

METHODOLOGICAL APPROACHES TO SUBSTANTIATING QUALITY AND SAFETY STANDARDS FOR VARIOUS TYPES OF WATER AND SOIL IN POPULATED AREAS USING THE *DANIO RERIO* BIOLOGICAL MODEL

Zaitseva NV, Zemlyanova MA, Kamenskikh DM[✉], Peskova EV

Federal Scientific Center for Medical and Preventive Health Risk Management Technologies of the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Perm, Russia

Today, environmental pollution with chemicals represents one urgent hygienic problem. In the context of intensification and modernization of the road transport complex and industrial production the development of methodology for hygienic rationing based on experimental research is relevant. The conventional use of small rodents (rats, mice) as biological models has a number of ethical, methodological and economic limitations, which necessitates the search for and implementation of alternative biological models in research practice. Our aim was to substantiate a unified methodological approach to establishing quality and safety standards for various types of water, except for technical water, and soil in populated areas using the *Danio rerio* (Hamilton, 1822) biological model. The reported method integrating existing international and national standards, confirms the possibility of using *D. rerio* as a valid and highly informative test system in biomedical research. The approach assumes prompt (1–1.5 months), reliable, cost-effective, humane yielding of results, which is necessary for the scientific substantiation of safety criteria and hygienic standards.

Keywords: rationing, safety, chemicals, water, soil, *Danio rerio*, acute toxicity, chronic toxicity, long-term effects

Author contribution: Zaitseva NV — study concept and design; Zemlyanova MA — study design, manuscript editing; Kamenskikh DM, Peskova EV — manuscript writing.

✉ **Correspondence should be addressed:** Daria M. Kamenskikh
Monastyrskaya, 82, Perm, 614045, Russia; teterina@fcrisk.ru

Received: 10.07.2026 **Accepted:** 07.08.2026 **Published online:** 07.09.2026

DOI: 10.24075/rbh.2026.171

Copyright: © 2026 by the authors. Licensee: Pirogov University. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

МЕТОДИЧЕСКИЕ ПОДХОДЫ К ОБОСНОВАНИЮ НОРМАТИВОВ КАЧЕСТВА И БЕЗОПАСНОСТИ РАЗЛИЧНЫХ ВИДОВ ВОД И ПОЧВЫ НАСЕЛЕННЫХ МЕСТ НА БИОЛОГИЧЕСКОЙ МОДЕЛИ *DANIO RERIO*

Н. В. Зайцева, М. А. Землянова, Д. М. Каменских[✉], Е. В. Пескова

Федеральный научный центр медико-профилактических технологий управления рисками здоровью населения Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека, Пермь, Россия

В настоящее время загрязнение объектов среды обитания химическими веществами представляет собой одну из значимых гигиенических проблем. В условиях интенсификации и модернизации дорожно-транспортного комплекса и промышленного производства актуальным является развитие методологии гигиенического нормирования, основанной на экспериментальных исследованиях. Традиционное использование в качестве биологической модели мелких грызунов (крыс, мышей) имеет ряд этических, методологических и экономических ограничений, что обуславливает необходимость поиска и внедрения альтернативных биологических моделей в исследовательскую практику. Целью работы было обосновать унифицированный методический подход к установлению нормативов качества и безопасности различных видов вод, кроме технической, и почвы населенных мест на биологической модели *Danio rerio* (Hamilton, 1822). Представленная методика, интегрирующая существующие международные и национальные стандарты, подтверждает возможность применения *D. rerio* в качестве валидной и высокоинформативной тест-системы в биомедицинских исследованиях. Подход предполагает оперативное (1–1,5 месяца), достоверное, финансово малозатратное, гуманное получение результатов, что необходимо для научного обоснования критериев безопасности и гигиенического нормирования.

Ключевые слова: нормирование, безопасность, химические вещества, вода, почва, *Danio rerio*, острая токсичность, хроническая токсичность, отдаленные эффекты

Вклад авторов: Н. В. Зайцева — концепция, дизайн исследования; М. А. Землянова — дизайн исследования, редактирование текста статьи; Д. М. Каменских, Е. В. Пескова — написание текста статьи.

✉ **Для корреспонденции:** Дарья Михайловна Каменских
ул. Монастырская, д. 82, г. Пермь, 614045, Россия; teterina@fcrisk.ru

Статья получена: 10.07.2026 **Статья принята к печати:** 07.08.2026 **Опубликована онлайн:** 07.09.2026

DOI: 10.24075/rbh.2026.171

Авторские права: © 2026 принадлежат авторам. Лицензиат: РНИМУ им. Н. И. Пирогова. Статья размещена в открытом доступе и распространяется на условиях лицензии Creative Commons Attribution (CC BY) (<https://creativecommons.org/licenses/by/4.0/>).

Issue of hygienic rationing of new chemical compounds

Pollution of environmental objects (water, soil, atmospheric air) represents one significant hygienic problem. The road transport complex intensification and modernization, as well as the industrial production expansion, lead to the entry of new chemical compounds into the environment as components of emissions and discharges. There are no experimentally verified maximum permissible concentrations (MPCs) and reference doses for chronic oral intake (RfD) for most such chemicals. New pollutants actually present in environmental objects can

negatively affect the exposed population health. In this regard, justification of quality standards (MPC) and safety standards (RfD) for new chemical compounds is an important aspect of ensuring the population sanitary and epidemiological well-being.

