UDC [615.9+612.014.46]: 546.43
DOI: 10.21668/health.risk/2022.2.17.eng

Read online

Research article

EXPERIMENTAL MODELS OF ANIMAL CHRONIC PATHOLOGY IN ASSESSING HEALTH RISKS FOR SENSITIVE POPULATION GROUPS

E.V. Drozdova[1], S.I. Sychik[1], V.A. Hrynchak[1], S.N. Rjabceva[2]

[1]Scientific and Practical Center for Hygiene, 8 Akademicheskaya Str., Minsk, 220012, Republic of Belarus
[2]Institute of Physiology of the National Academy of Sciences of Belarus, 28 Akademicheskaya Str., Minsk, 220072, Republic of Belarus

The methodology for health risk assessment and hygienic standardization of chemicals often neglects such a vulnerable population group as people with chronic non-communicable diseases. According to the data provided by the WHO, the prevalence of such pathologies is high in many European countries; therefore, when a disease burden in a certain population is unaccounted for, this may result in lower accuracy of accomplished assessments. On the other hand, introduction of too conservative safe factors when hygienic standards are being developed for chemicals in various media leads to high uncertainty and excessive limitations.

Our research goal was to provide scientific substantiation for a methodology for using experimental pathology models to improve reliability of hygienic standardization and accuracy of health risk assessments for sensitive population groups (people suffering from non-chronic communicable diseases) under exposure to naturally occurring chemicals. Another goal was to test this methodology by performing a case study on drinking water. The testing results indicate that a chronic 6-month exposure to model substances produced more apparent toxic effects on experimental animals with model pathologies (spontaneous hypertension and experimental gentamicin-induced nephropathy) in comparison with "healthy" animals.

This allowed us to recommend using experimental models of congenital and induced animal pathology bearing in mind target organs for toxic effects produced by the analyzed chemicals to substantiate hygienic standards, health risks taken into account. This should be done at the stage when dose-dependent reactions are identified (determination of no-effect and / or threshold levels) in addition to studies performed on "healthy" animals. It is most appropriate to use this approach when the following conditions are met: 1) a research object is naturally occurring chemicals that are widely spread in the environment due to its natural formation; 2) pathologies of organs (systems) that are targets for biological effects produced by the tested chemicals are widely spread in a population (circulatory diseases, diseases of the excretory system, etc.).

Keywords: *experimental pathology models, nephropathy, spontaneously hypertensive rats, risk assessment, hygienic standardization, sensitive population groups, methodological approaches, barium, total mineralization.*

It is a common practice to use an uncertainty factor when developing hygienic standards for chemicals in environmental objects. This factor is used to achieve proper transfer to standardized values from threshold or no-effect doses (concentrations) determined through experiments on laboratory animals, mathematical modeling, or epidemiological studies. Uncertainty factor is assumed to consider all basic uncertainties including intraspecies and interspecies variation as well as database deficiencies (availability or absence of data on chronic and specific toxicity, reproductive toxicity or other remote effects; scales of an experiment or an epidemiological study are also impor-

Elena V. Drozdova – Candidate of Medical Sciences, Associate Professor, Deputy Director for Science (e-mail: drozdovaev@mail.ru; tel.: +7 (017) 370-50-15; ORCID: https://orcid.org/0000-0002-3032-0895).

Sergey I. Sychik – Candidate of Medical Sciences, Associate Professor, director (e-mail: rspch@rspch.by; tel.: +7 (017) 347-73-70; ORCID: https://orcid.org/0000-0002-5493-9799).

Vitali A. Hrynchak – Candidate of Medical Sciences, Head of the Laboratory for Applied Toxicology and Safety of Medical Products (e-mail: grinchakva@gmail.com; tel.: +7 (017) 399-44-52; ORCID: https://orcid.org/0000-0002-4119-1793).

Svetlana N. Rjabceva – Candidate of Medical Sciences, Associate Professor, Head of the Laboratory "Center for Electron and Light Microscopy" (e-mail: sveta.rjabceva@tut.by; tel.: +7 (017) 357-22-72; ORCID: https://orcid.org/0000-0001-5960-3656).

tant). This approach is widely used in national, regional (the EAEU countries) and the best international practices [1, 2].

According to several researchers, when a conservative uncertainty factor equal to 10 is applied for intraspecific variation, this may guarantee covering only 80–95 % of variability inherent to human population if we remember about differences in metabolism of xenobiotics [3]. Later it was noted, that this analysis did not include certain population sub-groups (age-related effects or those produced by genetic polymorphism were neglected etc.). In addition, a large group of potentially vulnerable people was neglected although these people are much more sensitive than "an average healthy adult person" is, for example, due to pathologies or functional disorders of an organ or a system of organs participating in toxicant metabolism [4–9]. If we allow for all the aforementioned indicators, then uncertainty factor with its established value equal to 10 can "protect" only 60 % of population [10, 11]. Besides, if people have certain chronic pathologies, they tend to take drugs for a long-term period. These drugs can make substantial alterations into a direct or indirect response given by the body to chemical exposure[1] [12, 13]. Preexisting kidney diseases undoubtedly play a significant role in occurring nephrotoxic disorders. Experiments involving laboratory animals provided convincing evidence that common human diseases (hypertension, kidney failure and kidney ischemia) enhanced renal toxic effects produced by cyclosporine and bacterial endotoxins. However, there are rather scarce scientific data on actual predictive value of clinical observations and results produced by experiments involving laboratory animals. Chemical toxicity is usually examined in experiments on healthy laboratory animals. This does not allow extrapolating their results on such vulnerable population groups as people suffering from chronic non-communicable diseases and / or taking drugs constantly.

