements in quantities that differed from those detected in tissues of animals from the control group, some being higher and some lower. Thus, the following elements were detected in higher concentrations in tissues of animals from the test group as compared with animals from the control group:

– chlorides (blood, liver, kidneys, heart, and thyroid gland);

– potassium (liver, kidneys, heart, lungs, thigh muscle, and thyroid gland);

– calcium (blood, liver, kidneys, and thigh muscle);

– selenium (heart and thyroid gland);

– bromine (liver, kidneys, lungs, and thyroid gland);

– rubidium (liver).

The following elements were detected only in lower concentrations in tissues of animals from the test group as compared with animals from the control group:

– iron (blood, lungs, and thigh muscle);

– nickel (heart, thigh muscle, and thyroid gland);

– copper (blood, liver, heart, and thyroid gland);

– molybdenum (blood, heart, and lungs).

The following elements were contained in certain internal organs in higher concentrations, and in other ones, in lower concentrations in animals from the test group against the control:

– sulfur (increased concentrations in kidneys and thigh muscle, but lower one in the thyroid gland);

– chromium (lower concentrations in blood, thigh muscle, and thyroid gland, and higher concentrations in the liver);

– manganese (lower concentrations in blood and thigh muscle, and higher concentrations in the kidneys);

– zinc (lower concentrations in the lungs, and higher concentrations in the kidneys and the thyroid gland);

– strontium (higher concentrations in thigh muscle, and lower ones in the thyroid gland);

– lead (higher concentrations in the lungs and lower ones in blood).

Obviously concentrations of the above-mentioned elements (except form antimony and arsenic) grow due to redistribution of elements that are contained in a body to organs that are "in need" after intoxication with antimony and arsenic. Therefore we can state that all the examined organs with increased concentrations of elements are susceptible to intoxication with arsenic and antimony; here we can mention blood (increased concentrations of chlorides and calcium), liver (increased concentrations of chlorides, potassium, calcium, chromium, zinc, bromine, and rubidium), heart (increased concentrations of chlorides and potassium), lungs (increased concentrations of sulfur, calcium, bromine, and lead), thigh muscle (increased concentrations of potassium, calcium, and strontium), thyroid gland (increased concentrations of chlorides, potassium, zinc, and bromine).

At the same time there is a well-known toxic effect produced by metals when they replace other metals and co-enzymes and displace them out of molecules. Hence the detected decreases in concentrations of chromium, manganese, iron, copper, molybdenum, selenium, zinc, nickel, and strontium in tissues are naturally caused by toxic damage done to the heart, liver, blood system, lungs, muscles, and thyroid gland. It is interesting to note that tissues of kidneys taken out of experimental animals didn't contain any of the above-mentioned elements in concentrations lower than standard ones. On the contrary, there were increased manganese concentrations in the kidneys and it was obviously due to their increased functional activity caused by occurring toxic effects produced by antimony and arsenic. Table 4 contains lists of elements that

were contained in organs of the experimental animals in concentrations being either higher or lower than standard ones.

Table 4

Distribution of elements in tissues of internal organs taken out of white mal Wistar rats from the test group against the control group in a sub-acute (21 day) experiment involving oral introduction of antimony and arsenic in doses equal to 0.061 mg/kg/day and 0.015 mg/kg/day

Organs	Decreased concentrations	Increased concentrations
Blood	Cr, Mn, Fe, Cu, Mo, Pb	Cl, Ca
Liver	Cu	Cl, K, Ca, Cr, Zn, Br, Rb
Kidneys		S, Cl, K, Ca, Mn, Br
Heart	Ni, Cu, Se, Mo	Cl, K
Lungs	Fe, Zn ,Mo	K, Br, Pb
Thigh muscle	Cr, Mn, Fe, Ni	S, K, Ca, Sr
Thyroid gland	Cr, S, Ni, Cu, Se, Sr	Cl, K, Zn, Br

We should note that increased concentrations of elements indicate that metabolic processes related to those elements are active; obviously, when concentrations go down, it means these processes are inactive. Consequently, elemental deficiency in most organs, except from the kidneys, means they are susceptible to intoxication and toxic effects influence biochemical processes that involve participation of chromium, manganese, iron, copper, molybdenum, nickel, selenium, and strontium.