Justification of MPC and RfD for new substances is based on the strictly regulated approaches to experimental research considering the acute, chronic and long-term effects of toxicants. This approach allows one to determine general patterns of toxicity of compounds, specific manifestations during long-term exposure, define the lowest effective (LOEC) and no effective concentrations (NOEC). Historically, such research involves

warm-blooded animals, including small rodents (rats, mice), as biological models [1, 2]. Despite proven effectiveness and widespread use in scientific research, the use of those is associated with some limitations. The ethical aspect comes down to the need to justify the sample size and compliance with the requirements of humane research, due to the implementation of invasive procedures, surgical interventions and prolonged painful treatment of laboratory animals. It is very difficult to extrapolate the data obtained in small rodent experiments to the human body due to pronounced interspecies metabolic, structural, and physiological differences. An additional limitation is the significant costs of keeping laboratory animals in specialized vivariums. These difficulties highlight the need to search for and implement into research practice a valid and highly informative biological model characterized by the high genetic and physiological homology with humans, economic accessibility, and compliance with ethical standards. In this context, *Danio rerio* (Hamilton, 1822), the tropical freshwater fish, is considered as one of the most promising model organisms due to a combination of biological, genetic, and methodological advantages. The *D. rerio* whole genome sequencing revealed significant similarities between its genetic structure and that of humans: 70% of human genes have orthologs in *D. rerio* and 82% of *D. rerio* genes are associated with human disorders. This determines comparability of the key biochemical and physiological processes [3–5]. The possibility of conducting diverse experimental studies within a single biological system ensures differentiated assessment of various types of toxic effects (lethal and sublethal). When testing the effects of toxicants using the *D. rerio* biological model, it is possible to simulate the conditions of a real complex exposure caused by the entry of substances from environmental objects (water, soil, air). Such an approach reflects the simultaneous entry of chemicals into the human body through three pathways: oral, transdermal, and inhalation ones. Similarity of metabolic profiles of adult *D. rerio* and humans (the ability to absorb, oxidize, and excrete metabolites of toxicants) makes it possible to effectively extrapolate the results to the human body [6, 7]. Along with the biological and genetic advantages, the use of *D. rerio* in experiments is fully consistent with the provisions of the 3R concept (Replacement, Reduction, Refinement) that are fundamental for experimental research [8]. Replacing warm-blooded animals (small rodents) with fish allows for a significant reduction of the number of mammal experiments, which simultaneously reduces the ethical burden of research and reduces the logistical costs associated with the purchase, maintenance, and disposal of laboratory animals. High reproductive capacity and a fast life cycle ensure high statistical significance and promptness of yielding results (1–1.5 months), thereby ensuring compliance with the reduction principle [9]. The refinement principle is realized through the optimization of methods and experimental conditions aimed at minimizing or completely eliminating pain and stress effects. Extracorporeal development and optical transparency of *D. rerio* embryos enable the real-time noninvasive monitoring of morphogenesis, and the introduction of chemicals is carried out by simply adding those to the aquatic environment, which eliminates injection manipulations [10, 11].

The *D. rerio* model represents a highly sensitive and methodologically accessible instrument for studying the toxicity of new chemicals in both acute and chronic experiments, which determines its importance for advances in hygienic research [12–14]. The experimental use of *D. rerio* is regulated by international guidelines (OECD) and national standards (GOST). They standardize the key testing parameters,

such as exposure time, controlled environmental conditions, fish development stages, and outcome assessment criteria. At the same time, there is a unified stage-by-stage research methodology, since the existing approaches to rationing use different experimental protocols, which can lead to discrepancies in results, decreased objectivity and quality of the MPC and RfD justification.

Our aim was to provide scientific and methodological substantiation of the unified integrated approach to establishing maximum permissible concentrations and reference doses for chronic oral intake of chemicals using the *D. rerio* biological model.

Concept of an integrated methodological approach to substantiating quality and safety standards for new chemical compounds

An integrated methodological approach to substantiating quality and safety standards for various types of water and soil using the *D. rerio* biological model represents a multilevel algorithm. Within its framework, a preparatory stage is consistently implemented, experimental studies are conducted that involve determining the parameters of acute and chronic toxicity, as well as delayed effects, including teratogenic, mutagenic, and reprotoxic effects during long-term exposure, establishing the lowest effective (LOEC) and no effective (NOEC) concentrations for *D. rerio* to substantiate the MPC of chemicals in water and soil and RfD for humans during chronic oral intake (Fig.).

The integrated methodological approach to establishing MPC and RfD is based on international methods and national standards: GOST 32428—2013 [15], GOST 32473—2013 [16], GOST 32538—2013 [17], GOST 32541—2013 [18], GOST 33774—2016 [19], GOST 32368—2013 [20], OECD Test Guideline No. 229 [21], OECD Test Guideline No. 425 [22], OECD Test Guideline No. 203 [23], OECD Test Guideline No. 236 [11], OECD Test Guideline No. 210 [24].