Recently, multiple research works have been accomplished with their focus on providing substantiation for methodical approaches to selecting the most optimal factors of intraspecific and interspecific uncertainty together with establishing the most relevant values of uncertainty factors. For example, so called chemical-specific adjustment factors (CSAFs) have been developed; they consider data on toxicokinetics and toxicodynamics of a chemical and its metabolites in the body [14, 15]. Use of CSAFs has already been formalized in international recommendations and implemented into risk assessment practices[2] [16]; however, this approach also covers only optimal conditions when all the organs are healthy and function properly.

Therefore, such a vulnerable population group as people with chronic diseases is neglected completely in assessing health risks caused by exposure to chemical factors and in hygienic standardizing. We should note that there are certain global trends in population health that are also typical for the Republic of Belarus and most countries in the WHO-Euro region. These trends include excessive prevalence of chronic non-communicable diseases that account for 86 % in mortality and 77 % in the overall disease burden. Diseases of the circulatory system and oncological diseases are priority ones; their etiology may be potentially associated with exposure to chemical factors [17].

When the disease burden in a given population is neglected, this undermines accuracy of health risk assessment and hygienic standardization based on the conventional approach. At the same time, introduction of too high uncertainty factors when establishing maximum permissible concentrations in various media leads to high uncertainty and

[1] Myasnikov A.L. Patogenez gipertonii [Pathogenesis of hypertension]. *Gipertoniya voennogo vremeni*. Leningrad, MSU VMF Publ., 1945, pp. 4–16 (in Russian).

[2] Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment. Geneva, World Health Organization, 2005, 96 p.

excessive limitations. A good example here can be impossibility to use water from certain sources in drinking water supply since it contains natural chemicals in concentrations higher than established in "strict" standards and its treatment requires significant costs (barium).

Given all the above-stated, we can conclude that it is vital to develop more reliable hygienic standards and to accomplish more accurate health risk assessment under exposure to chemicals allowing for sensitive population groups. The issue is especially interesting when it comes down to substantiating hygienic standards for natural chemicals that occur in environmental objects in elevated concentrations due to regional peculiarities.

In the present study, we suggest a new methodical approach to reduce uncertainties associated with neglected potentially higher sensitivity of a vulnerable population group (people suffering from chronic non-communicable diseases or taking certain drugs constantly) in assessing health risks caused by exposure to naturally occurring chemicals and in their hygienic standardization.

Obviously, experimental pathology models, including spontaneously hypertensive rats (SHR), have been used for quite a long time to assess pharmacological properties of drugs [18–24] and to examine influence exerted on a developing pathology by certain food products [25, 26]. Such models are also applied in some toxicological studies, for example, a hypertension model to assess small doses of pollutants in ambient air or acute exposure to ethanol in small doses [27, 28]. However, there is no available methodology for systemic use of pathology models in assessing health risks and substantiating hygienic standards for naturally occurring chemicals in the environment.

Our research goal was to provide scientific substantiation for a methodology for using experimental pathology models considering the most common target organs and biological effects produced by the analyzed chemicals and to test this methodology. Its main purpose is to improve reliability of hygienic standardization and accuracy of health risk assessments for sensitive population groups under exposure to naturally occurring chemicals.

To achieve the goal, the following tasks were set:

1) to examine the existing experimental pathology models (exemplified by hypertension models and nephropathy models) and to substantiate their selection for further experimental testing of their use in hygienic standardization and health risk assessment (on the example of drinking water and its model chemical indicators typical for hydrochemical conditions in the republic);

2) to analyze and comparatively assess biological effects produced by model chemical indicators under different levels of exposure on healthy animals (average or standardized models) and experimental pathology models (risk group models);

3) to substantiate methodical approaches to applying experimental pathology models in hygienic standardization of naturally occurring chemicals based on health risk assessment considering sensitive population groups.

Materials and methods. Within the present study, we selected two experimental pathology models, namely, hypertension (spontaneous) and nephropathy (experimental gentamicin-induced) to test the suggested methodical approaches. Our choice is well grounded by performed analysis considering a whole set of relevant criteria.

A model chemical (barium) and a generalized indicator (total mineralization) were selected based on the following criteria: 1) relevance for regional hygienic standardization is confirmed by their prevalence in drinking water in the republic due to natural peculiarities of underground water-bearing horizons; 2) the cardiovascular and excretory system are exposure targets for model chemicals; their biological effects have been proven by multiple experiments and their threshold effects were considered in providing substantiation for national and foreign safety standards and levels recommended by

the WHO[3]; 3) non-communicable diseases of organs (systems) that are target ones for biological effects produced by the analyzed chemicals are widely spread in population.

The suggested models were tested in a 6-month chronic experiment on laboratory animals. The animals drank water containing the analyzed chemicals in different concentrations; they had free access to water during the whole experiment and no limitations were imposed on drinking. Chemical concentrations were substantiated allowing for toxicometric parameters used in establishing standards for the analyzed chemicals at the national and international levels. These parameters were non-effective concentrations and concentrations that would certainly produce biological effects on healthy laboratory animals: water with barium concentration 1.3 mg/l and 70 mg/l and total mineralization being 1500 and 10,000 mg/l accordingly.

