MEDICAL AND BIOLOGICAL ASPECTS RELATED TO ASSESSMENT OF IMPACTS EXERTED BY RISK FACTORS

UDC 616-099+616.24-005.98
DOI: 10.21668/health.risk/2024.4.10.eng

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Research article

DETERMINING AN INFORMATIVE VALUE OF METHODS FOR RESEARCHING MANIFESTATIONS OF LONG-TERM EFFECTS PRODUCED BY ACUTE INTOXICATION WITH NEUROTROPIC TOXICANTS IN AN EXPERIMENT

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Information characteristics of models are rarely determined in biomedical research, which is largely due to their assessments not being mandatory, poor standardization of biomedical research, and wide variability of methods employed to model and assess a condition of animals. Nevertheless, stricter requirements to implementation of GLP standards in preclinical studies lead to an increase in importance of evaluating information indicators in toxicological and pharmacological studies.

An experimental study was accomplished on modeling long-term consequences of acute neurotoxicant poisoning. In this experiment, animals were intoxicated with model neurotoxicants. By the end of 4th week, 100 % of laboratory animals intoxicated with lead acetate and 20 % of laboratory animals intoxicated with methanol were found to have signs of long-term consequences of acute poisoning. These signs were manifested as components of psycho-organic and asthenic syndromes including impaired responses to light and an electric current stimulus, inability to perform intense physical activity in the treadmill running test, a decrease in ability to reproduce skills of passive pain avoidance. These changes in the functional state of animals were statistically significant.

The study revealed that indicators, even those characterized by high differentiating ability (specificity) in relation to individual manifestations of long-term consequences of acute poisoning, were characterized with insufficient selectivity, accuracy and informational significance. A comprehensive assessment of the experimental model based on a criteria-based approach provides higher informational significance than the indicators taken separately. However, the discriminant model is the closest to the optimal system for detecting long-term consequences of acute poisoning with neurotropic toxicants.

Keywords: *biological modeling, intoxication, poisoning, neurotoxicant, long-term effects, lead acetate, informativeness, multifactorial analysis.*

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Biological modeling of pathological processes is a powerful instrument employed by contemporary healthcare. It allows investigating pathways of disease development, testing new drugs and treatment methods, as well as developing diagnostic and preventive procedures. The more accurate a biological model is, the more realistic results can be yielded by using it and more effective medical technologies can be created based on it. At present, the number of available diagnostic and prognostics tests is growing; therefore, it is necessary to estimate their diagnostic informative value [1]. It is worth noting that predicting an outcome of a disease (damage) requires as reliable diagnostics as only possible both when a patient is admitted to a hospital and later on in dynamics during the whole treatment. Biological modeling helps achieve better diagnostics and reduce a number of ungrounded prescriptions thereby ensuring more effective medical aid. It also makes it possible to detect pathogenetically grounded indicators that have the highest prognostic significance [2].

Estimation of information characteristics of created models is the key moment in toxicological and pharmaceutical research; unfortunately, it is very often neglected. This happens due to several factors:

– mandatory requirements are absent: in most cases, it is not a mandatory procedure to estimate information characteristics of created models;

– poor standardization: great variability is typical for biomedical research as regards applied methods including modeling techniques and assessing conditions of animals. Absence of standardized approaches makes it difficult to compare results reported in different research works and to assess information indicators;

– complexity and labor intensiveness: assessment of information characteristics of models requires specific knowledge and methods as well as additional time and resources.

As stricter requirements are being set to GLP (Good Laboratory Practice) standards in pre-clinical research, assessment of informative value is becoming more relevant. GLP standards are aimed at ensuring quality and reliability of obtained results. They regulate all stages in pre-clinical research including selection of a model, research techniques, data analysis and documentation.