Samples (water, soil) are prepared in accordance with the provisions of current national standards. Soil samples are collected in accordance with GOST 17.4.3.01—83 [25] and GOST 17.4.4.02—84 [26], depending on the test substance. When isolating chemicals from soil, standardized extraction procedures are used (e.g., obtaining aqueous extracts or extraction with organic solvents) [27]. Critical parameters include the solid-to-liquid ratio, duration, and temperature of extraction. Water sampling is conducted in accordance with GOST R 59024—2020 [28] regulating the localization of sampling sites taking into account the hydrological characteristics of the reservoir or watercourse and the potential impact of pollution sources. Isolation of test chemicals from the aqueous medium is carried out using sorption (for example, solid-phase extraction) or liquid-liquid extraction methods, selected based on the concentration. Special attention is paid to preventing the loss of volatile compounds by controlling the temperature regime.

Statistical processing of the experimental study results is carried out considering the nature of the distribution of the data set, estimated using the Kolmogorov–Smirnov test. When random variables of the test indicators are normally distributed, parametric statistical methods are used and the following characteristics are calculated: mean (M); standard error (m), Student's t -test when assessing significance of intergroup differences ($p \leq 0.05$), Fischer's exact test ($F > 3.96$) when assessing differences in variances, and the determination coefficient (R^2).

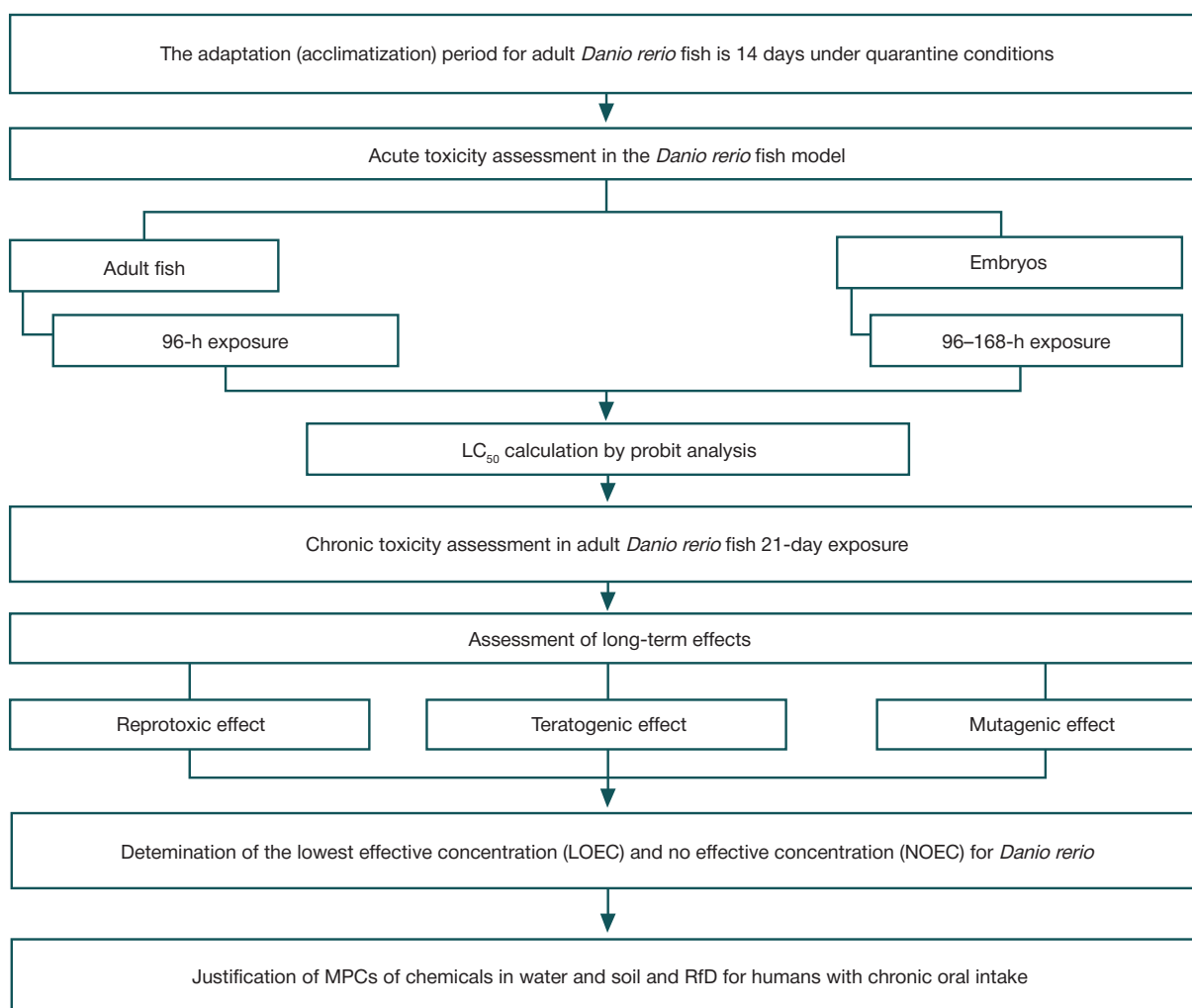


Fig. Integrated methodological approach to substantiating quality and safety standards for various types of water and soil in populated areas using the *Danio rerio* biological model

Modeling and evaluation of acute toxicity at different stages of *Danio rerio* ontogenesis

A unified integrated approach is implemented from the preparatory stage. *D. rerio* fish are kept in quarantine conditions for 14 days, during which the water hydrochemical parameters and the laboratory animals' health status are monitored daily in accordance with GOST 32473—2013 [16]. After 14 days, healthy sexually mature fish are randomly selected to form representative samples that ensure statistical significance of the results. Inclusion criteria include the fact of having no external signs of disease (fin damage, corneal clouding, color changes, scale ruffling), adequate response to external stimuli, and the fact of showing no abnormal motor activity. Such selection ensures minimization of false positives associated with the baseline heterogeneity of experimental groups.