To test experimental pathology models, we created five groups, 10 randomly bred white male rats in each. The control group had free access to drinking water without any limitations on its consumed amount and the remaining four test groups drank water with the relevant concentrations of analyzed model chemicals ("control", "Ba 1.3", "Ba 70", "M 1500" and "M 10,000" groups in the classical model).

To model nephropathy, we created five groups of rats, 10 animals in each. Prior to the experiment, gentamicin was administered intraperitoneally in a dose 70 mg/kg/day for 10 days. After nephropathy developed, one group ("control") had free access to water in unlimited quantities and the remaining four test groups drank water with the analyzed substances in relevant concentrations ("EINP control", "EINP Ba 1.3", "EINP Ba 70", "EINP M 1500", "EINP M 10,000" groups in the experimentally induced nephropathy (EINP) model).

To test the hypertension model, we created three groups, 10 male SHR (spontaneously hypertensive rats) in each. The animals had persistent elevated blood pressure. The control group and two test groups had free access to the initial water and water with barium concentrations being 1.3 and 70 mg/l accordingly ("SHR control", "SHR Ba 1.3", "SHR Ba 70" in the hypertension model).

All our experiments were performed on randomly bred male white rats from the vivarium of the Scientific and Practical Center for Hygiene and SHR from the vivarium of the Institute for Bioorganic Chemistry of the Belarus National Academy of Sciences. Prior to the experiments, animals underwent a two-week quarantine period. Experimental animals had to be active, with good appetite, smooth and shiny fur and proper coloring of visible mucosa. They also had to be accustomed to standard nutrition provided in the vivariums.

Water with relevant barium (Ba) concentration and mineralization (M) was prepared by dissolving powder 2-water barium chloride ("VEKTON" LLC, Russia, State Standard GOST 4108-72) and a powder mineral additive called "Severyanka" ("Eko-proekt" LLC, Russia) in required proportions accordingly.

The experimental animals were weighed daily during the whole 6-month experiment; we also assessed their daily water consumption and noted any clinical signs of intoxication or deaths. To determine whether hypertension as an analyzed critical effect was developing, we took systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in all the rates prior to the experiment and after it was over. The indicators were measured by

[3] Guidelines for Drinking-water Quality (4th ed. with adds). Geneva, WHO, 2017, 631 p.; Barium in Drinking Water: Guideline Technical Document for Public Consultation. *Health Canada*, 2018, 52 p. Available at: https://www.canada.ca/content/dam/hc-sc/documents/programs/consultation-barium-drinking-water/document-eng.pdf (November 20, 2021); Barium in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality. WHO, 2016, 21 p. Available at: https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/barium-background-jan17.pdf?sfvrsn=9a2355a1_4 (November 20, 2021); Toxicological profile for barium and barium compounds. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, 2007, 231 p. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp24.pdf (November 20, 2021); Barium and Compounds. CASRN 7440-39-3. US Environmental Protection Agency, National Center for Environmental Assessment, 2005, 34 p. Available at: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0010_summary.pdf (November 20, 2021).

using "Systola" system for non-invasive blood pressure measurement in rats and "Flogiston" platform manufactured by "Nuerobotiks" LLC, Russia.

White rats were taken from the experiment by decapitation followed by autopsy that involved determining relative mass coefficients (RMC) of internal organs. To examine morphofucntional state of experimental animals' bodies, we applied various analysis techniques. We assessed relevant biochemical indicators of blood serum including urea, lactate dehydrogenase (LDG), cholesterol, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, creatinine, total bilirubin and total protein, α-amylase, glucose, uric acid, high and low density lipoproteins (HDLP and LDLP), phosphor, iron and magnesium. These indicators as well as functional indicators of the urinary excretion system were examined with Accent 200 automated biochemical analyzer (Poland). We examined morphofucntional composition of peripheral blood by flow cytometry performed with Mythic 18 hematology analyzer (Switzerland); morphological structure of animals' internal organs was examined using conventional procedures.

Experimental animals were cared for in conformity with the ethical principles of good laboratory practice[4].

All the experimental data were statistically analyzed with parametric and non-parametric procedures that are conventionally applied in medical and biological research. The analysis was performed in Statistica 10 and MS Excel software packages. The critical significance level for testing statistical hypotheses was taken at $p < 0.05$.

Results and discussion. Functional changes detected in the rats from "EINP control" indicated that the model pathology (chronic nephropathy) was developing in them in contrast to white rates that were not given any gentamicin ("control"). We detected a statistically significant decrease in phosphor concentration in blood, by 13.5 %; LDLP, by 1.9 times; urea, by 2.3 times ($p < 0.001$); total protein, by 16.9 % ($p < 0.01$); albumin, by 18.1 % ($p < 0.003$). There was a growth in concentrations of uric acid (by 29.8 %) ($p < 0.04$); creatinine (by 25.4 %); glucose (by 22.9 %); AST (by 13.6 %) ($p < 0.001$); ALT, by 43.9 % ($p < 0.007$). We also detected a decline in daily diuresis by 19.1 % ($p < 0.004$) and multidirectional shifts in protein and mineral metabolism. Thus, we established that secretion of total protein was by 1.2 times higher ($p < 0.008$); phosphor concentration was by 2.3 times lower; magnesium concentration, by 2.6 times lower; urea and creatinine concentration in urine were by 1.4 and 1.3 times lower accordingly (at $p < 0.001$). We also detected that leukocyte levels were by 12.1 % higher in the animals with induced nephropathy in comparison with healthy animals, including neutrophils (by 20.0 %), monocytes (by 2.2 times), eosinophils (by 29.3 %) and basophils (by 1.4 times) ($p < 0.01$).

