

SUBSTANTIATING THE MAXIMUM PERMISSIBLE CONCENTRATION OF CYLINDROSPERMOPSIN IN WATER FOR DRINKING AND HOUSEHOLD USE TO MINIMIZE HUMAN HEALTH RISKS

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Increasing human-induced pollution of water objects, climate change, creation of reservoirs, and a decrease in water flow rates have accelerated saturation of water bodies with biogenic substances, which in turn has caused massive growth and spread of cyanobacteria. Intensification of “blooming” processes in water bodies is observed almost everywhere. In respect to human health hazards, the issue of massive growth of toxic cyanobacteria in surface water bodies used for drinking and recreational purposes is related to the possibility of various cyanotoxins forming in the water. This includes cylindrospermopsin (CYN), which is classified by the World Health Organization as a high-priority environmental pollutant, and this highlights high relevance of studying it. Assessing the experience gained by foreign researchers in studying the content of cyanobacteria in water bodies, it should be noted that there is a fairly wide species diversity of blue-green algae, depending on regional prevalence.

In many countries across the globe, the list of substances for drinking water quality control has been expanded to include not only the content of cyanobacteria metabolic products, but also, in some cases, the content of their specific species composition. A number of studies by foreign and Russian researchers have shown the need for timely measures aimed at raising drinking water safety by regulating metabolic products of cyanobacteria.

The aim of this study was to substantiate the maximum permissible concentration (MPC) of CYN content in water for household and drinking water use, as well as for recreational needs.

The study included analysis of the results obtained by research on the physicochemical properties and toxicity of CYN, as well as a subchronic experiment to investigate general toxic effects and specific ones including neurotoxic, embryotoxic, and teratogenic effects upon conditions intragastric administration to animals.

As a result, we established parameters of toxic effects produced by CYN and its hazard category and substantiated the MPC for the chemical in water equal to 1.0 µg/l, the sanitary-toxicological indicator of harm, and the hazard category.

Keywords: cyanotoxins, cylindrospermopsin, general toxic effect, embryotoxic effect, teratogenic effect, drinking water, maximum permissible concentration.

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Increasing human-induced pollution of water objects, climate change, creation of reservoirs, and a decrease in water flow rates have accelerated saturation of water bodies with biogenic substances. This, in its turn, has caused massive growth and spread of cyanobacteria (CB). The World Health Organization (WHO) ranks global development of toxic CB in surface water objects used for drinking water supply and recreational needs as a priority challenge for population health protection [1].

'Blooming' in water objects is being observed practically everywhere and the process is intensifying. A.V. Bakaev and others believe that rapid development of blue-green algae in water objects can be often considered an emergency [2].

The WHO classifies cyanotoxins per their predominant effects on the human body and primarily identifies hepatotoxins, nephrotoxins and neurotoxins. Cyanobacteria of the *Cylindrocapsa raciborskii* species are considered to produce toxic substances, including cylindrospermopsin (CYN), which primarily affects the liver, gastrointestinal tract and kidneys when entering the human body.

In Russia, CYN levels in drinking water, which are safe for human health, have not been established so far although many studies by Russian researchers give evidence of the issue being truly vital¹ [3–7].

The aim of this study was to substantiate the maximum permissible concentration (MPC) of CYN in water for household and drinking water use, as well as for recreational needs.

Materials and methods. Results reported in studies on CYN physical and chemical properties as well as its toxicity were searched

in e-library, PubMed, Web of Science, Jstor, Open Access Button, Russian state Library, and MedLine; in addition, we analyzed regulatory and methodical documents valid in Russian and international legislation and devoted to regulating cyanotoxins in water.

The analytical CYN standard produced in Spain, CAS 143545-90-8, was used as the research object. The molecular formula was $C_{15}H_{21}N_5O_7S$; the molar mass, 399.42 g/mol.

Effects produced by CYN on self-purification in water objects were examined and the subsequent substantiation for CYN MPC in water was provided in conformity with the Methodical Guidelines MUK 2.1.5.720-98. Substantiation of safe standards for chemical levels in water supplied from water objects used for drinking and household purposes².

Concentrations of 1.0 $\mu\text{g}/\text{dm}^3$, 10.0 $\mu\text{g}/\text{dm}^3$, and 100.0 $\mu\text{g}/\text{dm}^3$ were selected to investigate CYN effects on water self-purification.

Bearing in mind the results reported by some foreign researchers in toxicological studies with their focus on CYN and available safe standards in other countries, we decided to shorten our experiment down to 2 months and to use prediction methods for establishing chronic MPC. Based on available literature data on CYN as well as guided by the MUK 2.1.5.720-98, the CYN doses of 0.1 – 1.0 – 10.0 $\mu\text{g}/\text{kg}$ of body weight were selected for experimental studies to simulate subchronic intragastric administration into white rats' bodies. These doses amount to 1 / 14 000 000, 1 / 1 400 000 and 1 / 140 000 proportion of LD_{50} respectively.

General toxic CYN effects were examined using conventional white rats. All animal

¹ Sinitsyna O.O., Turbinsky V.V., Kuz N.V., Ryashentseva T.M., Pushkareva M.V., Masaltsev G.V., Veshchemova T.E., Vostrikova M.V. Toksichnost' anatoksin-a pri 3-kh mesyachnom vnutrizheludochnom vvedenii v organizm belykh kryss: svidetel'stvo o registratsii bazy dannykh [Toxicity of anatoxin-a upon 3-month intragastric administration into white rats]: certificate of database registration, Registration Number: RU 2023625000; published on December 25, 2023. Application No. 2023624658 dated December 08, 2023.

² MU 2.1.5.720-98. Obosnovanie gigienicheskikh normativov khimicheskikh veshchestv v vode vodnykh ob'ektov khoziaistvenno-pit'evogo i kul'turno-bytovogo vodopol'zovaniya: metodicheskie ukazaniya, utv. i vved. v deistvie Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii 15 oktyabrya 1998 goda [MU 2.1.5.720-98. Substantiation of safe standards for chemical levels in water supplied from water objects used for drinking and household purposes: Methodical guidelines, approved and enacted by the RF Chief Sanitary Inspector on October 15, 1998]. *KODEKS: electronic fund for legal and reference documentation*. Available at: <https://docs.cntd.ru/document/1200006903> (March 10, 2025) (in Russian).

experiments were accomplished in conformity with the principles fixed in the Guide R 1.2.3156-13 Assessment of Toxicity and Hazard of Chemicals and Their Mixtures for Human Health³, and in conformity with the approval of the Biomedical Ethics Committee of the Federal Scientific Center of Hygiene named after F.F. Erisman dated November 21, 2022, the Meeting Report No. 03/22.