Indicators of informative value are quantitative characteristics of a diagnostic or analytical procedure. They are employed to estimate significance of its practical use and allow comparing homogenous research techniques or measurements. They do not have dimensionality, which makes it possible to use them for characterizing various procedures. These indicators are relative values that describe how frequently errors occur in their practical use. Sensitivity and specificity are main indicators of a method's informative value; accuracy and predictive value of positive and negative results are auxiliary ones [3–9]. The concept of clinical informative value of diagnostic tests, estimation indicators and criteria are known, standardized and even declared mandatory within description of medical diagnostic and screening technologies. However, they are rarely used in Russian publications (not more than 10 % of clinical publications with their focus on new diagnostic procedures) [10–14].

The aim and tasks of this study. The aim of this study was to assess informative value of research procedures applied to investigate long-term effects of acute intoxications with neurotropic intoxicants in an experiment.

In order to achieve this aim, the following algorithm had to be accomplished:

1. To create a list of research methods based on analyzing findings of experimental research that focused on creating a biomedical model for investigating long-term effects of acute intoxication with neurotropic toxicants; another task was to create a list of indicators for these methods that allowed authentic differentiation of manifestations of long-term effects.

2. To determine criteria eligible for assigning research results obtained by using selected methods and indicators into a category 'long-term effects of acute intoxication with neurotropic intoxicants'.

3. To create a training sample of research objects for identifying signs typical for a group

of objects in a multi-dimensional space with manifestations of long-term effects of acute intoxication with neurotropic intoxicants.

4. To classify research objects into several groups per presence of long-term effects of acute intoxication and to verify their occurrence.

5. To assess whether it was possible to assign an object to a group of objects 'which have long-term effects of acute intoxications' per specific signs and integral approaches.

6. To estimate classification type I and type II errors when using different approaches to assigning an object to a group of objects, 'which have long-term effects of acute intoxications' and to calculate numeric characteristics (sensitivity, specificity, and accuracy) of the procedure and its total informative value[1].

Materials and methods. Research was conducted using healthy non-linear white male rats with their body weight equal to 180–220 grams at the beginning of the experiment. The lab animals were kept under standard conditions in accordance with the State Standards GOST 33215-2014 Guide for the Care and Use of Laboratory Animals / The Rules for Housing Design and Organization of Procedures and GOST 33044-2014 Good Laboratory Practice [14]. Comparability of the experimental group was achieved by random selection of animals that were deemed eligible for inclusion into research. LD_{50} of the analyzed toxicants was established in preliminary research using the procedure developed by V.B. Prozorovskii [15].

The research was approved by the Commission on Bioethics of Golikov Research Clinical Center of Toxicology (the meeting proceedings No. 4/24 dated April 04, 2024). The lab animals were kept under standard conditions in conformity with the State Standard GOST 33215-2014 issued on July 01, 2016 and the Recommendations of the Eurasian Economic Commission Board dated November 14, 2023 No. 33 Guide on Use of Laboratory (Experimental) Animals in Preclinical (Non-Clinical) Research.

After quarantine, animals that were included into the main and 'satellite' research were divided into four groups for each research area, one control and three test groups. In the main research, the control group was made of 8 male rats and each test group that was to be exposed to a toxicant was made of 15 animals.

Three neurotropic toxicants were selected to model long-term effects of acute intoxications:

– phenyl carbamate was toxicant No. 1; it was administered once intraperitoneally at a dose of 1.6 mg/kg as 0.1 % water solution;

– organic solvent methanol was toxicant No. 2; it was administered once intragastrically at a dose of 11.5 g/kg as 75 % water solution;

– toxicant No. 3 was represented by an organic salt of a heavy metal, lead acetate; it was administered once intraperitoneally at a dose of 300 mg/kg as 4.8 % water solution.

Doses of toxicants, which corresponded to average lethal dose for a selected administration way, were identified within preliminary research. All solutions were prepared on the same day when they were administered. The day when toxicants were administered was considered Day 1 in the experiment.

Animals in all control groups were not exposed to toxicants; instead, water for injection was administered intraperitoneally.