After the adaptation period completion, the first stage of the study is carried out according to standardized protocols (OECD Test Guideline No. 203 [23] and GOST 32473—2013 [16]). The study continues for 96 h (adults and embryos) with an extension of up to 168 h (for embryos) to determine acute toxic effects. The selection of test substance concentrations is regulated by the specified standards and is based on testing to determine the concentration range. It is necessary to identify the sensitivity threshold and the maximum possible concentration of a substance during its single administration in order to rule out the development of negative effects caused

by nonspecific factors. When the appropriate range is obtained, the exposure ensured using at least five concentrations that form a geometric progression with a denominator of no more than 2.2.

Adult animals are exposed to toxicants in the semistatic mode under controlled conditions in accordance with GOST 32473—2013 [16]. Visual recording of outcomes is performed 3, 6, 24, 48, 72, and 96 h from the beginning of the experiment. The number of dead individuals, the time of death, apparent behavioral anomalies, and intoxication signs are recorded. Reliability of the results obtained confirmed by the low mortality rate in the control group (less than 10%), adherence to constant experimental conditions, and maintenance of optimal test concentrations throughout the entire exposure period in accordance with GOST 32473—2013 [16].

The intact parent stock is the source of embryos required for assessing the acute toxicity of chemicals. The spawning procedure is compliant with GOST 32541—2013 [18]. The resulting spawn is treated with a solution of methylene blue to prevent fungal and bacterial contamination. Then a visual inspection is carried out to assess the quality of the eggs and remove unfertilized and damaged eggs. Criteria for selection of viable fertilized spawn that are compliant with GOST 33774—2016 [19] include transparency of the chorion, the presence of a perivitelline space between the egg wall and the embryo, the onset of cleavage (4–64 blastomere stage), and no signs of coagulation. According to the above standard, viable spawn

at the early cleavage stage is transferred using sterile plastic pipettes into 24-well plates for cultivation and distribution into experimental groups.

The test concentration range is determined by OECD Test Guideline No. 236 [11]. Preliminary testing is performed to justify the concentration range, similar to that used for the acute toxicity assessment in adult *D. rerio*.

The mortality of embryos is assessed daily at the same time (every 24 h) using an inverted microscope. The indicators are recorded in absolute and relative units based on the presence of embryo coagulation, non-separation of the tail part from the yolk sac, absence of somite formation and heartbeat. The detection of any of the listed signs indicates the embryo death. In addition, the hatching rate (number of free-swimming larvae) is recorded in the experimental groups starting from 48 h of exposure. The experimental results are considered reliable provided that the total level of fertilized spawn in the batch is at least 70%, the hatching rate and survival rate of embryos in the negative control at the end of the 96-h exposure exceeds 80%, the temperature during incubation of the plates is maintained at the level of 26 ± 1 °C, pH of test solutions is within the range of 7.5 ± 0.5 in accordance with GOST 33774—2016 [19]. An in-depth assessment of toxic effects of chemical compounds in early *D. rerio* ontogenesis is carried out under conditions of a prolonged acute experiment (up to 168 h) in accordance with the guidelines for assessing toxicity in the early fish life [19]. Such an approach makes it possible to register acute lethal and sublethal (delayed) effects in embryos, prelarvae and larvae (the stage of transition to exogenous feeding and active swimming).

At the end of the exposure, based on the results of the concentration-effect (lethality) relationship assessment, the mean lethal concentration (LC_{50}) is calculated for adult animals and embryos using probit analysis [29], which makes it possible to justify the choice of concentrations for subsequent experiments to assess chronic toxicity. When no lethality is reported, the highest concentration tested is taken as LC_{50} .

Modeling and assessment of chronic toxicity and long-term effects of chemicals

Chronic toxicity is assessed at the second stage of the study. The test substance concentrations are determined based on the average lethal concentration values obtained (LC_{50}). According to GOST 32538—2013 [17], at least two concentrations of test substances are selected: $1/100 LC_{50}$ and $1/1000 LC_{50}$. *D. rerio* fish are exposed for 21 days in accordance with the guidelines [30] considering the *D. rerio* species-specific features, and GOST 32428—2013 [15]. Every 24 hours from the start of the experiment, the condition of the fish is monitored, and the indicators regulated by the state standard are recorded. The number of dead animals, the time of death, apparent behavioral anomalies, and signs of intoxication are recorded.

The assessment of the long-term effects of toxicants involves assessment of reprotoxic, mutagenic, and teratogenic effects. The choice of test concentrations is similar to that used in chronic toxicity assessment.

The study of the toxic effect of compounds on the reproductive potential of fish is carried out in accordance with the regulated methodology [21]. The assessment is based on qualitative and quantitative indicators of the *D. rerio* reproductive function state according to GOST 32368—2013 [20].