CYN was administered intragastrically into the experimental animals every day for 60 days; the control group was given distilled water in the same volume. The animals' health, water and food consumption was monitored throughout the experiment; any changes in body weight were registered in dynamics and any clinical signs of exposure to the tested substance were fixed on the 15th, 45th and 60th day in the experiment. On the same days, hematological and biochemical blood indicators were checked as well as physiological signs of the animals' health including behavioral aspects.

When the experiment was completed, the animals were euthanized; we performed post-mortem studies and macro-pathological studies of internal organs as well as histological analysis of specimens.

Blood serum was examined using ChemWell® 2910 biochemical analyzer to establish levels of alanine aminotransferase, albumin, alpha-amylase, aspartate aminotransferase, glucose, creatinine, lactate dehydrogenase, choline esterase, uric acid, urea, total protein, triglycerides, chlorides, cholesterol, and alkaline phosphatase.

Hematological studies were performed using Abacus Vet 5 Junior analyzer to establish counts of leucocytes, lymphocytes, monocytes,

neutrophils, the leucocyte formula, erythrocytes, hemoglobin, PCV, MCV, MCHC, and RDW.

Morphofunctional studies of internal organs (thyroid, thymus, heart, lungs, stomach, liver, spleen, pancreas, ileum, large intestine, kidneys, adrenals, and testicles) were accomplished in conformity with [8] using morphological, morphometric and stereometric methods.

Effects on the animals' motor activity, investigative activity and cognitive functions were examined using the Open Field Test (the motor component) and the Burrowing test (orientation and investigative reactions)⁴.

Hippocampus-dependent (explicit) and associative (implicit) memory was estimated in rats using the T-shaped labyrinth and the virtual labyrinth (What? Where? When? test) respectively.

In the T-shaped labyrinth test, the rats were trained in the experimental labyrinth for 3 days using food as reward. Next, the animals were tested 1 and 2 months later. Each testing session involved 10 runs made by the animals. Data per each 10 runs taken separately for each animal were averaged. The run was considered correct in case the animal went into a branch where food had been previously located.

To estimate associative (implicit) memory in the What? Where? When? test, each animal was placed into an individual automated chamber; containers with attractive and indifferent smells for rats were located in its two opposite areas.

Two hours later the animal was again placed in this individual automated chamber with different stimuli in its opposite corners,

³ R 1.2.3156-13. Otsenka toksichnosti i opasnosti khimicheskikh veshchestv i ikh smesei dlya zdorov'ya cheloveka: rukovodstvo, utv. vrio Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 27 dekabrya 2013 g. [R 1.2.3156-13. Assessment of Toxicity and Hazard of Chemicals and Their Mixtures for Human Health: guidelines, approved by the acting RF Chief Sanitary Inspector on December 27, 2013]. *KODEKS: electronic fund for legal and reference documentation*. Available at: <https://docs.cntd.ru/document/1200115595?ysclid=ma319lp49885704016> (March 10, 2025) (in Russian).

⁴ Metodicheskie rekomendatsii po ispol'zovaniyu povedencheskikh reaktsii zhivotnykh v toksikologicheskikh issledovaniyakh dlya tselei gigenicheskogo normirovaniya [Methodical guidelines on using animals' behavioral reactions in toxicological studies for establishing safe standards]. Kiev, Kievskii NII GT and PZ Publ., 1980, 43 p. (in Russian); Pavlenko S.M. Primenenie summatsionno-porogovogo pokazatelya v toksikologicheskom eksperimente na belykh kryсах [Use of the total threshold indicator in a toxicological experiment on white rats]. *Metodiki sanitarno-toksikologicheskogo eksperimenta [Methods of sanitary-epidemiological experimentation]: collection of research works by the Federal Scientific Center of Hygiene named after F.F. Erisman*, Moscow, 1975, pp. 5–7 (in Russian).

indifferent (area 1) and attractive (area 2) smells. The animals from all experimental groups were trained for 3 days in a row together with registering time of a contact between the animals and an area where this or that container was located. The automated chambers were washed with 1:1 water-spirit solution after each testing to remove all smells.

Each training session lasted for 6 minutes. More frequent visits to the area 2 (attractive smell) indicated training was successful. On the 4th day, smell sources, which had been previously located in the opposite corners, were removed to estimate short-term memory. After that, we traced the rats' routes and the number of times they approached those corners where attractive and indifferent smell sources had been located previously.

Embryotoxic and teratogenic effects produced by ATC-a were examined using conventional white rats, totally 40 females and 20 males. All animal experiments were conducted in conformity with the principles fixed in the Guide R 1.2.3156-13⁵.

Females were coupled with healthy males in the ratio 2:1 prior to exposure. Pregnancy was diagnosed in females when sperm cells were found in vagina smears. The females in the test group were orally exposed to CYN at doses equal to 0.1, 1.0 and 10.0 µg/kg of body weight every day starting from the beginning of pregnancy and up to its 20th day; the females in the control group were given distilled water.

The animals' health as well as food and water consumption was monitored during the

whole experiment; we registered changes in body weight in dynamics and fixed any clinical signs of exposure outcomes. The animals were euthanized on the 20th day of pregnancy and postmortem studies were accomplished to identify scope of embryotoxic and teratogenic effects using the following indicators: the number of fetuses; the total weight of the litter; the number of yellow bodies; embryos' length and weight; placentas' weight and diameter. Complex indicators were calculated using conventional formulas by A.M. Malashenko and I.K. Egorov⁶.

Two embryos were taken from each litter to identify absolute and relative masses of internal organs (thymus, heart, lungs, liver, and kidneys). The remaining embryos were divided into equal groups, which were used as experimental materials to study teratogenic effects according to the Wilson – Dyban method⁷, which involved assessing general signs of fetus maturity (auricle sticking, eye closing, extremities and tail build); the second group was placed in 96 % ethanol for 7 days for subsequent examination of their skeletons per the Dawson method⁸.

Primary data were analyzed using Microsoft Office Excel 2013; statistical data analysis was performed in SPSS Statistics v. 22.0. Significance of differences between the analyzed indicators was estimated using the Student's t-test at $p < 0.05$ ($t > 2$). The results were given as $M \pm m$ (where M is the mean value, m is the standards error of mean). We used the Shapiro – Wilk test to establish normalcy of distribution; Levene's test was used to check for the equality of dispersions. Occurrence of statistical

⁵ R 1.2.3156-13. Otsenka toksichnosti i opasnosti khimicheskikh veshchestv i ikh smesei dlya zdorov'ya cheloveka: rukovodstvo, utv. vrio Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 27 dekabrya 2013 g. [R 1.2.3156-13. Assessment of Toxicity and Hazard of Chemicals and Their Mixtures for Human Health: guidelines, approved by the acting RF Chief Sanitary Inspector on December 27, 2013]. *KODEKS: electronic fund for legal and reference documentation*. Available at: <https://docs.cntd.ru/document/1200115595?ysclid=ma319lzp49885704016> (March 10, 2025) (in Russian).