Therefore, the results we obtained in our experiment allow us to conclude that:

1. Arsenic in tissues of the liver, kidneys, muscles, thyroid gland and whole blood of white rats and antimony in whole blood of white rats are markers of oral exposure to antimony and arsenic contained in a complex mixture.

2. Sub-acute combined oral exposure of white male Wistar rats to arsenic in a dose equal to 15 μg/kg and antimony in a dose equal to 61 μg/kg introduced into a body as components in a complex mixture results in increased concentrations of chlorine, potassium, sulfur, calcium, rubidium, zinc, manganese, and chromium in the liver and kidneys, and decreased concentrations of chromium, manganese, iron, copper, molybdenum, nickel, selenium, and strontium in blood, heart, thyroid gland, thigh muscle, and lungs; all these metals are elemental markers of exposure.

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References

1. Koval'skii V.V. Biogeokhimicheskie provintsii SSSR i metody ikh izucheniya [Biogeochemical provinces in the USSR and techniques applied to study them]. *Trudy Biogeokhimicheskoi laboratorii ANSSSR*, Moscow, 1960, 11 p. (in Russian).

2. Koval'skii V.V. Problemy biogeokhimiimi kroelementov i geokhimicheskoi ekologii [Issues related to biogeochemistry of microelements and geochemical ecology]. In: Yu.V. Koval'skii ed. Moscow, Rossel'khozakademiya Publ., 2009, 357 p. (in Russian).

3. Ermakov V.V. Geochemical ecology and biogeochemical criteria for estimating the ecologic state of biospherictaxons. *Geokhimiya*, 2015, no. 3, pp. 203–221 (in Russian).

4. D'yakovich M.P., Rukavishnikov V.S., Kazakova P.V., Finogenko I.A., Bokmel'der E.P., Solov'eva I.Yu. Kachestvo zhizni, svyazannoe so zdorov'em: otsenka i upravlenie: monografiya [Health-related life quality: assessment and management: a monograph]. In: V.S. Rukavishnikov ed. Irkutsk, Nauchnyi tsentr rekonstruktivnoi i vosstanovitel'noi khirurgii Sibirskogo otdeleniya RAMN Publ., 2012, 168 p. (in Russian).

5. Zaitseva N.V., Ustinova O.Yu., Zemlyanova M.A. Medical and preventive technologies of the management of the risk of health disorders associated with exposure to adverse environmental factors. *Fundamental'nye issledovaniya,* 2014, no. 10–4, pp. 665–670 (in Russian).

6. Gurvich V.B., Plotko E.G., Kuzmin S.V., Selyankina K.P., Ryzhov V.V., Makarenko N.P., Nadeenko V.G. Aktual'nye problemprofilakticheskoi meditsiny v Ural'skom regione [Vital issues of preventive medicine in the Urals]. *Sbornik nauchnykh trudov i nauchno-prakticheskikh rabot, posvyashchennyi 80-letiyu gossanepidsluzhby Rossii*, Ekaterinburg, 2002, pp. 76–81 (in Russian).

7. Avtsyn A.P., Zhavoronkov A.A., Rish M.A., Strochkova L.S. Mikroelementozy cheloveka: etiologiya, klassifikatsiya, organopatologiya: monografiya [Microelemental disorders in a human body: etiology, classification, and organopathology: a monograph]. Moscow, Meditsina Publ., 1991, 496 p. (in Russian).

8. Bulatova E.M., Gabrusskaya T.V., Bogdanova N.M., Yalfimova E.A. Current view on physiologic role of calcium in human organism. *Pediatriya*, 2007, vol. 86, no. 5, pp. 117–124 (in Russian).

9. Beggs M.R., Alexander R.T. Intestinal absorption and renal reabsorption of calcium throughout postnatal development. *Exp. Biol. Med.*, 2017, vol. 242, no. 8, pp. 840–849. DOI: 10.1177/1535370217699536

10. Titan v organizme cheloveka: rol', istochniki, nekhvatka i izbytok. [Titanium in a human body: role, sources, deficiency and excess]. *Zdorov'eipsikhologiya: vse o fizicheskom i dushevnomzdorov'e. Blog Alekseya Rybaka.* Available at: http://zdips.ru/zdorovoe-pitanie/mineraly/1635-titan-v-organizme-cheloveka.html (15.12.2018) (in Russian).