Research results under exposure to the model chemicals indicated absence of any significant changes in overall health of animals from all 13 experimental groups throughout the chronic experiment. Water consumption remained at reference levels. A daily dose for laboratory animals that were given barium solutions in concentrations equal to 1.3 and 70 mg/l amounted to 0.05 and 2.7–2.9 mg/kg accordingly (Table 1).

We examined functional indicators of laboratory animals after the experiment was over and established statistically significant disorders occurring in some organs and systems. Thus, chronic consumption of water with barium concentration being 70 mg/l made for a statistically significant rise in blood pressure (both systolic and diastolic) in all experimental groups. White rats had SBP by 5.5 % ($p < 0.003$) and DBP by 9.4 % ($p < 0.009$) higher; animals with induced neuropathy, SBP by 9.7 % ($p < 0.001$) and DBP by 9.3 % ($p < 0.005$) higher; hypertensive rats, SBP by 14.8 % ($p < 0.003$) and DBP by 18.6 % ($p < 0.02$) higher (Table 2). Hypertension developed most apparently under exposure to

[4] Guide for the care and use of laboratory animals. Washington, D.C., National Academies Press, 1996, 154 p.

Table 1

A consumed dose and water consumption by rats under exposure to solutions with different barium concentrations and mineralization levels in the chronic experiment, Me (P_{25}–P_{75})

Experiment groups		Indicators, measuring units	
Animals	Group	Water consumption, ml	Dose, mg/kg a day
White rats	"control"	84.6 (71.4–97.8)	–
	"Ba1.3"	84.8 (71.6–98.0)	0.05 (0.05–0.05)
	"Ba 70"	84.7 (71.5–97.9)	2.70(2.68–2.72)
	"M 1500"	84.6 (71.4–97.8)	–
	"M 10,000"	85.0 (71.8–98.2)	–
White rats with neuropathy (EINP)	"EINP control"	85.2 (71.9–98.3)	–
	"EINP Ba 1.3"	85.0 (71.7–98.0)	0.05 (0.05–0.05)
	"EINP Ba 70"	85.3 (72.0–98.3)	2.71 (2.57–2.75)
	"EINP M 1500"	91.6 (72.8–103.5)	–
	"EINP M 10,000"	84.5 (78.1–97.1)	–
SHR (spontaneously hypertensive rats)	"SHR control"	105.0 (91.7–120.7)	–
	"SHR Ba 1.3"	106.3 (93.0–119.3)	0.05(0.05–0.06)
	"SHR Ba 70"	107.4 (94.1–120.4)	2.91(2.68–3.17)

Table 2

Blood pressure and heart rate of SHR under exposure to solutions with different barium concentrations in the chronic experiment, Me (P_{25}–P_{75})

Experimental groups	Blood pressure, measuring units					
	Initial			After 6 months		
	SDP, mm Hg	DBP, mm Hg	Heart rate, str./min	SDP, mm Hg	DBP, mm Hg	Heart rate, str./min
"SHR control"	140 (138–144)	123 (118–125)	484 (471–490)	189 (177–201)	167 (160–190)	534 (520–556)
"SHR Ba 1.3"	142 (133–147)	122 (113–123)	483 (473–491)	189 (184–202)	175 (163–186)	544 (532–555)
"SHR Ba 70"	145 (139–148)	123 (111–129)	486 (479–491)	217 (210–226)*	198 (193–206)*	534 (528–545)

Note: * means statistically significant differences at $p < 0.02$.

barium in a concentration equal to 70 mg/l in SHR since their blood pressure on average grew by 30 mm Hg with DBP growth being the most apparent. Heart rate did not change in any experimental group.

We detected a statistically significant growth in rats' body mass in the experiment that involved chronic exposure to barium in drinking water in a concentration equal to 70 mg/l. Body mass of white rats grew by 2.8 % ($p < 0.003$); rats with nephropathy, by 6.1 % ($p < 0.02$); and hypertensive rats, by 5.1 % ($p < 0.01$) whereas there was no growth in body mass of rats from all the other test groups against the control ones.

When animals with kidney failure and without any renal pathology were exposed to barium in a concentration equal to 70 mg/l, this made for developing leukocytosis. It became apparent via growing levels of leukocytes and neutrophils, by 19.9 and 32.0 % ($p < 0.003$) against the control groups and by 14.6 and 16.7 % ($p < 0.01$) accordingly against the control group with nephropathy (EINP control). We also detected a 10.9 % decrease in thrombocytes ($p < 0.006$) and lower hemoglobin contents in erythrocytes (by 7.2 %) ($p < 0.001$), which determined its level in the test animal being by 4.2 % ($p < 0.001$) lower than in the control animals with nephropathy.

Toxic effects produced by barium on animals with nephropathy became obvious due to a more apparent decline by 12.5 % in total protein contents in blood ($p < 0.006$), and a

5.0 % growth in creatinine concentration ($p < 0.001$). Changes detected in blood serum of experimental animals with model pathology that were exposed to solutions with mineralization equal to 10,000 mg/l became obvious since mineral metabolism was apparently impaired. It was confirmed by statistically significantly lower phosphor and magnesium concentrations, by 19.7 and 12.5 % accordingly, as well as a 2.2 time decrease in uric acid concentration against the control group of white rats that were given gentamicin.