⁶ Malashenko A.M., Egorov I.E. Dominantnye letali u inbrednykh myshei pod deistviem etilenimina [Dominant lethals in inbred mice upon exposure to ethylenimine]. *Genetika*, 1967, no. 3, pp. 59–68 (in Russian).

⁷ Dyban A.P., Puchkov V.F., Chebotar N.A. [et al.]. Metodicheskie ukazaniya po izucheniyu embriotoksicheskogo deistviya farmakologicheskikh veshchestv i vliyanie ikh na reproduktivnuyu funktsiyu [Methodical guidelines on examining embryotoxic effects of pharmaceuticals and their influence on the reproductive function]. Moscow, USSR Ministry of Health Publ., 1986, 21 p. (in Russian).

⁸ Dyban A.P., Baranov V.S., Akimova I.M. Osnovnye metodicheskie podkhody k testirovaniyu teratogennoi aktivnosti khimicheskikh veshchestv [Basic methodological approaches to testing teratogenic activity of chemicals]. *Arkhiv anatomii, gistologii i embriologii*, 1970, vol. 59, no. 10, pp. 89–100 (in Russian).

outliers was checked by the boxplot method⁹. Intergroup comparisons were accomplished by one-factor dispersion analysis with a posterior comparisons per Bonferroni (F-test, parametric indicators) or by using the Kruskal – Wallis non-parametric test^{9,10}. We checked for trend occurrence in investigations (in case intergroup differences were significant) by using the Spearman's rank correlation method (two-side analysis)¹¹.

Results and discussion. We conducted a literature review, analyzed available research works using e-library, PubMed and Web of Science and studied regulatory and methodical documents valid in the Russian and international legislation with their focus on identifying cyanotoxins in water, assessing physical and chemical properties and toxicity of some cyanotoxins, investigating CYN toxicity upon intragastric administration into warm-blooded animals in acute, short-term experiments; we also analyzed data on occurrence of remote exposure effects. As a result we established the following:

CYN is an alkaloid produced by some CB species. The following structural variants can be found in natural conditions: 7-epi-CYN, 7-desoxi-CYN (Figure), 7-desoxidesulfo-CYN and 7-desoxidesulfo-12-acetyl-CYN. The molecular formula is $C_{15}H_{21}N_5O_7S$.

Upon entering water, CYN does not change its aesthetic properties; does not influence water color or smell; is not a volatile compound; is not decomposed by boiling; is relatively stable in dark and under the temperature between 4 and 50 °C for up to 5 weeks; is persistent to changes in pH and remains stable for a period up to 8 weeks when pH is equal to 4, 7 and 10 [1].

Inhalation exposure is possible only when the substance is sprayed, for example, during irrigation or a storm. In the environment, drinking water consumption is the basic way

of human exposure to CYN in case this water comes from untreated or insufficiently treated surface water sources. Another exposure way is using water in lakes, rivers, or nearshore areas in the sea for recreation.

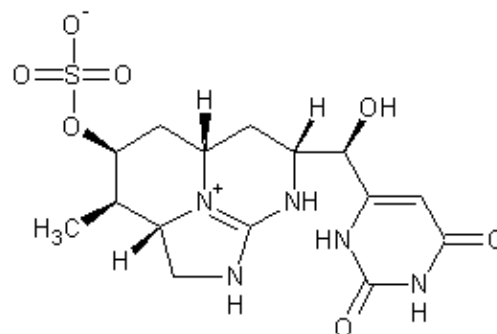


Figure. The structural formula of 7-desoxi-cylindrospermopsin

Acute CYN toxicity reported in foreign studies is described with the following values: intraperitoneal acute toxicity (DL_{50}), 20–65 mg/kg of body weight; acute toxicity upon oral administration is identified at 1400 mg/kg of body weight. [9–11]. Some authors reported lower lethal doses upon other exposure ways (intratracheal, for example) [12–14].

Several foreign researchers established the threshold dose in sub-acute experiments involving CYN administration in experimental animals with drinking water or using a gastric tube for 21 and 14 days, 66 µg/kg of body weight and 150 µg/kg of body weight respectively [15, 16].

According to available research publications, the liver and other gastrointestinal tract organs as well as the kidneys are primary targets upon CYN exposure under longer administration into animals with drinking water at doses ranging from 60 to 657 µg/kg of body weight [11, 17–19]. Intragastric CYN administration for 11 weeks in doses equal to 60–240 µg/kg of body weight led to an increase in the relative mass of the kidneys and liver. Necrotic or in-

⁹ Khalafyan A.A. STATISTICA 6. Statisticheskii analiz dannykh [STATISTICA 6. Statistical data analysis]: manual, the 3rd ed. Moscow, OOO 'Binom-Press' Publ., 2007, 512 p. (in Russian).

¹⁰ Abdi H. The Bonferroni and Šidák corrections for multiple comparisons. In book: *Encyclopedia of measurement and statistics*. USA, SAGE Publ., 2007, no. 3, pp. 103–107.

¹¹ Corder G.W., Foreman D.I. Nonparametric statistics: A step-by-step approach, 2nd ed. USA, Wiley Publ., 2014, 288 p.; Agresti A. Categorical data analysis, 2nd ed. USA, Wiley Publ., 2002, 734 p.

flammatory foci were identified in the liver of 60 % mice upon exposure to a dose equal to 120 µg/kg of body weight and in 90 % mice upon exposure to 240 µg/kg of body weight [11].

Fatal outcomes were detected in pregnant mice, which were given purified CYN by intraperitoneal injections thrice a day at a dose of 64 µg/kg of body weight. The relative weight of the liver was considerably increased in those mice that survived; however, the authors did not report any influence on fetuses' weight, death rate, or changes in skeletal or soft tissues [20]. Some researchers reported hemorrhagic lesions in certain tissues including the gastrointestinal tract [21].

The authors did not establish genotoxicity in ovary cells of Chinese hamsters (CHO-K1) exposed to CYN in concentrations equal to 0.5 and 1 µg/l in spite of induction of non-cytotoxic effects on cell morphology and microtubule structures [22].

Some foreign researchers (E. Bazin et al., 2010) established mutagenic and carcinogenic effects upon exposure to high CYN doses. Still, investigations aimed at determining bacterial mutagenicity using *Salmonella Typhimurium* TA98, TA100, TA1535, TA1537 strains and *Escherichia coli* WP2 uvrA and WP2 [pKM101] strains did not reveal any mutagenic CYN activity. Studies on genotoxicity for mammalian cells showed mutagenic effects, in particular, cellular lines of human hepatocytes showed elevated numbers of multinuclear and binucleated cells [23].