Quantitative indicators are assessed after spawning. The spawning and spawn processing procedures comply with

the requirements of GOST 32541—2013 [18]. After collecting spawn, a visual inspection is carried out to control quality and the total number of fertilized and unfertilized eggs is counted in absolute and relative units (abs./%). Criteria of viable fertilized spawn are transparency of the chorion, the presence of a perivitelline space between the egg wall and the embryo, the onset of cleavage (4–64 blastomere stage), and no signs of coagulation. Unfertilized and damaged eggs are removed after counting.

The viable spawn is used to assess teratogenic effects in accordance with OECD Test No. 236 [11], GOST 33774—2016 [19], and GOST 32541—2013 [18]. The duration of the study is 168 h (7 days), embryos and larvae are not exposed to negative effects.

Every 24 h, lethal effects are recorded using an inverted microscope according to the criteria specified in GOST 33774—2016 [19] and GOST 32541—2013 [18]: coagulation (opalescence, turbidity) of the embryo, no somite formation (abnormal segmentation), failure of the tail to separate from the yolk sac, no heartbeat. The detection of any of the listed signs indicates the death of the embryo. Additionally, the hatching rate (number of free-swimming larvae) is recorded starting from 48 h of exposure. Sublethal effects indicating the presence of a teratogenic effect are assessed according to the following criteria: the presence of deformation of the chord and tail section; delayed hatching (starting from 48 h); early postembryonic (post hatching) lethality; development of postembryonic edema. Additionally, the presence of combined morphological anomalies in specific embryos is assessed. The criteria for normal development of prelarvae in the control group are complete or almost complete yolk sac resorption, swim bladder fullness, active locomotion, and no signs of distress.

The experimental results are considered reliable when these meet the validity criteria: the overall fertilization rate in the collected batch of spawn is at least 70%, and the embryo survival rate and hatching rate in the negative control at the end of the 96-h exposure is at least 80%.

After completion of the 96-h period of recording lethal effects, monitoring the surviving prelarvae is extended to 168 hours (7 days) in accordance with GOST 32541—2013. The method allows for a comprehensive assessment of lethal and sublethal effects. The coping environment remains unchanged. Lethal effects are assessed daily (every 24 h) using an inverted microscope according to the above criteria. The assessment of teratogenic effects is completed after 168 h (7 days).

After spawning, fish are euthanized for further assessment of morphological alterations. According to the international guidelines on euthanasia of laboratory animals, the methods used must ensure rapid and irreversible death with minimization of pain and psycho-emotional stress in animals [31]. In this regard, an anesthetic substance called eugenol (clove oil) pre-dissolved in 96% ethyl alcohol in a volume ratio of 1 : 9 (1 cm³ of eugenol per 9 cm³ of alcohol) is used. The resulting solution is added to the water gradually (1 cm³ every 10 min) until deep anesthesia is achieved. The criteria for the onset of death are the gill cover motor activity cessation and no response to external stimuli. After that the fish are dried with filter paper and weighed with an accuracy of 0.1 g. The samples obtained are labeled. The gonadal histopathology assessment (identification of structural abnormalities in the tissues of the ovaries and testes) is performed. Histological slices are prepared by conventional methods.

The study and assessment of the mutagenic effect of chemicals is carried out using a micronucleus test in accordance with the Guideline (international standard) ISO 21427-1:2006

[32] representing the generally accepted methodology for assessment of the aquatic environment genotoxicity, including using fish as test objects. After 21 days of exposure, the fish are euthanized in the above manner. Blood is collected from the caudal artery by amputating the caudal peduncle distal to the anal fin. One drop of blood (5–10 µL) is immediately applied to the clean, degreased glass slide and spread in an even layer. Smears are fixed and dried in air at room temperature. The fixed smears are May–Grünwald–Giemsa stained. In each smear, erythrocytes are examined microscopically using an immersion lens.

The lowest effective concentration (LOEC) and no effective concentration (NOEC) of chemicals are determined based on the chronic toxicity assessment results. These parameters are used for subsequent justification of MPC in water and soil and RfD for humans with chronic oral intake.

Benefits of introducing the integrated approach into the hygienic rationing practice

The reported methodology, which integrates existing approaches to assessing acute and chronic toxicity in the *D. rerio* biological model into a single algorithm, makes it possible to use different model organism development stages (adult animals and embryos). In terms of methods this is different from the similar study [33], in which toxic properties were assessed at the embryonic stage only. The simultaneous use of model organisms at different stages of development allows

for the implementation of a comprehensive approach to the study of pathogenetic mechanisms underlying negative effects of test chemicals and for confirmation of the hygienic hypothesis about the living systems specific sensitivity depending on the development stage [13, 14].

In terms of hygiene, the exposure period extension to 168 h is a critically important step that makes it possible to record long-term negative effects of toxicants not detected during the standar 96-h biotesting. This fact is fully consistent with the conclusions that the abridged toxicology testing protocols may significantly underestimate the true hazard of compounds with high cumulative potential [34]. The reported step-by-step design allows for increased accuracy and objectivity in determining doses and ineffective concentrations of new chemical substances during chronic oral intake by humans.