Exposure to barium in a concentration equal to 70 mg/l initiated weak leukocytosis in SHR as well but there were no other statistically significant changes in morphofucntional indicators of SHR blood (Table 3).

We compared biochemical blood indicators under exposure to barium in a concentration equal to 70 mg/l in the white rats control and SHR. The comparison revealed lower contents of total protein, by 20.7 % ($p < 0.004$) and 16.2 % ($p < 0.001$); and a higher AST concentration, by 21.4 % ($p < 0.001$) and 10.6 % ($p < 0.003$) accordingly. We should note that there were multi-directional shifts in mineral metabolism of hypertensive rats exposed to barium in a concentration equal to 70 mg/l such as an increase in phosphor concentration in blood serum by 25.7 % ($p < 0.001$) and a decline in magnesium concentration by 7.8 % ($p < 0.02$).

Chronic exposure to barium in a concentration equal to 70 mg/l induced proteinuria and its signs were more apparent in animals with nephropathy. Spontaneously hypertensive rats, in contrast to other animals, had statistically significant disorders of mineral metabolism and nitrogen-containing products of protein metabolism in their urine under exposure to barium in a concentration equal to 70 mg/l (Table 4).

We did not detect any changes in functional state of white rats' kidneys after they were exposed to drinking water with mineralization being 1500 mg/l. Still, drinking water with mineralization being 10,000 mg/l induced functional disorders of the urinary excretion system. We detected a statistically significant decrease in phosphor and magnesium contents in urine, by 42.4 and 35.7 % accordingly together with elevated urea excretion; these signs were even more apparent in animals with chronic nephropathy. We should also note that α-amylase excretion with urine fell by 19.4 % ($p < 0.01$) in comparison with the control white rats with kidney pathology.

Table 3

Morphofunctional indicators of SHR blood under exposure to solutions with different barium concentrations in the chronic experiment, Me (P_{25}–P_{75})

Indicators, measuring units	Experimental groups, exposure levels, mg/l		
	Control	Ba 1.3	Ba 70
Leukocytes, $\times 10^9$ cells/l	14.2 (13.6–14.9)	14.5 (12.9–15.5)	16.7 (15.9–17.2)*
Neutrophils, $\times 10^9$ cells/l	2.7 (2.5–2.9)	2.6 (2.4–2.8)	3.2 (2.9–3.5)*
Lymphocytes, $\times 10^9$ cells/l	9.4 (8.5–10.2)	9.1 (8.4–9.9)	9.3 (8.3–9.9)
Monocytes, $\times 10^9$ cells/l	1.2 (0.9–1.6)	1.0 (0.7–1.5)	1.1 (0.9–1.3)
Eosinophils, $\times 10^9$ cells/l	0.87 (0.80–0.91)	0.87 (0.81–0.91)	0.89 (0.83–0.95)
Basophils, $\times 10^9$ cells/l	0.18 (0.11–0.19)	0.17 (0.16–0.20)	0.18 (0.15–0.21)
Erythrocytes, $\times 10^{12}$ cells/l	8.2 (8.0–8.4)	8.1 (8.0–8.3)	8.1 (7.9–8.6)
Hemoglobin concentration, g/l	142 (139–143)	140 (139–141)	143 (142–143)
Hematocrit, l/l	0.39 (0.37–0.41)	0.38 (0.37–0.39)	0.39 (0.39–0.40)
Average erythrocyte volume, fl	51.4 (51.0–51.8)	51.1 (50.2–51.7)	51.5 (49.8–52.1)
Average hemoglobin contents in erythrocytes, pg	18.3 (18.1–18.7)	18.3 (18.1–18.7)	18.5 (17.8–18.7)
Average hemoglobin concentration in erythrocyte, g/l	363 (362–364)	363 (362–364)	364 (362–368)
Thrombocytes, $\times 10^9$ cells/l	849 (823–935)	853 (729–1015)	863 (844–900)
Average thrombocyte volume, fl	6.2 (6.1–6.3)	6.2 (6.1–6.3)	6.3 (6.1–6.3)

N o t e : * means statistically significant differences at $p < 0.001$.

Table 4

Functional state of SH rats' kidneys under exposure to solutions with different barium concentrations in the chronic experiment, Me (P_{25}–P_{75})

Indicators, measuring units	Experimental groups, exposure levels, mg/l		
	Control	Ba 1.3	Ba 70
Total protein, g/l	5.4 (5.2–6.0)	5.6 (5.4–5.8)	6.6 (6.1–7.0)*
Phosphor, mmol/l	33.9 (22.5–43.0)	33.3 (28.7–38.3)	17.9 (14.8–20.0)*
Iron, μmol/l	17.4 (17.1–17.5)	17.3 (16.4–17.5)	17.3 (16.4–18.7)
Magnesium, mmol/l	0.87 (0.54–0.98)	0.92 (0.81–1.00)	0.51 (0.46–0.54)*
Urea, mmol/l	251 (230–329)	232 (213–258)	192 (177–224)*
Uric acid, μmol/l	3700 (3303–3706)	3568 (3310–3689)	2916 (2791–3048)*
α-amylase, units/l	1185 (986–1234)	1197 (1074–1293)	1134 (1026–1247)
Creatinine, μmol/l	5244 (3480–6476)	5305 (4848–5935)	2753 (2359–3073)*
Glucose, mmol/l	0.99 (0.83–1.05)	1.02 (0.97–1.07)	0.92 (0.82–0.98)
Diuresis, l^{-3}/days	13.8 (13.0–14.1)	14.4 (14.0–15.2)	13.6 (13.1–13.9)
pH, pH units	7.1 (7.0–7.2)	6.9 (6.7–7.0)	7.0 (6.7–7.2)

N o t e : * means statistically significant differences at $p < 0.01$.