An experiment was performed on Swiss albino mice by foreign researchers who found neoplastic changes in mice exposed to CYN at doses of 2.75 or 8.25 mg/kg of body weight. However, those changes were not significant as compared to the control group. The authors believe that neoplastic changes detected in various target organs of the experimental ani-

mals are rather controversial evidence of CYN carcinogenicity at the moment [23, 24].

It is worth noting that pathways of toxic effects produced by long-term oral exposure to small CYN doses have not been examined to the full so far.

Many countries across the globe have extended the list of indicators used to control drinking water quality, both per CB levels in it and products of their life activity. In doing so, they usually emphasize the significance of research conducted within a country and show other countries the necessity to take relevant measures aimed at making drinking water safer by regulating levels of CB and cyanotoxins, CYN included. Our analysis has established that Australia, Brazil, and New Zealand employ their national research as criteria for establishing safe standards for drinking water quality per levels of CB and toxins (Table 1); they do not limit themselves to following only WHO guidelines¹² [1, 25–27].

Table 1

Permissible cylindrospermopsin concentrations in drinking water established in various countries

Country	Safe standard	Permissible concentration
Australia	<i>Cylindrospermopsin</i>	1 µg/l
Brazil	<i>Cylindrospermopsin</i>	15 µg/l
New Zealand	<i>Cylindrospermopsin</i>	1 µg/l

In Russia, the only MPC for cyanotoxins that has been scientifically substantiated and implemented in practical activities is MPC for Microcystin-LR in water objects used for drinking and household water supply as well as recreational needs. It is equal to 0.001 mg/l as the substance is assigned Hazard Category 1, sanitary-toxicological harm per SanPiN 2.1.3685-21¹³.

¹² NHMRC, NRMCC. National Water Quality Management Strategy. Australian Drinking Water Guidelines 6. Australia, Canberra, Australian Government, 2011.

¹³ SanPiN 1.2.3685-21. Gигиенические нормативы и требования к обеспечению безопасности и (или) безвредности для человека факторов среды обитания: санитарные правила и нормы (с изменениями на 30 декабря 2022 года), утв. постановлением Главного государственного санитарного врача Российской Федерации от 28 января 2021 года № 2 [SanPiN 1.2.3685-21. Hygienic standards and requirements to providing safety and (or) harmlessness of environmental factors for people: sanitary rules and norms (last edited as of December 30, 2022), approved by the Order of the RF Chief Sanitary Inspector dated January 28, 2021 No. 2]. KODEKS: electronic fund for legal and reference documentation. Available at: <https://docs.cntd.ru/document/573500115> (March 12, 2025).

Investigations with their focus on water self-purification in model water objects were conducted using three CYN concentrations: 1.0, 10.0 and 100.0 µg/l. They did not establish any influence on biochemical oxygen consumption during 5 days.

No spontaneous animal deaths were detected in a sub-chronic experiment that involved exposure to CYN at doses of 0.1; 1.0; and 10.0 µg/kg of body weight; we also did not establish any significant differences in body weight growth, any authentic differences in absolute or relative masses of internal organs between the tests and controls.

Hematological tests of animal blood were performed on the 30th, 45th, and 60th day in the experiment; they did not establish any significant differences between the analyzed indicators of the animals exposed to CYN at doses of 0.1; 1.0 and 10 µg/kg of body weight and the controls in all observation periods.

Biochemical blood serum tests were conducted on the 60th day in the experiment and established significant deviations in some indicators among those animals exposed to CYN

at a dose of 10.0 µg/kg of body weight against the controls. We established authentic elevated levels of alanine aminotransferase, aspartate aminotransferase, glucose, creatinine, and triglycerides and an authentic decline in levels of albumin, uric acid, urea and total protein (Tables 2 and 3).

Increased motor activity in the Open Field test and investigative activity in the Burrowing test were established in the animals exposed to CYN at a dose of 10 µg/kg of body weight by the end of the experiment against the controls (Table 4).

The animals exposed to CYN at a dose of 10.0 µg/kg of body weight had fewer correct runs in the T-shaped labyrinth test; time spent on accomplishing them was also longer against the controls (Table 5).

We did not establish any effects produced by CYN on implicit (associative) memory in the What? Where? When? test using the indicator of the successfully completed training. This was evidenced by the most frequent visits to the area 2 (attractive smell) by the animals from all experimental groups prior to CYN administration (background).

Table 2

Changes in biochemical indicators of rats' blood serum, liver function indicators

CYN dose (µg/kg of body)	Mean ± standard error of mean				
	Alanine aminotransferase (U/l)	Albumin (g/l)	Aspartate aminotransferase (U/l)	Glucose (mol/l)	Creatinine (µmol/l)
15 days					
Control	72.74 ± 5.88	39.24 ± 0.43	158.53 ± 9.87	7.70 ± 0.26	109.15 ± 3.16
0.1	72.63 ± 5.48	38.68 ± 0.66	161.52 ± 10.84	7.23 ± 0.20	115.64 ± 2.75
1.0	64.10 ± 5.08	35.60 ± 0.65	143.27 ± 7.75	6.85 ± 0.26	108.10 ± 4.20
10.0	82.14 ± 3.49	39.42 ± 0.42	179.01 ± 9.97	7.56 ± 0.28	121.02 ± 4.71
45 days					
Control	60.79 ± 4.55	39.03 ± 0.68	222.15 ± 12.42	9.09 ± 0.47	116.46 ± 4.32
0.1	58.85 ± 2.76	39.29 ± 0.30	211.68 ± 8.51	9.15 ± 0.16	126.09 ± 3.90
1.0	59.68 ± 2.73	38.62 ± 0.64	229.07 ± 11.26	8.63 ± 0.25	118.06 ± 1.84
10.0	65.10 ± 4.23	40.44 ± 0.50	233.96 ± 15.67	9.40 ± 0.33	130.11 ± 5.89
60 days					
Control	59.23 ± 3.08	38.35 ± 0.68	123.83 ± 6.12	8.01 ± 0.23	108.06 ± 3.67
0.1	62.56 ± 3.83	38.63 ± 0.51	120.64 ± 4.67	7.94 ± 0.16	102.18 ± 3.25
1.0	57.81 ± 3.01	37.04 ± 0.70	121.82 ± 7.31	8.56 ± 0.27	106.23 ± 3.41
10.0	↑75.03 ± 4.44*	↓27.08 ± 0.22*	↑158.97 ± 5.52*	↑9.26 ± 0.25*	↑140.48 ± 4.77*

Note: *significant at $p < 0.05$.

