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CHEMICAL PHYSICS OF ECOLOGICAL PROCESSES

Chemical Safety of Aquatic Ecosystems: Biological Methods of Control

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Abstract—A comparative analysis of biological methods for monitoring the toxic properties of water in the context of ensuring the chemical safety of aquatic ecosystems is carried out. The analysis is based on the time scale of response to the toxic impact, possibility of identifying sources of chemical pollution, quantitative assessment of toxicity effects, and sensitivity. The method of bioassaying the genotoxic properties of native (without preliminary treatment) samples of natural and waste water by recording changes in the number of chromosomal aberrations in transplantable mammalian cell cultures is described in detail as the most informative. The sensitivity of the method makes it possible to detect the genotoxic effects of hydrophobic toxicants at a concentration of less than 1 μg/dm^3. Analysis of ecotoxicological situations in surface water bodies indicates that water-soluble compounds not controlled by hydrochemical monitoring can play a key role in the formation of toxic properties of aquatic media.

Keywords: pollutants, benzo[a]pyrene, polychlorinated organic compounds, reduced sulfur compounds, toxicity, control methods, bioassaying, cytogenetic effects

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INTRODUCTION

The water protection requirements in the Russian Federation are largely aimed at regulating the quality of the environment in controlled pollution components. The requirements are based on the concept of maximum permissible concentration (MPC) of chemicals in household-and-drinking and cultural-and-community water objects [1] or in fishery water bodies [2].

It is believed that, if the MPC regulations are complied with for all pollutants (PO), the chemical composition of natural water is harmless. Note, however, that, on the one hand, it is practically impossible to control the entire list of standardized chemicals in water, including more than 1000 names, whereas on the other hand, because of the possible presence in the water of chemicals for which MPC levels have not yet been established or whose MPC values are below the limit of their detection by modern methods of analytical control, any list cannot be exhaustive to guarantee the safety of drinking water for human health or the safety of the natural aquatic environment for aquatic ecosystems [3].

It is becoming increasingly obvious that an integrated approach is needed to adequately assess the quality of water, taking into account the whole set of pollution factors of the aquatic environment, both anthropogenic and natural. In fact, if natural water is regarded as a habitat of aquatic organisms, it should have a biological full-value, the ability to ensure the normal development and reproduction of the main components of the aquatic ecosystem [4].

The aim of the present work is to examine biological methods of water quality control in relation to the problem of chemical safety of water objects of the Russian Federation.

BIOLOGICAL INDICATORS OF THE STATE OF WATER ECOSYSTEMS

The most important biological indicators of the state of the aquatic ecosystem are its species biodiversity, balance between production–destruction processes, and fish productivity.

Species Biodiversity

To assess the degree of pollution of the aquatic environment, various kinds of indices of species diversity are used [5]. The values of the indices are rather subjective and are largely determined by the skills of the specialist.

The dependence of the values of the species diversity indices on the degree of pollution of the aquatic environment is typically nonlinear. Against the background of seasonal and interannual changes in the natural state of the aquatic ecosystem, the isolation of the anthropogenic contribution to the change in species biodiversity requires long-term observations; with a multifactorial anthropogenic impact, the identification of the source of negative changes is problematic.

Bioindication

A traditional method for assessing the state of aquatic ecosystems is the bioindication of water quality in terms of the presence of indicator organisms in the aquatic ecosystem in a controlled area (exposed to anthropogenic impact) and a "background" (reference) area wherein the natural state of the water body is not disturbed, with the biological characteristics of the ecosystem being close to natural [6].

An important role in assessing the balance of production–destruction processes is played by information on the state of phytoplankton [7]. The change in the species composition and abundance of microalgae under the influence of anthropogenic factors can have a significant effect on the productivity of the subsequent links in the trophic chain. One of the most important components of the aquatic ecosystem for bioindication is zooplankton [8], which is a staple food for fish.