Every day survival rates were estimated and the animals were examined; food and water consumption and laboratory animals' weights were estimated every week.

Cognitive functions, locomotor and sensory reactions were estimated on Day 2, 15, and 29 by using behavioral tests (Open-field [16, 17], RotaRod [18], Grip Strength [17], and Sensory Reaction Test per TSE Startle Response System methodology [19]). On Days 6–7 and 28–29, Passive Avoidance Test was applied to assess the same indicators [20]. Motor activity and endurance were estimated on

[1] Rukovodstvo po provedeniyu doklinicheskikh issledovanii lekarstvennykh sredstv. Chast' 1 [Guide on conducting pre-clinical tests of drugs. Part 1]. In: N.D. Bunyatyan, A.N. Vasiliev, O.L. Verstakova, M.V. Zhuravleva, V.K. Lepakhin, N.V. Korobov, V.A. Merkulov, S.N. Orekhov eds. Moscow, Grif i K Publ., 2012, 944 p. (in Russian).

Days 2, 15, and 29; resistance to hypoxia and hyperthermia was estimated on Days 30 and 34.

Results and discussion. Analysis of the experimental results revealed several indicators among various procedures for dynamic investigation of statistically authentic changes established in animals that were able to survive for 28 days after severe acute intoxication. Interpretation of these revealed indicators may indicate occurrence of certain long-term effects of survived intoxication (Table 1).

Dynamics of other analyzed indicators does not allow predicting their informative value to be high for assessing long-term effects of acute intoxication with neurotropic toxicants.

To determine what criteria should be used in assigning research results to a specific type of a body state, we applied an approach based on S-like scaling that involved using features of normal statistical distribution of a random value[2]. It was implemented technically by building an experimental curve of frequency distribution of an analyzed indicator's values and a cumulative curve with its knees being boundaries of respective normalized (that is, approximate to normal distribution) ranges. The results obtained by this graphic analysis are shown in Figures 1–5.

Table 2 provides boundaries of respective ranges identified graphically for the analyzed indicators and shown in Figures 1–5.

Table 1

Indicators that may characterize occurrence of certain long-term effects of acute intoxication

Test	Indicator	Level, changes	Interpretation of changes	Significance
Water consumption	Daily consumption, ml/day	Low	Drinking and eating behavior disorder, impaired endocrine balance	0.05
Passive Avoidance Test	Tome spent in a dark chamber, sec	High	Disrupted reproduction of conditioned reflexes	0.05
Running on a treadmill	Duration of running at a speed of 43 m/min	Refusal or extremely low	Coordination disorders, affected endurance and motivation	0.05
TSE Startle Response System	Duration of response to light, msec	High	Polysynaptic chain inhibition	0.004
	Amplitude of response to stimulation by current	Low	Insufficiency (depletion) of activation effects	0.003

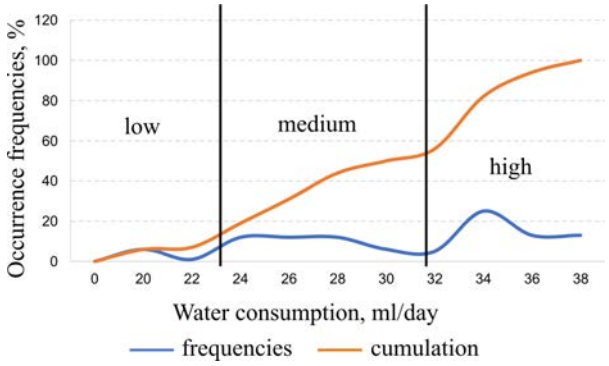

Figure 1. Boundaries of ranges that describe water consumption

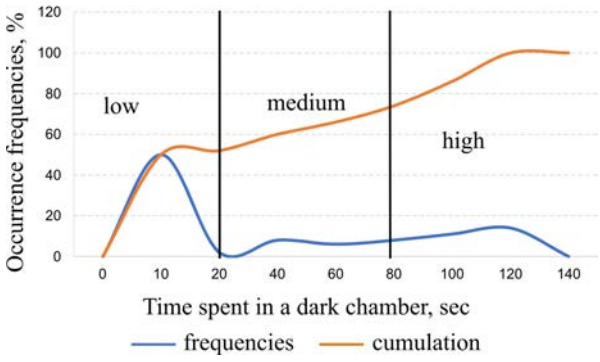

Figure 2. Frequencies of ranges that describe time spent in a dark chamber, Passive Avoidance Test