Table 3

Changes in biochemical indicators of rats' blood serum, kidney function indicators

CYN dose ($\mu\text{g/kg}$ of body)	Mean $\pm$ standard error of mean			
	Uric acid ($\mu\text{mol/l}$)	Urea (mol/l)	Total protein (g/l)	Triglycerides (mol/l)
15 days				
Control	132.01 ± 17.97	7.24 ± 0.33	68.98 ± 1.08	0.57 ± 0.05
0.1	135.57 ± 20.86	6.66 ± 0.38	70.39 ± 0.98	0.43 ± 0.02
1.0	148.98 ± 17.32	7.71 ± 0.32	71.16 ± 1.27	0.47 ± 0.04
10.0	141.06 ± 5.63	7.82 ± 0.68	70.45 ± 0.77	0.48 ± 0.03
45 days				
Control	45.50 ± 5.57	7.11 ± 0.32	70.06 ± 0.68	0.78 ± 0.10
0.1	54.75 ± 9.37	7.47 ± 0.52	70.19 ± 0.63	0.68 ± 0.09
1.0	48.58 ± 10.16	7.44 ± 0.35	69.97 ± 0.93	0.62 ± 0.05
10.0	47.04 ± 11.63	6.81 ± 0.40	69.70 ± 0.76	0.63 ± 0.06
60 days				
Control	60.54 ± 10.13	6.30 ± 0.28	67.90 ± 1.05	0.69 ± 0.08
0.1	69.40 ± 9.86	6.04 ± 0.40	68.26 ± 0.89	0.54 ± 0.05
1.0	60.54 ± 7.20	7.30 ± 0.50	68.12 ± 0.86	0.65 ± 0.08
10.0	$\downarrow 39.28 \pm 6.98^*$	$\downarrow 4.23 \pm 0.28^*$	$\downarrow 48.19 \pm 0.58^*$	$\uparrow 0.82 \pm 0.06^*$

Note: *significant at $p < 0.05$.

Table 4

Levels of animals' motor and investigative activity established by Open Field and Burrowing tests

CYN dose ($\mu\text{g/kg}$ of body)	Background	30 days	60 days
Open Field (distance covered, cm)			
Control	1648 ± 71.52	533.99 ± 150.30	578.93 ± 116.65
0.1	1472.38 ± 101.99	570.48 ± 112.03	686.33 ± 119.05
1.0	1453.09 ± 112.98	562.77 ± 177.51	959.73 ± 202.67
10.0	1638.84 ± 145.40	895.40 ± 235.10	$1303.30 \pm 67.07^*$
Burrowing test (number of events)			
Control	46.30 ± 7.22	8.10 ± 2.34	8.80 ± 2.20
0.1	43.20 ± 5.26	13.20 ± 3.65	11.40 ± 2.10
1.0	37.30 ± 3.34	9.90 ± 3.02	10.50 ± 3.63
10.0	41.70 ± 4.04	15.90 ± 3.83	$20.60 \pm 1.18^*$

Note: * significant at $p < 0.05$.

Table 5

Indicators of animals' hippocampus-dependent (explicit) memory in the T-shaped labyrinth test

CYN dose ($\mu\text{g/kg}$ of body)	Background	30 days	60 days
Number of correct runs, %			
Control	26.67 ± 9.69	36.80 ± 10.52	33.33 ± 11.11
0.1	23.37 ± 7.14	23.33 ± 8.69	37.04 ± 10.31
1.0	26.73 ± 9.73	26.67 ± 10.89	40.00 ± 8.31
10.0	30.03 ± 9.27	$13.27 \pm 5.42^*$	36.67 ± 9.23
Time spent on a run, sec			
Control	42.05 ± 13.86	47.18 ± 9.06	59.22 ± 18.59
0.1	39.20 ± 12.38	35.86 ± 24.45	48.99 ± 11.54
1.0	64.80 ± 2.96	48.14 ± 16.65	27.06 ± 6.63
10.0	47.00 ± 11.72	$102.00 \pm 17.01^*$	25.87 ± 8.82

Note: * significant at $p < 0.05$.

Microscopic examination of histological specimens of various organs taken from the animals exposed to CYN at a dose of 10.0 µg/kg of body weight established authentic morphofunctional changes in the following organs. Fat dystrophy and micronecroses were increased in the liver; the glomerule alteration index and glomerule necrosis were higher in the kidney; gland hypersecretion was identified in the stomach and large intestine; more layers of spermatogenic cells moved away from the basement membrane of the testicular tubules in the testicle and we also observed rarer spermatids and sperm cells; thyrocyte desquamation was observed in the thyroid.

Experimental studies with their focus on embryotoxic and teratogenic CYN effects did not establish any spontaneous animal deaths in all experimental groups. We did not find authentic changes in body weight of pregnant female rats; average body weight growth during pregnancy was the same in the test groups and the control. We did not reveal any significant changes in the absolute or relative mass of embryos' internal organs as well as authentic changes in embryogenesis indicators in all test groups as compared to the control. Average numbers of yellow bodies, implantation sites, live fetuses, the overall weight of fetuses in a litter, crown-rump length of fetuses, placenta diameters and weights did not have significant differences in the tests against the controls. Pre-implantation, post-implantation and total death rates among offspring were the same in the test groups as in the parallel control without any significant differences between them.

Intravital microscopic examination of fetuses did not find any severe malformations. Subsequent micro-post mortem study of fixed fetus material and analysis of cross sections of the brain and other internal organs revealed that fetuses' internal organs had normal locations and structures in the test groups and their structure

and topography did not have any differences from the control. Detected embryogenesis disorders were not diverse, were not numerous and were similarly rare in all animal groups; this did not allow suspecting any associations between them and CYN effects on offspring formation and intrauterine development.

Our assessment of fetus skeletons with clear soft tissues was performed in the test and control groups and did not find any osteogenesis disorders or desynchronized ossification of the cartilage anlagen evidencing absence of any teratogenic CYN effects.

Discussion of the results and substantiation of CYN MPC in water. When assessing materials on regulation and control of CYN contents in water objects, we should remember that available foreign regulatory standards are based exclusively on studying toxic properties in experimental conditions. Russian practice of substantiating safe standards for chemicals in water is based not only on establishing concentrations with no observed toxic effects on laboratory animals but also, in conformity with the Methodical Guidelines 2.1.5.720-98¹⁴, includes establishing threshold levels of chemicals' effects on water organoleptic properties and the overall sanitary condition of a water object; a safe standard is fixed per a limiting harm indicator with the lowest no-effect or threshold concentration.

When substantiating CYN MPC in water from water objects used for drinking and household water supply, we did not establish threshold concentrations per its effects produced on water organoleptic properties since available literature data give evidence that CYN does not influence water aesthetic properties. We also thought that involving volunteers in an experiment with a highly toxic substance with poorly studied potential carcinogenic effects could create substantial risks of adverse health impacts for them.