The greatest attention in the ecological monitoring of Earth's surface waters is paid to macrozoobenthos, which is due to the widespread occurrence and large numbers of benthic organisms, their relatively large size, and a lifetime long enough to accumulate appreciable amounts of chemically resistant pollutants. To assess the level of pollution, various indexes of the status of macrozoobenthos are used, but there is no universal index that would reflect the level of pollution or the well-being of the aquatic environment.

Fish species are also used as indicator species, since the presence of toxic substances in water can be accompanied by physiological disturbances in the fish body. For example, the subject of numerous studies is the physiological response in populations of fish inhabiting areas polluted by pulp-and-paper industry wastewater [9]. This response consists, in particular, in an increase in the monooxygenase (mixed-function oxygenase, MFO) activity of the liver of fish. This effect (MFO-induction) is an early indicator of the potential sublethal toxicity of the aquatic environment. For a long time, it was thought that the toxic effect on fish was due to the presence of polychlorinated organic compounds (POCs) in pulp-and-paper industry wastewater, which are formed because of use of chlorine in cellulose bleaching.

Despite an extremely low content in the aqueous phase, due to the ability to be overaccumulated when moving through food chains, hazardous chemicals (HCs), such as polychlorinated dibenzodioxins (PCDD), dibenzofurans (PCDF), and phenols are concentrated in fish in appreciable amounts [10, 11]. Among chlorophenols, tetrachloroveratrol demonstrates a particularly high degree of bioconcentration (25000), which is formed during the biomethylation of tetrachloroguaiacol in the course of biological purification of wastewater from pulp-and-paper mills wastewater.

It has been established that PCDD/PCDF, accumulating in the fish body, can indeed produce MFO-induction effect, with an increased MFO activity persisting for a long time after fish has moved from contaminated water to clean water [12]. At the same time, chlorophenols, including tetrachloroguaiacol, do not cause MFO-induction effect [13], so fish with an elevated level of MFO activity living in wastewater discharge ponds of pulp-and-paper mills quickly recover to normal one when placed in clean water [9].

Thus, the MFO activity test is not specific for hydrophobic POCs. Accordingly, the MFO-induction effect in the zone of influence of wastewater from pulp-and-paper mills is not associated with exposure to dioxins or other hydrophobic toxicants.

The main drawback of bioindication methods is their post-factum detection of negative changes in the ecosystem, long after the impact. In addition, as in the assessment of biodiversity, a "background" water body with characteristics identical to the affected object is required; it is necessary to quantify the state of the population of species-indicators (indices) before the onset of the impact, with the values of the indices, dependent on the qualification of the hydrobiologist, being subjective.

Bioassaying

The methods of bioassaying are free of the above drawbacks. They are used to assess the toxicity of wastewater, individual chemicals, and mixtures thereof, water extracts from bottom sediments, wastes, etc. [14–19]. However, within the framework of the current water protection legislation of the Russian Federation, the use of bioassaying methods in the water quality control system is only recommendatory.

Luminous bacteria [20] and microalgae [21], as well as infusoria, typically *Tetrahymena pyriformis* [18] and *Paramecium caudatum* [22], are widely used in the rapid analysis of the toxicity of water samples and water extracts [20]. Using luminescent bacteria, microalgae, and infusoria as test organisms, offers the possibility of instrumental control of the toxicity of the aquatic environment.

Traditionally, *Daphnia magna Straus* is used in the toxicological control of aquatic environments and the classification of chemicals [23]; the criterion of acute toxicity is the survival rate of less than 50% of the individuals after 96 h of incubation, whereas the criterion of chronic toxicity is a decrease in fertility compared to

the control after 30 days. The widespread use of daphniae is due to the simplicity of their maintenance in the laboratory and the ease of determining toxicity from simple visual observations.

More sensitive and short-term bioassays are *Ceriodaphnia dubia* and *Ceriodaphnia affinis* [18, 24], which have a short life cycle. A test for chronic toxicity by fertility of the ceriodaphniae in three generations takes 5 days.