[2] Rukovodstvo po provedeniyu doklinicheskikh issledovanii lekarstvennykh sredstv. Chast' 1 [Guide on conducting pre-clinical tests of drugs. Part 1]. In: N.D. Bunyatyan, A.N. Vasiliev, O.L. Verstakova, M.V. Zhuravleva, V.K. Lepakhin, N.V. Korobov, V.A. Merkulov, S.N. Orekhov eds. Moscow, Grif i K Publ., 2012, 944 p. (in Russian).

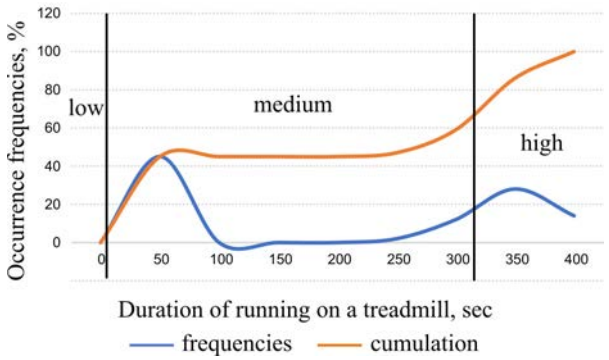

Figure 3. Boundaries of ranges that describe duration of running on a treadmill, band speed 43 m/min

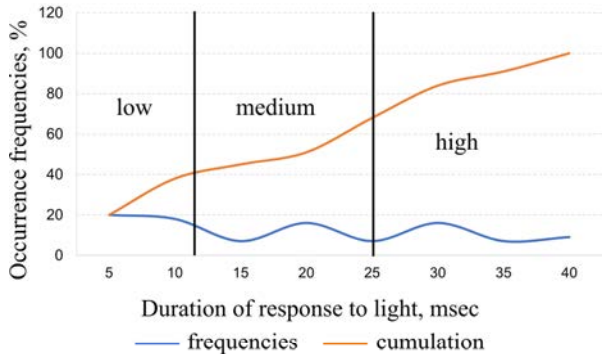

Figure 4. Boundaries of ranges that describe duration of response to light (msec), TSE Startle Response System test

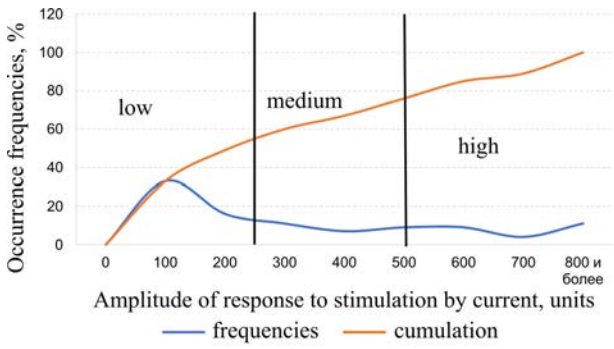

Figure 5. Boundaries of ranges that describe amplitude of response to stimulation by current (units), TSE Startle Response System test

To create a training sample, we analyzed test results obtained for 32 animals that had survived severe acute intoxication. In case an indicator was considered a manifestation of long-term effects of intoxication according to the criteria provided in Table 2, the animal got 1 score (for such indicators as water consumption, time spent in a dark chamber, duration of running on a treadmill at high speed) or 2 scores (duration of response to light or amplitude of response to stimulation by current). Differences in score estimates were associated with a different level of validity of differences against the control per indicators. The score sum identified for the animals was within the 0–6 range. Figure 6 provides the structure of total score estimates.