¹⁴ MU 2.1.5.720-98. Obosnovanie gigenicheskikh normativov khimicheskikh veshchestv v vode vodnykh ob'ektov khozyaistvenno-pit'evogo i kul'turno-bytovogo vodopol'zovaniya: metodicheskie ukazaniya, utv. i vved. v deistvie Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii 15 oktyabrya 1998 goda [MU 2.1.5.720-98. Substantiation of safe standards for chemical levels in water supplied from water objects used for drinking and household purposes: Methodical guidelines, approved and enacted by the RF Chief Sanitary Inspector on October 15, 1998]. *KODEKS: electronic fund for legal and reference documentation*. Available at: <https://docs.cntd.ru/document/1200006903> (March 10, 2025) (in Russian).

Investigations in model water objects showed no influence on water self-purification, which is consistent with opinions expressed by other Russian researchers. They consider that biocoenosis in 'blooming' water objects is more resistant to cyanometabolites and explain it by the result of co-evolutionary co-existence with toxic cyanobacteria in natural conditions¹⁵.

Guided by the MUK 2.1.5.720-98¹⁴, we substantiated exposure doses of 0.1 – 1.0 – 10.0 µg/kg of body weight when conducting our sub-chronic experiment aimed at establishing a threshold CYN concentration per its general toxic effects as well as at revealing potential remote effects (embryotoxic and teratogenic ones).

This sub-chronic experiment established several changes in biochemical blood serum indicators upon exposure to CYN at a dose of 10 µg/kg of body weight. These changes indicated impairments of enzyme-forming liver function, protein and carbohydrate metabolism; we also revealed some changes in kidney functional indicators, which is consistent with multiple studies by foreign researchers [15, 16, 18, 19, 28].

In addition, an increase in motor activity (Open Field test) and investigative activity (the Burrowing test) was established in the animals exposed to CYN at a dose of 10 µg/kg of body weight against the control by the end of the subchronic experiment.

These changes can be explained by the fact that the basic pathway of CYN effects is inhibiting protein synthesis, including synthesis of glutathione. This leads to oxidative stress, which, in its turn, enhances lipid peroxidation and induces DNA damage¹⁶ [28–33]. CYN is able to penetrate through the blood-brain barrier [34, 35] and reduce the number of synapses; consequently, it is able to produce a neurotoxic effect. Still, the very neurotoxic effects produced by CYN have not been studied sufficiently so far [36]. Therefore, we made an effort in this study to estimate CYN

influence on animals' cognitive functions in tests aimed at revealing disorders in ability to memorize, recognize and reproduce as well as in investigative activity.

Explicit memory disorders in animals were of the greatest interest to us since this memory is associated with activities of the brain hippocampus and is conscious. We revealed a decline in the number of correct runs and longer time spent on doing them in the T-shaped labyrinth test, which indicates disorders of hippocampus-dependent memory in rats exposed to CYN at a dose of 10 µg/kg of body weight. This disorder might result from changes in the body described in literature such as declining acetyl cholinesterase activity and growing lipid peroxidation levels with accompanying histopathological changes in the brain of animals under subchronic CYN exposure [34]. Recovery of indicators up to their levels identified in the controls by the end of the second months of CYN exposure might result from activation of compensatory mechanisms.

Implicit memory disorders can lead to 'accumulated forgetting' of visits to sites in the Open Field test, which is the reason why a covered distance becomes longer; this can also cause a growth in the number of time when rats peep in burrows in the Burrowing test after CYN administration due to the described damage to neurons at the synapsis level [35].

Examinations of histological specimens of organs taken from the animals exposed to CYN at a dose of 10 µg/kg of body weight found considerable changes in the liver such as fat dystrophy up to micro-necrosis, which is consistent with [11, 15, 16, 19, 28]; glomerule necrosis was established in the kidneys and gland hypersecretion in the stomach and large intestine, which is consistent with [18]. Established morphofunctional changes in animals' internal organs primarily give evidence that the liver, kidneys and the gastrointestinal tracts do not function correctly.

¹⁵ Semenova A.S. Koevolutsiya tsianobakterii i zooplanktona: zashchitnaya rol' tsianotoksinov [Co-evolution of cyanobacteria and zooplankton: protective role of cyanotoxins]: Grant No. 15-04-04030. Moscow, Russian Foundation for Basic Research (RFBR), 2015.

¹⁶ Terao K., Ohmori S., Igarashi K., Ohtani I., Watanabe M.F., Harada K.I., Ito E., Watanabe M. Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green-alga *Umezakia natans*. *Toxicon*, 1994, vol. 32, no. 7, pp. 833–843. DOI: 10.1016/0041-0101(94)90008-6

Our experimental study of embryotoxic CYN effects did not find any authentic changes in the absolute and relative mass of fetuses' internal organs in the test groups against the control. Embryogenesis indicators established in the test groups did not reveal any authentic changes against the controls either, which means CYN does not have any embryotoxic effects.

We did not find any changes in the number of bones in fetuses or in their bone structure, or any significant malformations of bone tissues. Some sporadic changes were identified in some animals when we investigated pathology of internal organs; however, their dependence on an administered CYN dose was not confirmed ($p > 0.05$). Analysis of these deviations from the control group makes it possible to conclude that all CYN doses tested in the experiment do not have teratogenic effects on the body.

Neoplastic changes in target organs of various animals, which are reported by some researchers, provide some ambiguous evidence of carcinogenicity; this requires additional research [29, 30].

Considering authentic changes in biochemical blood indicators, changes in animals' motor and investigative activity, as well as morphofunctional lesions of internal organs, we estimate CYN dose of 10 µg/kg of body weight as an effective one per general toxicity

in a sub-chronic experiment; doses of 0.1 and 1.0 µg/kg of body weight are considered as no-effect ones.

To substantiate CYN MPC in water for drinking and household water use, according to the Item 10.3 MUK 2.1.5.720-98 and Table 10.2, we selected the coefficient value J ($J_1 = 20$) to establish the threshold dose for chronic exposure depending on how well toxic effects of a substance are known and a structural group it belongs to. CYN is assigned the Hazard Category 1 and its MPC for chronic exposure is established at the level of 3.3 µg/kg of body weight per the ratio DL_{50} / MPC_{IEC} .

Bearing in mind that CYN is a stable substance, a safety factor 5 is introduced in accordance with Table 5.2, MUK 2.1.5.720-98, and the MPC for chronic exposure is 0.66 µg/kg of body weight.

According to the Item 11.7 in MUK 2.1.5.720-98, the maximum no-effect concentration (MEC) for chronic exposures has been established in conformity with Table 11.1 using a safety factor equal to 10. Considering this safety factor, chronic CYN MEC upon oral administration to warm-blooded animals' bodies is 0.06 µg/kg of body weight.

Table 6 provides indicators used in complex assessment of hazards posed by CYN contents in water.