CONCLUSION

The paper considers the use of the *Danio rerio* (Hamilton, 1822) freshwater fish as a highly informative, valid model organism in experimental studies to substantiate quality and safety standards for various types of water, except for industrial water, and soil in populated areas. An integrated approach that implements short-term and long-term studies in a single experimental design allows for the establishment, with a high degree of reliability, of quantitative parameters necessary for calculating maximum permissible concentrations and reference doses for chronic oral intake of chemical compounds by humans.

References

- Gajdaj EA, Gajdaj DS. Geneticheskoe raznoobrazie eksperimental'nykh myshej I krys: istoriya vozniknoveniya, sposoby polucheniya i kontrolya. Laboratornye zhivotnye dlya nauchnykh issledovaniy. 2019; (4): 78–85 (in Rus.). DOI: 10.29296/2618723X-2019-04-09.
- Rydell-Törmänen K, Johnson JR. The applicability of mouse models to the study of human disease. Methods Mol Biol. 2019; 1940: 3–22. DOI: 10.1007/978-1-4939-9086-3_1.
- Kachanov DA, Lakeenkov NM, Levikin KE, Blazhenko AA, Lapkina gYa, Bagaturiya GO, et al. *Danio rerio* (zebrafish) kak universal'nyy model'nyj ob"ekt v doklinicheskikh issledovaniyakh. Forcipe. 2018; 1 (1): 49–54 (in Rus.).
- Lashchenova LI, Perfilova VN, Snigur GL, Kaluev AV. Ispol'zovanie ryb zebbradano (Zebrafish) v kachestve model'nykh ob"ektov dlya izucheniya vozrast-assotsirovannykh zabolevanij (obzor). Nauchnye rezul'taty biomeditsinskikh issledovaniy. 2025; 11 (1): 120–41 (in Rus.). DOI: 10.18413/2658-6533-2025-11-1-0-7.
- Lepanto P, Zolessi FR, Badano JL. Studying human genetic variation in zebrafish. In: Cellular and animal models in human genomics research. Elsevier, 2019; 89–117.
- de Souza Anselmo C, Sardela VF, Matias BF, de Carvalho AR, de Sousa VP, Pereira HMG, et al. Is zebrafish (*Danio rerio*) a tool for human-like metabolism study? Drug Test Anal. 2017; 9 (11-12):1685–94. DOI: 10.1002/dta.2318.
- Prado E, Matos RR, de Lima G, Geovana M, de Sã CBL, da Costa N, et al. Metabolism of synthetic cathinones through the zebrafish water tank model: a promising tool for forensic toxicology laboratories. Forensic Toxicol. 2021; 73–88. DOI: 10.1007/s11419-020-00543-w.
- Russell WMS, Burch RL. The principles of humane experimental technique. Wheathampstead (UK): Universities Federation for Animal Welfare, 1959 (as reprinted 1992).
- Mitchell B, Chernick M, Rittschof D, Jayasundara N. Embryonic exposure to irgarol impacts development and larval and juvenile swimming in zebrafish (*Danio rerio*). Biofouling. 2026; 42 (2): 122–35. DOI: 10.1080/08927014.2025.2595131.
- Hoo JY, Kumari Y, Shaikh MF, Hue SM, Goh BH. Zebrafish: A versatile animal model for fertility research. Biomed Res Int. 2016; 2016: 1–2.
- OECD, Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 2025. DOI: 10.1787/9789264203709-en.
- Polistovskaya PA, Karpenko IYu, Makhnin IA, Azhikina O. Yu. Dekhorionizatsiya embrionov Danio rerio dlya otsenki ostroj toksichnosti organomineral'nykh khelatnykh kompleksov. Mezhdunarodnyj vestnik veterinarii. 2024; (1): 99–105 (in Rus.). DOI: 10.52419/issn2072-2419.2024.1.9.
- Zub AV, Zagrebin VL, Dvoryashina IA, Terentev AV. Vozmozhnost' ispol'zovaniya biologicheskoy modeli presnovodnoj ryby Danio Rerio v doklinicheskikh issledovaniyakh. Vestnik VolIGMU. 2020; 1 (73): 10–13 (in Rus.). DOI: 10.19163/1994-9480-2020-1(73)-10-13.
- Parg C, Seng WL, Semino C, McGrath P. Zebrafish: a preclinical model for drug screening. Assay Drug Dev Technol. 2002; (1): 41–8.
- GOST 32428–2013 "Metody ispytaniy khimicheskoy produktsii, predstavlyayushchej opasnost' dlya okruzhayushchej sredy. Opredelenie khronicheskoy toksichnosti dlya ryb: 14-dnevnyj test: mezhgosudarstvennyj standart".
- GOST 32473–2013 "Metody ispytaniy khimicheskoy produktsii, predstavlyayushchej opasnost' dlya okruzhayushchej sredy. Opredelenie ostroj toksichnosti dlya ryb: mezhgosudarstvennyj standart".
- GOST 32538—2013 "Metody ispytaniy khimicheskoy produktsii, predstavlyayushchej opasnost' dlya okruzhayushchej sredy. Opredelenie biokontsentratsii na rybkakh v protochnykh akvariumakh: mezhgosudarstvennyj standart".
- GOST 32541—2013 "Metody ispytaniy khimicheskoy produktsii, predstavlyayushchej opasnost' dlya okruzhayushchej sredy. Kratkosrochnoe ispytanie toksichnosti na embrionakh i predlichinkakh ryb: mezhgosudarstvennyj standart".
- GOST 33774—2016 "Metody ispytaniy khimicheskoy produktsii, predstavlyayushchej opasnost' dlya okruzhayushchej sredy. Ostraya toksichnost' dlya embrionov ryb: mezhgosudarstvennyj standart".
- GOST 32368—2013 "Metody ispytaniy khimicheskoy produktsii, predstavlyayushchej opasnost' dlya okruzhayushchej sredy.