We analyzed relative mass coefficients (RMC) of internal organs taken from the experimental animals 6 months after gentamicin was administered in them. The analysis revealed that the liver mass grew by 5.3 % ($p < 0.02$) and the stomach mass grew by 16.4 % ($p < 0.001$) whereas kidney RMC went down by 6.8 % ($p < 0.03$). The heart mass grew statistically significantly in all animal groups (SHR, rats with nephropathy, white rats without kidney pathology) under exposure to water solutions with mineralization being 10,000 mg/l and barium in a concentration equal to 70 mg/l. Barium in this concentration also made for a decrease in liver and stomach RMC by 6.8 % ($p < 0.04$) and 21.2 % ($p < 0.001$) accordingly in animals with nephropathy and mineralization equal to 10,000 mg/l induced a decrease in the stomach mass by 20.0 % at $p < 0.001$.

The aforementioned changes in relative mass components as well as functional disorders detected in internal organs of experimental animals are confirmed by morphological examinations. Thus, after white rates were chronically exposed to barium in a concentration equal to 70 mg/l, we detected slight focal diffuse dystrophic changes in their cardiac muscle, signs of myocarditis, focal chronic hepatitis with slight hepatocytes dystrophy (1/3 parts of the lobule periphery), pyelitis and moderate dystrophic and necrobiotic changes in the epithelium of the proximal renal tubules. Chronic exposure to water solutions with mineralization being 10,000 mg/l resulted in kidney, heart and liver lesions in experimental animals. We detected focal diffuse dystrophic changes in the cardiac muscle and myocarditis, focal chronic hepatitis with hepatocytes dystrophy (2/3 parts of the lobule periphery), pyelitis and focal dystrophic changes in the epithelium of the proximal renal tubules, chronic slight active atrophic-hyperplastic gastritis.

After gentamicin was administered into experimental animals from "EINP control" group, we detected the following changes in their internal organs: slight liver and kidney dystrophy, signs of pyelitis together with glomerule lesions and signs of hepatitis. We did not detect any pathological changes in the heart and stomach. However, after white rats were exposed to barium in a concentration equal to 1.3 mg/l, we detected slight disorders, such as changes in the kidneys together with pyelitis but there were no toxic effects produced on the heart or stomach. After exposure to barium in a concentration equal to 70 mg/l, the animals had apparent changes in the kidneys, cardiac muscle lesions with developing myocarditis, and slight hepatocytes dystrophy. There was also chronic active gastritis with signs of hyperplasia and gland epithelium atrophy. Mineralization equal to 1500 mg/l induced slight hepatocytes dystrophy, signs of

hepatitis, and slight changes in the kidneys of the experimental animals with nephropathy without any toxic effects produced on the heart or stomach. However, after these animals were exposed to water solutions with mineralization being 10,000 mg/l, we established moderate lesions of the cardiac muscle and kidneys, signs of developing pyelitis and myocarditis, moderate hepatocytes dystrophy as well as chronic active gastritis with hyperplasia and gland atrophy together with epithelium hypersecretion.

After SH rats underwent chronic exposure to barium in water in a concentration being equal to 1.3 mg/l, we did not detect any signs of toxic effects produced on the heart, liver or stomach of the experimental animals. There were slight changes in the epithelium of proximal renal tubules typical for such laboratory animals. After hypertensive rats were exposed to barium in a concentration equal to 70 mg/l, they had apparent changes in the epithelium of the proximal renal tubules, developing glomerulonephritis and pyelitis; there were also moderate lesions of the liver and heart. We detected chronic active gastritis with signs of both hyperplasia and gland atrophy and epithelium hypersecretion and desquamation.

Therefore, our test results indicate that toxic effects produced by the model chemicals under chronic 6-month experimental exposure were more apparent in the animals with modeled experimental pathologies (spontaneous hypertension and experimentally gentamicin-induced nephropathy) than in the classical (healthy) animals. Morphofucntional disorders detected in the urinary excretion system, cardiovascular system and hepatobiliary system proved that pathologies were developing in the experimental animals under chronic exposure to the analyzed chemicals in certainly effective concentrations. Toxic effects produced by barium in a concentration equal to 70 mg/l became apparent through developing leukocytosis, impaired protein metabolism, elevated blood pressure, morphofucntional changes in the heart with its mass growing obviously, as well as signs of chronic hepatitis, pyelitis, and dystrophic changes in the epithelium of the proximal renal tubules. After white rats were exposed to water with mineralization being 10,000 mg/l, this led to impaired mineral and protein metabolism as well as developing slight lesions of the kidneys, heart and liver. Barium in a concentration equal to 1.3 mg/l and mineralization being 1500 mg/l can be considered no-effect doses in this chronic experiment on rats.

Conclusions. We have suggested methodical approaches to applying experimental pathology models to assess health risks and to establish hygienic standards for chemicals. The approaches consider sensitive population groups (people suffering from chronic non-communicable diseases or taking certain drugs constantly). The methodology was tested on drinking water in the chronic experiment on model animals with spontaneous hypertension and experimentally induced chronic nephropathy. The model chemical indicators (barium and total mineralization) were selected for the experiment since they are typical for water-bearing horizons in the Republic of Belarus. The results confirmed our assumption that pathology models were authentically more sensitive and susceptible to toxic effects produced by the analyzed chemicals than classical models ("healthy" animals).