Frequency analysis established three ranges of the total score estimates:

0–1 scores – any manifestations of long-term effects are absent;

2 scores – some initial manifestations of long-term effects or the CNS state cannot be considered optimal;

3–6 scores – manifestations of long-term effects produced by severe acute intoxications are present and their intensity is proportionate to the score estimate.

Table 3 provides distribution of the foregoing ranges of total score estimates typical for various toxicants.

Obviously, risks of long-term effects produced by acute intoxications to a great extent depend on a toxicant if we assess their likely occurrence in the analyzed period: exposure to phenyl carbamate did not lead to development of any long-term effects; long-term effects developed in 20 % of cases after methanol

Table 2

Boundaries of ranges identified for indicators that can potentially differentiate occurrence of long-term effects produced by acute intoxication

Indicator	Units	Value ranges		
		low	medium	high
Water consumption	ml/day	24.0 and below*	24.1–34.0	34.1 and above
Time spent in a dark chamber, Passive Avoidance Test	sec	20.0 and less	20.1–80.0	80.1 and more*
Duration of running on a treadmill, 43 m/min	sec	Not capable to run*	46–350	351 and more
Duration of response to light	msec	5.0 and less	5.1–25.0	25.1 and more*
Amplitude of response to stimulation by current	units	200 and below*	201–500	501 and above

Note: * means ranges that describe occurrence of long-term effects produced by acute intoxication with neurotoxicants.

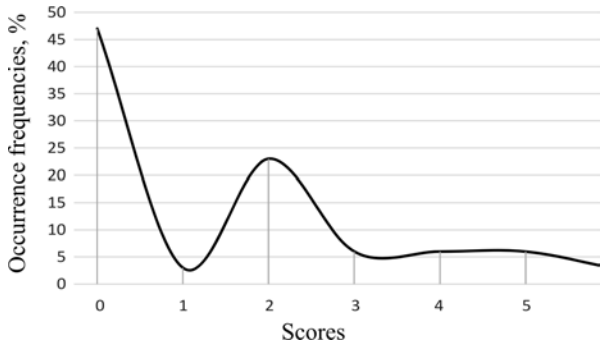

Figure 6. Frequency of occurrence of the total score estimates that describe intensity of manifestations of long-term effects produced by acute intoxication in the lab animals on Day 28 in the experiment

Table 3

Distribution of long-term effects of severe acute intoxications per groups of lab animal exposed to specific neurotoxicants (frequency of occurrence, %)

Group	No manifestations	Not optimal condition	Manifestations of long-term effects
Control ($n = 8$)	75	25	0
Phenyl carbamate ($n = 9$)	89	11	0
Methanol ($n = 10$)	40	40	20
Lead acetate ($n = 5$)	0	0	100

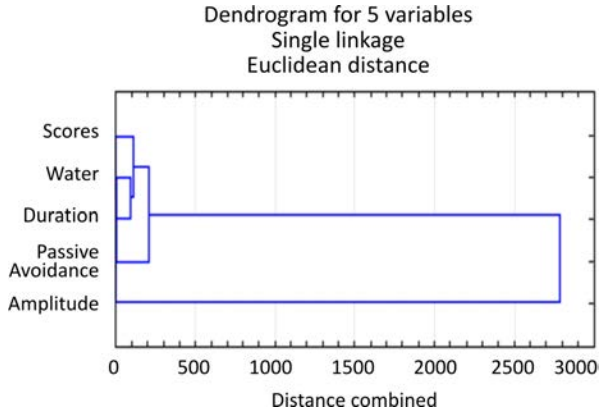

Figure 7. Dendrogram of linkages between indicators that describe long-term effects of acute intoxications

intoxication; long-term effects developed in 100 % of animals that had survived intoxication with lead acetate. That is, relative risks are equal to 0, 0.2 and 1.0 respectively.