- Otsenka reproduktivnoy sposobnosti ryb: mezhgosudarstvennyy standart".
21. OECD, Test No. 229: Fish Short Term Reproduction Assay. OECD Publishing, Paris, 2009. DOI: 10.1787/9789264076211-en.
 22. OECD, Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris, 2022. DOI: 10.1787/9789264071049-en.
 23. OECD, Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 2025. DOI: 10.1787/9789264069961-en.
 24. OECD, Test No. 210: Fish, Early-life Stage Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 2025. DOI: 10.1787/9789264203785-en.
 25. GOST 17.4.3.01—83 "Okhrana prirody. Pochvy. Obshchie trebovaniya k otboru prob: mezhgosudarstvennyy standart".
 26. GOST 17.4.4.02—84 "Okhrana prirody. Pochvy. Metody otbora i podgotovki prob dlya khimicheskogo, bakteriologicheskogo, gel'mintologicheskogo analiza: mezhgosudarstvennyy standart".
 27. FR1.39.2007.03221 "Biologicheskie metody kontrolya. Metodika opredeleniya toksichnosti vody i vodnykh vytyazhek iz pochv, osadkov stochnykh vod, otkhodov po smertnosti i izmeneniyu plodovitosti tseriodafnij: metodika vypolneniya izmerenij".
 28. GOST R 59024—2020 "Voda. Obshchie trebovaniya k otboru prob: natsional'nyy standart Rossijskoj Federatsii".
 29. Finney DJ. Probit analysis (2nd ed). J Inst Actuar. 1952; 78 (3): 388–90. DOI: 10.1017/S0020268100052938.
 30. OECD, Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 1984. DOI: 10.1787/9789264069985-en.
 31. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Schaumburg, IL: American Veterinary Medical Association, 2020; 121 p.
 32. ISO International Standard. Water quality—Evaluation of genotoxicity by measurement of the induction of micronuclei—Part 1: Evaluation of genotoxicity using amphibian larvae. ISO 21427-1, ICS: 13.060.70. Genova—CH, 2006.
 33. Nilén G, Obamwonyi OS, Liem-Nguyen V, Engwall M, Larsson M, Keiter SH. Observed and predicted embryotoxic and teratogenic effects of organic and inorganic environmental pollutants and their mixtures in zebrafish (*Danio rerio*). Aquat Toxicol. 2022; 248: 106175. DOI: 10.1016/j.aquatox.2022.106175.
 34. Perrichon P, Le Bihan F, Bustamante P, Le Menach K, Budzinski H, Cachot J, et al. Influence of sediment composition on PAH toxicity using zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*) embryo-larval assays. Environ Sci Pollut Res Int. 2014; 21 (24): 13703–19. DOI: 10.1007/s11356-014-3502-7.