11. Biologicheskaya rol' titana. Dioksid titana TiO_2 (dvuokis' titana, titanium dioxide) [Biological role of titanium. Titanium dioxide TiO_2]. Available at: http://www.titandioxide.ru/articles/art_21.php (15.12.2018) (in Russian).

12. Oganyan A.A., Neelova O.V. Biologicheskaya rol' khroma, primenenie dikhromata kaliya v farmatsevticheskom analize [Biological role of chromium and application of potassium dichromate in pharmaceutical analysis]. *Uspekhi sovremennogo estestvoznaniya*, 2011, no. 8, pp. 227–227 (in Russian).

13. Mamyrbaev A.A. Toksikologiya khroma i ego soedinenii: monografiya [Toxicology of chromium and its compounds: a monograph]. Aktobe, 2012, 284 p. (in Russian).

14. Dobson A., Erikson K.M., Aschner M. Manganese Neurotoxicity. *Annals of the New York Academy of Science*, 2004, vol. 1012, pp. 115–128. DOI: 10.1196/annals.1306.009

15. Zhelezo v organizme, regulyatsiya [Iron in a body and its regulation]. Availableat: http://biohimik.net/prichiny-anemii/zhelezo-v-organizme/zhelezo-v-organizme-regulyatsiya (15.12.2018) (in Russian).

16. Obmen medi v organizme i egonarushenie [Metabolism of copper in a body and its disorders]. Available at: http://sunmuseum.ru/patofiziologiya/1527-obmen-medi-v-organizme-i-ego-narushenie.html (15.12.2018) (in Russian).

17. Rish M.A. Geneticheskii kontrol' mikroelementozov u cheloveka i zhivotnykh. Geokhimicheskaya ekologiya i biogeokhimicheskoe raionirovanie biosfery: materialy vtoroi Rossiiskoi shkoly [Genetic control over microelemental disorders in human and animals. Geochemical ecology and biogeochemical zoning in the biosphere: the materials collected by the second all-Russian school]. Moscow, 1999, pp. 205–207 (in Russian).

18. Goncharova E.I., Rossman T.G. A role for metalothionein and zinc in spontaneous mutagenesis. *Cancer Res.*, 1994, vol. 54, no. 20, pp. 5318–5323.

19. Stefanidou M., Maravelias C., Dona A., Spiliopoulou C. Zinc: a multipurpose trace element. *Arch. Toxicol.*, 2006, vol. 80, no. 1, pp. 1–9.

20. Selenvorganizme [Selenium in a body]. *Biologiya i meditsina.* Available at: http://medbiol.ru/medbiol/har/0055c9fd.htm (15.12.2018) (in Russian).

21. Gosse J.A., Taylor V.F., Jackson B.P., Hamilton J.W., Bodwell J.E. Monomethylated trivalent arsenic species disrupt steroid receptor interactions with their DNA response elements at non-cytotoxic cellular concentrations. *J. Appl. Toxicol.*, 2014, vol. 34, no. 5, pp. 498–505. DOI: 10.1002/jat.2898

22. Mak Dermott M. Sekrety endokrinologii [Secrets of endocrinology]. 2-nd edition. Sankt-Peterburg: Binom – Nevskii Dialekt Publ., 2001, 464 p.

23. Sur'ma «Rvotnyi kamen'» [Antimony or "vomitive emetic stone"]. *Farmakognoziya*. Available at: http://pharmacognosy.com.ua/index.php/makro-i-mikro-chudesa/surma-rvotniy-kamen (12.11.2018) (in Russian).

24. Klinicheskaya mikroelementologiya. Sur'ma [Clinical microelementology. Antimony]. *Mikroelement. Nauchno-meditsinskii tsentr*. Available at: www.microelement.ru/uslovno-toksichnye/99-surma.html (15.12.2018) (in Russian).

Franovskii S.Yu., Turbinskii V.V., Oks E.I., Bortnikova S.B. Elemental markers of exposure under combined oral introduction of chemical mixtures with prevalent antimony and arsenic into white wistar rats. *Health Risk Analysis, 2019, no. 3, pp. 94–103. DOI: 10.21668/health.risk/2019.3.11.eng*

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