Interestingly, we found some rats in the control group with minimal manifestations of the CNS dysfunction. This may indicate that the animal population provided by a farm was not homogenous; so, when conducting neurotoxicological or neuropsychopharmacological studies, it is necessary to use a specific procedure as a mandatory inclusion criterion, which involves spotting out unhealthy animals with some boundary symptoms.

Therefore, according to the obtained analysis results, we created the following training samples for subsequent multidimensional statistical analysis:

– Group 1 made of animals without any manifestations of long-term effects produced by acute intoxications (11 animals);

– Group 2 made of animals with manifestations of long-term effects produced by acute intoxications (7 animals);

– Group 3 for verifying rules of multiparametric classification (12 animals). It was necessary to create this group due to the requirement to verify decision rules for classification of objects into groups obtained by methods of multi-parametric statistics.

We had to exclude duration of running on a treadmill at the band speed of 43 m/min from 5 indicators selected for multi-parametric analysis. This was due to this indicator being qualitative in its essence as shown by inability to perform the test. The remaining indicators were quantitative and could be included into statistical analysis.

Cluster analysis of indicator classification (Figure 7) showed that amplitude of response to stimulation by current could be considered an independent characteristic in predicting long-term effects of acute intoxications whereas such indicators as water consumption, time spent in a dark chamber in Passive Avoidance Test and duration of response to light created a new variable for estimation together with score estimates of various manifestations.

The method of principal components was applied to show that 2 orthogonal factors were identified in the multidimensional space of manifestations. Factor loads for them are provided in Table 4.

In total, these identified principal components cover 73 % of the variability of the analyzed indicators. This is quite sufficient for obtaining statistically correct description of a biomedical model. Based on the obtained results, we can consider the results of Passive Avoidance Test an independent indicator. It is not directly linked to long-term effects of acute intoxications and describes some qualities that are interpreted independently such as preservation of conditioned responses related to cognitive functions.

Equations that characterize a possibility to obtain score estimates for intensity of manifestations of long-term effects were derived by multidimensional linear regression analysis. Their description is provided in Table 5. Step-by-step analysis established the 4-parametric regression model to be the most optimal.

Since we were able to create clearly separated groups of animals with and without manifestations of long-term effects produced by acute intoxications, we decided to solve the task of making decision rules for assigning objects into these groups by using discriminant analysis.

When 5 indicators that described long-term effects of acute intoxications were included in a model, a formula was derived that described the following linear discriminant function (Formula 1):

$$LDF1 = 0.46X_1 - 0.66X_2 + 0.8X_3 - 1.12X_4 + 1.19X_5, \quad (1)$$

where X_1–X_5 represent Z-scores of indicator values (water consumption, time spent in a dark chamber, duration of response to light, amplitude of response to stimulation by current, and scores of manifestation intensity) respectively.

If the *LDF* value is below 0, an object is assigned into Group 1 (without any manifestations of long-term effects produced by acute intoxications); if it is above 0, an object is assigned into a group with some manifestation of long-term effects of acute intoxications. *LDF* values within the (-0.1)–(+0.1) range indicate an indistinguishability zone;

Table 4

Factor loads identified for principal components in multifactorial analysis of indicators that describe long-term effects produced by severe acute intoxications

Indicator	Principal component 1	Principal component 2
Water consumption	0.87	0.23
Tome spent in a dark chamber, Passive Avoidance Test	-0.08	0.93
Duration of response to light	-0.74	0.36
Amplitude of response to stimulation by current	0.52	-0.36
Scores of long-term effects	-0.884373	0.34
Proportion in the total dispersion	**0.474**	**0.260**

Table 5

Description of linear regression models for calculating score estimates for intensity of manifestations of long-term effects produced by acute intoxications