Литература

1. Гайдай Е. А., Гайдай Д. С. Генетическое разнообразие экспериментальных мышей и крыс: история возникновения, способы получения и контроля. Лабораторные животные для научных исследований. 2019; (4): 78–85. DOI: 10.29296/2618723X-2019-04-09.
2. Rydell-Törmänen K, Johnson JR. The applicability of mouse models to the study of human disease. Methods Mol Biol. 2019; 1940: 3–22. DOI: 10.1007/978-1-4939-9086-3_1.
3. Качанов Д. А., Лакеенков Н. М., Левикин К. Е., Блаженко А. А., Лапкина Г. Я., Багатурия Г. О. и др. *Danio rerio* (zebrafish) как универсальный модельный объект в доклинических исследованиях. Forcipe. 2018; 1 (1): 49–54.
4. Лашенцова Л. И., Перфилова В. Н., Снигур Г. Л., Калыев А. В. Использование рыб зебраданио (*Zebrafish*) в качестве модельных объектов для изучения возраст-ассоциированных заболеваний (обзор). Научные результаты биомедицинских исследований. 2025; 11 (1): 120–41. DOI: 10.18413/2658-6533-2025-11-1-0-7.
5. Lepanto P, Zolesi FR, Badano JL. Studying human genetic variation in zebrafish. In: Cellular and animal models in human genomics research. Elsevier, 2019; 89–117.
6. de Souza Anselmo C, Sardela VF, Matias BF, de Carvalho AR, de Sousa VP, Pereira HMG, et al. Is zebrafish (*Danio rerio*) a tool for human-like metabolism study? Drug Test Anal. 2017; 9 (11-12): 1685–94. DOI: 10.1002/dta.2318.
7. Prado E, Matos RR, de Lima G, Geovana M, de Sã CBL, da Costa N, et al. Metabolism of synthetic cathinones through the zebrafish water tank model: a promising tool for forensic toxicology laboratories. Forensic Toxicol. 2021; 73–88. DOI: 10.1007/s11419-020-00543-w.
8. Russell WMS, Burch RL. The principles of humane experimental technique. Wheathampstead (UK): Universities Federation for Animal Welfare, 1959 (as reprinted 1992).
9. Mitchell B, Chernick M, Rittschof D, Jayasundara N. Embryonic exposure to irgarol impacts development and larval and juvenile swimming in zebrafish (*Danio rerio*). Biofouling. 2026; 42 (2): 122–35. DOI: 10.1080/08927014.2025.2595131.
10. Hoo JY, Kumari Y, Shaikh MF, Hue SM, Goh BH. Zebrafish: A versatile animal model for fertility research. Biomed Res Int. 2016; 2016: 1–2.
11. OECD, Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 2025. DOI: 10.1787/9789264203709-en.
12. Полистовская П. А., Карпенко Л. Ю., Махнин И. А., Ажикина О. Ю. Дехорионизация эмбрионов *Danio rerio* для оценки острой токсичности органоминеральных хелатных комплексов. Международный вестник ветеринарии. 2024; (1): 99–105. DOI: 10.52419/issn2072-2419.2024.1.9.
13. Зуб А. В., Загребин В. Л., Дворяшина И. А., Терентьев А. В. Возможность использования биологической модели пресноводной рыбы Данио Рерио в доклинических исследованиях. Вестник ВолГМУ. 2020; 1 (73): 10–13. DOI: 10.19163/1994-9480-2020-1(73)-10-13.
14. Parrig C, Seng WL, Semino C, McGrath P. Zebrafish: a preclinical model for drug screening. Assay Drug Dev Technol. 2002; (1): 41–8.
15. ГОСТ 32428—2013 «Методы испытаний химической продукции, представляющей опасность для окружающей среды. Определение хронической токсичности для рыб: 14-дневный тест: межгосударственный стандарт».
16. ГОСТ 32473—2013 «Методы испытаний химической продукции, представляющей опасность для окружающей среды. Определение острой токсичности для рыб: межгосударственный стандарт».
17. ГОСТ 32538—2013 «Методы испытаний химической продукции, представляющей опасность для окружающей среды. Определение биоконцентрации на рыбах в проточных аквариумах: межгосударственный стандарт».
18. ГОСТ 32541—2013 «Методы испытаний химической продукции, представляющей опасность для окружающей среды. Краткосрочное испытание токсичности на эмбрионах и предличинках рыб: межгосударственный стандарт».
19. ГОСТ 33774—2016 «Методы испытаний химической продукции, представляющей опасность для окружающей среды. Острая токсичность для эмбрионов рыбы: межгосударственный стандарт».
20. ГОСТ 32368—2013 «Методы испытаний химической продукции, представляющей опасность для окружающей среды. Оценка репродуктивной способности рыб: межгосударственный стандарт».
21. OECD, Test No. 229: Fish Short Term Reproduction Assay. OECD Publishing, Paris, 2009. DOI: 10.1787/9789264076211-en.
22. OECD, Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris, 2022. DOI: 10.1787/9789264071049-en.
23. OECD, Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 2025. DOI: 10.1787/9789264069961-en.
24. OECD, Test No. 210: Fish, Early-life Stage Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 2025. DOI: 10.1787/9789264203785-en.
25. ГОСТ 17.4.3.01—83 «Охрана природы. Почвы. Общие требования к отбору проб: межгосударственный стандарт».

26. ГОСТ 17.4.4.02—84 «Охрана природы. Почвы. Методы отбора и подготовки проб для химического, бактериологического, гельминтологического анализа: межгосударственный стандарт».
27. ФР 1.39.2007.03221 «Биологические методы контроля. Методика определения токсичности воды и водных вытяжек из почв, осадков сточных вод, отходов по смертности и изменению плодовитости цериодафний: методика выполнения измерений».
28. ГОСТ Р 59024—2020 «Вода. Общие требования к отбору проб: национальный стандарт Российской Федерации».
29. Finney DJ. Probit analysis (2nd ed). J Inst Actuar. 1952; 78 (3): 388–90. DOI: 10.1017/S0020268100052938.
30. OECD, Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, 1984. DOI: 10.1787/9789264069985-en.
31. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Schaumburg, IL: American Veterinary Medical Association, 2020; 121 p.
32. ISO International Standard. Water quality—Evaluation of genotoxicity by measurement of the induction of micronuclei—Part 1: Evaluation of genotoxicity using amphibian larvae. ISO 21427-1, ICS: 13.060.70. Geneva—CH, 2006.
33. Nilén G, Obamwonyi OS, Liem-Nguyen V, Engwall M, Larsson M, Keiter SH. Observed and predicted embryotoxic and teratogenic effects of organic and inorganic environmental pollutants and their mixtures in zebrafish (*Danio rerio*). Aquat Toxicol. 2022; 248: 106175. DOI: 10.1016/j.aquatox.2022.106175.
34. Perrichon P, Le Bihanic F, Bustamante P, Le Menach K, Budzinski H, Cachot J, et al. Influence of sediment composition on PAH toxicity using zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*) embryo-larval assays. Environ Sci Pollut Res Int. 2014; 21 (24): 13703–19. DOI: 10.1007/s11356-014-3502-7.