This allowed us to recommend using experimental models of congenital and induced animal pathology bearing in mind target organs for toxic effects produced by the analyzed chemicals to substantiate hygienic standards, health risks taken into account. This should be done at the stage when dose-dependent reactions are identified (determination of no-effect and/or threshold exposure levels) in addition to conventional studies performed on "healthy" animals. It is most appropriate to use this approach when the following conditions are met: 1) a research object is naturally occurring chemicals that are widely spread in the environment due to its natural formation; 2) pathologies of organs (systems) that are targets for biological effects produced by the tested chemicals are widely spread in a population (circulatory diseases, diseases of the excretory system, etc.).

It is also quite appropriate to examine biological effects produced by chemicals to determine their no-effect concentrations (doses) for reference effects in a chronic experiment on animals with model pathologies. Model pathologies should be selected bearing in mind organs that are targets for toxic effects produced by analyzed chemicals. When such experiments are performed in addition to conventional ones on "healthy" animals, this allows achieving more precise health risk assessment and creating more reliable hygienic standards for chemical concentrations in the environmental objects. This gives an opportunity to consider sensitive population groups and can also "enhance" an evidence base when hygienic standards for chemicals in various media are being revised, regional peculiarities taken into account, to make them less "strict" (risk-based standardization).

The suggested methodical approaches have been formalized in the instructions "The procedure for hygienic standardization of chemicals in drinking water as per health risk".

Funding. The study has been accomplished within the grant provided by the Belarus Republican Fund for Fundamental Research No. M20-071 "Experimental pathology models in assessing health risks associated with exposure to chemical factors for sensitive population groups".

Competing interests. The authors declare no competing interests.

References

1. Uncertainty factors: Their use in human health risk assessment by UK Government. Leicester, Institute for Environment and Health Publ., 2003, 73 p.

2. Shur P.Z., Khasanova A.A. Analytical review of approaches to providing safety when substantiating hygienic standards for chemicals contents in ambient air. *Health Risk Analysis*, 2021, no. 2, pp. 156–167. DOI: 10.21668/health.risk/2021.2.15.eng

3. Calabrese E.J. Uncertainty factors and interindividual variation. *Regul. Toxicol. Pharmacol.*, 1985, vol. 5, no. 2, pp. 190–196. DOI: 10.1016/0273-2300(85)90032-7

4. Hattis D., Erdreich L., Ballew M. Human variability in susceptibility to toxic chemicals--a preliminary analysis of pharmacokinetic data from normal volunteers. *Risk Anal.*, 1987, vol. 7, no. 4, pp. 415–426. DOI: 10.1111/j.1539-6924.1987.tb00479.x

5. Dourson M.L., Felter S.P., Robinson D. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol.*, 1996, vol. 24, no. 2, pp. 108–120. DOI: 10.1006/rtph.1996.0116

6. Scheuplein R., Charnley G., Dourson M.L. Differential sensitivity of children and adults to chemical toxicity: I. Biological basis. *Reg. Toxicol. Pharmacol.*, 2002, vol. 35, no. 3, pp. 429–447. DOI: 10.1006/rtph.2002.1558

7. Dourson M.L., Charnley G., Scheuplein R. Differential sensitivity of children and adults to chemical toxicity: II. Risk and regulation. *Reg. Toxicol. Pharmacol*., 2002, vol. 35, no. 3, pp. 448–467. DOI: 10.1006/rtph.2002.1559

8. Hines R.N., Sargent D., Autrup H., Birnbaum L.S., Brent R.L., Doerrer N.G., Cohen Hubal E.A., Juberg D.R. [et al.]. Approaches for assessing risks to sensitive populations: lessons learned from evaluating risks in the pediatric population. *Toxicol. Sci.*, 2010, vol. 113, no. 1, pp. 4–26. DOI: 10.1093/toxsci/kfp217

9. Haber L.T., Maier A., Gentry P.R., Clewell H.J., Dourson M.L. Genetic polymorphisms in assessing interindividual variability in delivered dose. *Regul. Toxicol. Pharmacol.*, 2002, vol. 35, no. 2 (pt 1), pp. 177–197. DOI: 10.1006/rtph.2001.1517

10. Dourson M.L., Gadagbui B., Onyema C., McGinnis P.M., York R.G. Data derived extrapolation factors for developmental toxicity: A preliminary research case study with perfluorooctanoate (PFOA). *Regul. Toxicol. Pharmacol.*, 2020, vol. 110, pp. 104502. DOI: 10.1016/j.yrtph.2019.104502

11. Dourson M.L., Gadagbui B., Onyema C., McGinnis P.M., York R.G. Data derived Extrapolation Factors for developmental toxicity: A preliminary research case study with perfluorooctanoate (PFOA). *Regul. Toxicol. Pharmacol.*, 2019, vol. 108, pp. 104446. DOI: 10.1016/j.yrtph.2019.104446

12. Grynchak V.A., Sychik S.I. Peculiarities of the toxic effect of diisononyl phthalate plasticizer phthalate on experimental models. *Gigiena i sanitariya*, 2018, vol. 97, no. 5, pp. 474–477. DOI: 10.47470/0016-9900-2018-97-5-474-477 (in Russian).