Table 6

Indicators used in complex assessment of hazards posed by CYN contents in water

Indicator	Values to be identified	Source	Result
Stability in water	Stability	[1]	stable
Effect on water organoleptic properties	Effects on color or smell	[1]	No effect
Effect on water self-purification*	MC_{san}		above 100.0 µg /l
Toxicological studies			
Acute experiment	LD_{50}	Ref. 6, 8, 9	1400 µg/kg of b.w.
Sub-acute experiment	MPC_{IEC}	[9, 10]	66.0 µg/kg of b.w.
Sub-chronic experiment*	Calculated chronic MPC Calculated chronic MEC Hazard Category	MUK 2.1.5.720-98	3,3 µg/kg of b.w. 0,06 µg/kg of b.w. hazard category 1
Remote effects			
Mutagenic		[11]	Upon exposure to high doses
Carcinogenic		[17, 18]	Upon exposure to high doses
Embryotoxic*	MPC_{sep}		above 10.0 µg/kg of b.w.
Teratogenic*	MPC_{sep}		above 10,0 µg/kg of b.w.

Note: *means our own research.

The maximum no-effect CYN concentration (MEC_{chr}) in water amounts to 1.2 $\mu\text{g/l}$ per the sanitary-toxicological indicator of harm and in conformity with the recalculation formula, which considers an average human body weight (60 kg) and daily water intake (3 l).

Conclusion. Therefore, considering that there is no threshold effect on water organoleptic properties established for CYN and absence of any influence on self-purification in concentrations below 100 $\mu\text{g/l}$, we recommend CYN MPC at the level of 1.0 $\mu\text{g/l}$ in water for drinking and household water use per the sani-

tary-toxicological indicator of harm, the Hazard Category 1.

Methodical Guidelines MUK Quantification of Cylindrospermopsin in Drinking and Natural Water by Immune Enzyme Analysis (the bottom limit of quantification is 0.00005 mg/l) have been developed for control of CYN contents in water.

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References

1. WHO. Guidelines for drinking-water quality, 4th ed. *WHO Documentation Centre*, 2011. Available at: https://iris.who.int/bitstream/handle/10665/44584/9789241548151_eng.pdf (January 11, 2025).
2. Bakaev A.V., Bakaeva E.N., Ignatova N.A. “Blooming” blue-green algae (cyanobacteria) a variety of emergency situations in reservoirs. *Inzhenernyi vestnik Dona*, 2012, vol. 23, no. 4–2, pp. 144 (in Russian).
3. Kuz' N.V., Zholdakova Z.I. The problem of the «flowering» of the water sources. Evaluation of the influence of water processing on the content of cyanobacterium in drinking water of economic-drinking water supply of Moscow. *ZNiSO*, 2017, no. 9 (294), pp. 35–39 (in Russian).
4. Egorova N.A., Kuz N.V., Sinitsyna O.O. Materials for the substantiation of the hygienic standard of microcystin-LR in water of water objects. *Gigiena i sanitariya*, 2018, vol. 97, no. 11, pp. 1046–1052. DOI: 10.18821/0016-9900-2018-97-11-1046-52 (in Russian).
5. Kuz N.V., Sinitsyna O.O., Turbinsky V.V. Flowering of water sources – new challenges to drinking water safety in the condition of climate change. *Zdorov'e – osnova chelovecheskogo potentsiala: problemy i puti resheniya*, 2022, vol. 17, no. 1, pp. 218–225 (in Russian).
6. Sinitsyna O.O., Turbinskii V.V., Pushkareva M.V., Kuz N.V., Masaltsev G.V., Shiryayeva M.A., Ryashentseva T.M., Valtseva E.A., Vostrikova M.V. Morphofunctional changes in the animal organisms under oral exposure to anatoxin-a in subchronic experimental settings. *Toksikologicheskii vestnik*, 2024, vol. 32, no. 6, pp. 336–347. DOI: 10.47470/0869-7922-2024-6-336-347 (in Russian).
7. Turbinsky V.V., Bragina I.V., Kuz N.V., Sinitsyna O.O., Pushkareva M.V. The problem of algal bloom in the source of drinking water supply for the population. *Gigiena i sanitariya*, 2024, vol. 103, no. 12, pp. 1466–1472. DOI: 10.47470/0016-9900-2024-103-12-1466-1472 (in Russian).
8. Belyaeva N.N., Rakitskii V.N., Nikolaeva N.I., Vostrikova M.V., Veshchemova T.E. Quantitative structural and functional assessment of various systems of the body of laboratory animals in hygienic studies. *Gigiena i sanitariya*, 2020, vol. 99, no. 12, pp. 1438–1445. DOI: 10.47470/0016-9900-2020-99-12-1438-1445 (in Russian).
9. Falconer I.R. An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Environ. Toxicol.*, 1999, vol. 14, no. 1, pp. 5–12.
10. Falconer I.R. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. *Phycologia*, 2001, vol. 40, no. 3, pp. 228–233. DOI: 10.2216/i0031-8884-40-3-228.1
11. Falconer I.R., Humpage A.R. Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins. *Environ. Toxicol.*, 2006, vol. 21, no. 4, pp. 299–304. DOI: 10.1002/tox.20194
12. Seawright J. Testing for necessary and/or sufficient causation: Which cases are relevant? *Political Analysis*, 2002, vol. 10, no. 2, pp. 178–193. DOI: 10.1093/pan/10.2.178
13. Norris R.L., Seawright A.A., Shaw G.R., Smith M.J., Chiswell R.K., Moore M.R. Distribution of ^{14}C cylindrospermopsin in vivo in the mouse. *Environ. Toxicol.*, 2001, vol. 16, no. 6, pp. 498–505.