Indicators	Equation	Significance assessment
X_3 – duration of response to light; X_4 – amplitude of response to stimulation by current	$Y = 0.892X_3 - 0.43X_4 + 4.772$	$R^2 = 0.779$ $p = 0.00003$
X_1 – water consumption; X_3 – duration of response to light; X_4 – amplitude of response to stimulation by current	$Y = -0.30X_1 + 0.425X_3 - 0.39X_4 + 4.02$	$R^2 = 0.83$ $p = 0.000002$
X_1 – water consumption; X_2 – time spent in a dark chamber, Passive Avoidance Test; X_3 – duration of response to light; X_4 – amplitude of response to stimulation by current	$Y = -0.34X_1 + 0.119X_2 + 0.333X_3 - 0.40X_4 + 4.772$	$R^2 = 0.841$ $p = 0.000007$

Table 6

Informative value of significant indicators

Indicator	Integral parameters			
	Se	*Sp*	*Ac*	*I*
Water consumption	0.33	1.00	0.63	0.71
Time spent in a dark chamber	0.27	0.93	0.56	0.65
Capability to run on a treadmill	0.67	0.87	0.72	0.76
Duration of response to light	0.60	1.00	0.75	0.80
Amplitude of response to stimulation by current	0.40	0.93	0.63	0.69
Criterion score (score sum)	0.47	1.00	0.92	0.83
Discriminant model	1.00	1.00	0.94	0.98

Note: *Se* stands for selectivity (sensitivity); *Sp*, specificity; *Ac*, accuracy; *I*, informative value.

in this case objects are assigned into Group 3 (boundary group) or into Group 1 since they cannot be assigned into a group with manifestations of long-term effects produced by acute intoxications.

Statistical verification of significance of the obtained discriminant model established its statistical significance to be high (the determination coefficient $D = R^2 = 0.976$, $p < 10^{-7}$).

Values of *Z(x)*-scores were derived by using the following formula 2:

$$Z(x) = \frac{X - M}{\sigma}, \qquad (2)$$

where $Z(x_i)$ is Z-score of the X_i;

X_i is the concrete value of the analyzed indicator;

M is mathematical expectation (a mean of sample);

σ is standard deviation for the indicator X.

Table 6 provides the results of calculating integral indicators of informative value for assessing intensity of long-term effects of acute intoxications. They were obtained by analyzing classification type I and II errors (true and false positive or negative results) with classification values per discriminant functions taken as reference ones.

Analysis of the data provided in Table 6 makes it possible to conclude that indicators even with high differentiating capacity (specificity) do not have sufficient selectivity, accuracy and informative value as regards some specific manifestations of long-term effects produced by acute intoxications. A comprehensive assessment of the experimental model based on a criteria-based approach provides higher informational significance than the indicators taken separately. However, the discriminant model is the closest to the optimal system for detecting long-term effects of acute intoxication with neurotropic toxicants.

Conclusion. In this study, we assessed informative value of various ways for identifying long-term effects of acute intoxication. The assessment established that separate indicators revealing changes in the functional state were characterized with relatively low sensitivity and prediction accuracy and higher specificity. In general, this does not allow establishing long-term effects using them as a basis in isolated analysis. Simultaneously, the criterion approach that involves calculating an integral score estimate of manifestations of long-term effects and using linear discriminant functions make it possible to estimate their formation by creating biological models with high informative value.

Research objects are divided into groups per absence or presence of long-term effects of acute intoxications most distinctly by using linear discriminant functions derived from training samples created by discriminant analysis. Such analysis allowed assessing risks of long-term effects that might develop in future depending on a toxicant's chemical essence.

Funding. The experiments within this study were accomplished as a part of the State Assignment of the Federal Medical-Biological Agency of the Russian Federation No. 388-00071-24-00 (the subject code 64.004.24.800).

Competing interests. The authors declare no competing interests.

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Received: 07.10.2024
Approved: 21.10.2024
Accepted for publication: 17.12.2024