13. Dorne J.L.C.M. Human variability in hepatic and renal elimination: implications for risk assessment. *Journal of Applied Toxicology*, 2007, vol. 27, no. 5, pp. 411–420. DOI: 10.1002/jat.1255

14. Burnett S.D., Karmakar M., Murphy W.J., Chiu W.A., Rusyn I. A new approach method for characterizing inter-species toxicodynamic variability. *J. Toxicol. Environ. Health A*, 2021, vol. 84, no. 24, pp. 1020–1039. DOI: 10.1080/15287394.2021.1966861

15. Bhat V.S., Meek M.E.B., Valcke M., English C., Boobis A., Brown R. Evolution of chemical-specific adjustment factors (CSAF) based on recent international experience; increasing utility and facilitating regulatory acceptance. *Crit. Rev. Toxicol.*, 2017, vol. 47, no. 9, pp. 729–749. DOI: 10.1080/10408444.2017.1303818

16. Murray F.J., Schlekat C.E. Comparison of Risk Assessments of Boron: Alternate Approaches to Chemical-Specific Adjustment Factors. *Human and Ecological Risk Assessment*, 2004, vol. 10, no. 1, pp. 57–68. DOI: 10.1080/10807030490280954

17. The public health impact of chemicals: knowns and unknowns. Geneva, World Health Organization, 2016. Available at: http://www.who.int/ipcs/publications/chemicals-public-health-impact/en/ (19.12.2021).

18. Nakamoto H., Soeda Y., Seki T., Watanabe T., Satoh M. Possible involvement of descending serotonergic systems in antinociception by centrally administered elcatonin in mice. *Biol. Pharm. Bull.*, 1999, vol. 22, no. 7, pp. 691–697. DOI: 10.1248/bpb.22.691

19. Nakamoto H., Nemoto H., Sugahara S., Okada H., Suzuki H. Nifedipine and arotinolol in combination for accelerated-malignant hypertension: results of one year follow-up. *Hypertens. Res.*, 1999, vol. 22, no. 2, pp. 75–80. DOI: 10.1291/hypres.22.75

20. Nakamoto H., Hasegawa M. Targeted inactivation of the gene psaK encoding a subunit of photosystem I from the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.*, 1999, vol. 40, no. 1, pp. 9–16. DOI: 10.1093/oxfordjournals.pcp.a029479

21. Zhuravlyov D.A. Hypertension models. Spontaneously hypertensive rats. *Arterial'naya gipertenziya*, 2009, vol. 15, no. 6, pp. 721–723. DOI: 10.18705/1607-419X-2009-15-6-721-723 (in Russian).

22. Okamoto K., Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn. Circ. J.*, 1963, vol. 27, pp. 282–293. DOI: 10.1253/jcj.27.282

23. Plotnikov M.B., Aliev O.I., Anishchenko A.M., Sidekhmenova A.V., Shamanaev A.Yu., Fedorova E.P. Dynamics of blood pressure and quantity indices of erythrocytes in SHR in early period of arterial hypertension forming. *Rossiiskii fiziologicheskii zhurnal im. I.M. Sechenova*, 2015, vol. 101, no. 7, pp. 822–828 (in Russian).

24. Sidekhmenova A.V., Aliev O.I., Anishchenko A.M., Shamanaev A.Yu., Fedorova E.P., Plotnikov M.B. Dynamics of indicators of platelets, white blood cells, and functional activity of the endothelium in young SHR rats. *Sibirskii meditsinskii zhurnal (Tomsk),* 2015, vol. 30, no. 3, pp. 61–65. DOI: 10.29001/2073-8552-2015-30-3-61-65 (in Russian).

25. Kovaleva M.A., Makarova M.N., Selezneva A.I., Makarov V.G. Expediency of application of animals with the spontaneous hypertensia for modelling of the metabolic syndrome. *Obzory po klinicheskoi farmakologii i lekarstvennoi terapii*, 2012, vol. 10, no. 4, pp. 91–94 (in Russian).

26. Cappelli-Bigazzi M., Rubattu S., Battaglia C., Russo R., Enea I., Ambrosio G., Chiariello M., Volpe M. Effects of high-cholesterol and atherogenic diets on vascular relaxation in spontaneously hypertensive rats. *Am. J. Physiol.*, 1997, vol. 273, no. 2, pp. H647–H654. DOI: 10.1152/ajpheart.1997.273.2.H647

27. Perez C.M., Hazari M.S., Ledbetter A.D., Haykal-Coates N., Carll A.P., Cascio W.E., Winsett D.W., Costa D.L., Farraj A.K. Acrolein inhalation alters arterial blood gases and triggers carotid body-mediated cardiovascular responses in hypertensive rats. *Inhal. Toxicol.*, 2015, vol. 27, no. 1, pp. 54–63. DOI: 10.3109/08958378.2014.984881

28. Abdel-Rahman A.A. Alcohol abolishes the hypotensive effect of clonidine in spontaneously hypertensive rats. *Hypertension*, 1994, vol. 24, no. 6, pp. 802–807. DOI: 10.1161/01.hyp.24.6.802

Drozdova E.V., Sychik S.I., Hrynchak V.A., Rjabceva S.N. Experimental models of animal chronic pathology in assessing health risks for sensitive population groups. Health Risk Analysis, 2022, no. 2, pp. 185–195. DOI: 10.21668/health.risk/2022.2.17.eng

Received: 03.02.2022
Approved: 20.05.2022
Accepted for publication: 21.06.2022