14. Cordeiro-Araújo M.K., Bittencourt-Oliveira M.C. Active release of microcystins controlled by an endogenous rhythm in the cyanobacterium *Microcystis aeruginosa*. *Phycological Research*, 2013, vol. 61, no. 1, pp. 1–6.
15. Reisner A.D. The common factors, empirically validated treatments, and recovery models of therapeutic change. *Psychol. Rec.*, 2005, vol. 55, pp. 377–399. DOI: 10.1007/BF03395517
16. Shaw G.R., Seawright A.A., Moore M.R., Lam P.K. Cylindrospermopsin, a cyanobacterial alkaloid: evaluation of its toxicologic activity. *Ther. Drug Monit.*, 2000, vol. 22, no. 1, pp. 89–92. DOI: 10.1097/00007691-200002000-00019
17. Humpage A.R., Falconer I.R. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.*, 2003, vol. 18, no. 2, pp. 94–103. DOI: 10.1002/tox.10104
18. Sukenik A., Reisner M., Carmeli S., Werman M. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in mice: Long-term exposure to low doses. *Environ. Toxicol.*, 2006, vol. 21, no. 6, pp. 575–582. DOI: 10.1002/tox.20220
19. Chernoff N., Hill D.J., Diggs D.L., Faison B.D., Francis B.M., Lang J.R., Larue M.M., Le T.-T. [et al.]. A critical review of the postulated role of the non-essential amino acid, β -N-methylamino-L-alanine, in neurodegenerative disease in humans. *J. Toxicol. Environ. Health B Crit. Rev.*, 2017, vol. 20, no. 4, pp. 1–47. DOI: 10.1080/10937404.2017.1297592
20. Merel S., Walker D., Chicana R., Snyder S., Baures E., Thomas O. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.*, 2013, vol. 59, pp. 303–327. DOI: 10.1016/j.envint.2013.06.013
21. Karlsson O., Roman E., Berg A.L., Brittebo E.B. Early hippocampal cell death, and late learning and memory deficits in rats exposed to the environmental toxin BMAA (beta-N-methylamino-L-alanine) during the neonatal period. *Behav. Brain Res.*, 2011, vol. 219, no. 2, pp. 310–320. DOI: 10.1016/j.bbr.2011.01.056
22. Geh E.N., Ghosh D., McKell M., de la Cruz A.A., Stelma G., Bernstein J.A. Identification of *Microcystis aeruginosa* Peptides Responsible for Allergic Sensitization and Characterization of Functional Interactions between Cyanobacterial Toxins and Immunogenic Peptides. *Environ. Health Perspect.*, 2015, vol. 123, no. 11, pp. 1159–1166. DOI: 10.1289/ehp.1409065
23. Bazin E., Mourot A., Humpage A.R., Fessard V. Genotoxicity of a freshwater cyanotoxin, cylindrospermopsin, in two human cell lines: Caco-2 and HepaRG. *Environ. Mol. Mutagen.*, 2010, vol. 51, no. 3, pp. 251–259. DOI: 10.1002/em.20539
24. Novak M., Hercog K., Žegura B. Assessment of the mutagenic and genotoxic activity of cyanobacterial toxin beta-N-methyl-amino-L-alanine in *Salmonella typhimurium*. *Toxicon*, 2016, vol. 118, pp. 134–140. DOI: 10.1016/j.toxicon.2016.04.047
25. Szlag D.C., Sinclair J.L., Southwell B., Westrick J.A. Cyanobacteria and Cyanotoxins Occurrence and Removal from Five High-Risk Conventional Treatment Drinking Water Plants. *Toxins (Basel)*, 2015, vol. 7, no. 6, pp. 2198–2220. DOI: 10.3390/toxins7062198
26. Kong Y., Lou I., Zhang Y., Lou C.U., Mok K.M. Using an online phycocyanin fluorescence probe for rapid monitoring of cyanobacteria in Macau freshwater reservoir. *Hydrobiologia*, vol. 741, pp. 33–49. DOI: 10.1007/s10750-013-1759-3
27. Hoagland P., Jin D., Beet A., Kirkpatrick B., Reich A., Ullmann S., Fleming L.E., Kirkpatrick G. The human health effects of Florida Red Tide (FRT) blooms: an expanded analysis. *Environ. Int.*, 2014, vol. 68, pp. 144–153. DOI: 10.1016/j.envint.2014.03.016
28. Chernoff N., Hill D.J., Chorus I., Diggs D.L., Huang H., King D., Lang J.R., Le T.-T. [et al.]. Cylindrospermopsin toxicity in mice following a 90-d oral exposure. *J. Toxicol. Environ. Health A*, 2018, vol. 81, no. 13, pp. 549–566. DOI: 10.1080/15287394.2018.1460787
29. Yang Y., Yu G., Chen Y., Jia N., Li R. Four decades of progress in cylindrospermopsin research: The ins and outs of a potent cyanotoxin. *J. Hazard. Mater.*, 2021, vol. 406, pp. 124653. DOI: 10.1016/j.jhazmat.2020.124653
30. Poniedziałek B., Rzymiski P., Kokociński M. Cylindrospermopsin: water-linked potential threat to human health in Europe. *Environ. Toxicol. Pharmacol.*, 2012, vol. 34, no. 3, pp. 651–660. DOI: 10.1016/j.etap.2012.08.005

31. Poniedziałek B., Rzymiski P., Kokociński M., Karczewski J. Toxic potencies of metabolite(s) of non-cylindrospermopsin producing *Cylindrospermopsis raciborskii* isolated from temperate zone in human white cells. *Chemosphere*, 2015, vol. 120, pp. 608–614. DOI: 10.1016/j.chemosphere.2014.09.067
32. Puerto M., Pichardo S., Jos Á., Gutiérrez-Praena D., Cameán A.M. Acute effects of pure Cylindrospermopsin on the activity and transcription of antioxidant enzymes in Tilapia (*Oreochromis niloticus*) exposed by gavage. *Ecotoxicology*, 2011, vol. 20, no. 8, pp. 1852–1860. DOI: 10.1007/s10646-011-0723-0
33. Žegura B., Gajski G., Štraser A., Garaj-Vrhovac V. Cylindrospermopsin induced DNA damage and alteration in the expression of genes involved in the response to DNA damage, apoptosis and oxidative stress. *Toxicon*, 2011, vol. 58, no. 6–7, pp. 471–479. DOI: 10.1016/j.toxicon.2011.08.005
34. Guzmán-Guillén R., Lomares I., Moreno I.M., Prieto A.I., Moyano R., Blanco A., Cameán A.M. Cylindrospermopsin induces neurotoxicity in tilapia fish (*Oreochromis niloticus*) exposed to *Aphanizomenon ovalisporum*. *Aquat. Toxicol.*, 2015, vol. 161, pp. 17–24. DOI: 10.1016/j.aquatox.2015.01.024
35. Rabelo J.C.S., Hanusch A.L., de Jesus L.W.O., Mesquita L.A., Franco F.C., Silva R.A., Sabóia-Morais S.M.T. DNA damage induced by cylindrospermopsin on different tissues of the biomonitor fish *Poecilia reticulata*. *Environ. Toxicol.*, 2021, vol. 36, no. 6, pp. 1125–1134. DOI: 10.1002/tox.23111
36. Hinojosa M.G., Prieto A.I., Muñoz-Castro C., Sánchez-Mico M.V., Vitorica J., Cameán A.M., Jos Á. Cytotoxicity and Effects on the Synapsis Induced by Pure Cylindrospermopsin in an E17 Embryonic Murine Primary Neuronal Culture in a Concentration- and Time-Dependent Manner. *Toxins (Basel)*, 2022, vol. 14, no. 3, pp. 175. DOI: 10.3390/toxins14030175

Sinitsyna O.O., Turbinsky V.V., Pushkareva M.V., Kuz N.V., Shiryayeva M.A., Masaltsev G.V., Safande V.V. Substantiating the maximum permissible concentration of cylindrospermopsin in water for drinking and household use to minimize human health risks. Health Risk Analysis, 2025, no. 2, pp. 16–29. DOI: 10.21668/health.risk/2025.2.02.eng

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