In the 1980–1990s, various methods of bioassaying were tested on a great number of large water bodies, small rivers, lakes, and reservoirs [25–27]. In terms of bioassaying indicators, almost all water bodies turned out to be toxic, but with a relatively low level of the controlled chemical pollution indicators. Note that a particularly high level of toxicity was observed in areas of water discharge from urban water treatment facilities. That there is a qualitative correlation between the toxicity level and the content of easily oxidizeable organic substances in water, as indicated by BOD_5 tests (5-day biological oxygen demand) suggests that the source of both toxic substances and easily oxidizeable organic compounds is municipal wastewater after its biological purification. At the same time, no correlation between the toxicity level and the content of water pollutants controlled by hydrochemical monitoring, was revealed [28].

Thus, the current system of water quality control, based on measuring the water content of a limited number of pollutants according to their MPCs, does not reflect the actual quality of the natural aquatic environment as the habitat of aquatic organisms.

Genotoxic Bioassays

For the bioassaying of natural and waste waters, the Ames test for mutagenic activity is widely used [29]. In this test, the strains of the salmonella bacteria (*Salmonella typhymurium*) specially designed by Ames are used.

A significant disadvantage of these microorganisms is that they lack enzymes capable of metabolic activation of xenobiotics; the impact of only direct mutagens, compounds directly interacting with the genetic material of the cell, is recorded. Most hydrophobic xenobiotics acquire mutagenic properties after having been metabolically activated in the monooxygenase system of cytochrome P-450.

A special place in bioassaying is occupied by cytogenetic methods based on assessing chromosomal changes in somatic cells of mammals [30]. In general, with a proper choice of test objects and test systems, bioassaying is the most suitable method for monitoring the current state of toxic pollution of the natural aquatic environment.

Below are the results of our comparative toxicological studies of natural and waste waters and of multicomponent industrial effluents performed using various test objects and model test systems.

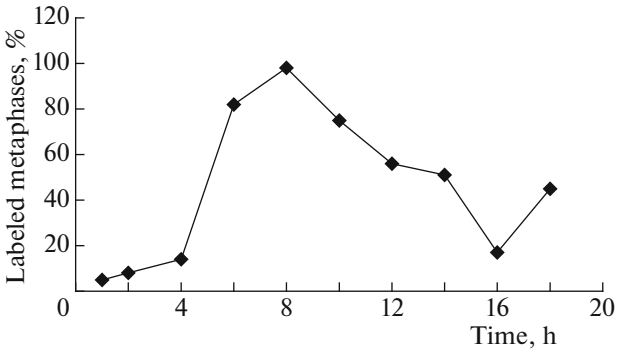

Fig. 1. Kinetics of the appearance of labeled metaphases after labeling cells CH 237 with ^{3}H-thymidine [32].

OBJECTS AND METHODS

The test objects were *Benekea harveyi* bacteria, a culture of green algae *Chlorella vulgaris*, infusoria *Tetrahymena pyriformis*, and ceriodaphnia *Ceriodaphonia diodia*. In addition, the Ames test, a modified test system based on modeling the peroxidation of lipids (LPO) [31], unsaturated residues of fatty acids (phospholipids), determining the stability of cellular biomembranes, as well as a cytogenetic method, using transplantable Chinese hamster cell cultures, ovarian cells (CHO) and lung cells (CH 237), as test objects [32].

Lecithin, a phospholipid of chicken yolk, was used as a model of the cell membrane. The product of the LPO reaction is malonic dialdehyde (MDA). Accordingly, from the amount of MDA formed, the rate of LPO reaction in the natural water sample in comparison with the control was determined.

To characterize the genotoxicity of the water sample, the total number of chromosomal aberrations per 100 cells and the mutagenic activity coefficient, calculated as the deviation of the frequency of aberrations in the induced sample from the frequency of aberrations in the control, were determined. An experimental substantiation of the cell cycle stage optimal for bioassaying was obtained based on the direct genotoxic effect of a number of chemical substances, such as sodium nitrite, hydrogen peroxide, 3,3',4,4'-tetrachlorobiphenyl (PCB 77), and benzo[a]pyrene (BP), both in the absence and in the presence of the metabolic activation system [32].

For all the substances studied, with different physicochemical and toxic properties, the S-stage of replicative DNA synthesis 8–10 h after exposure, turned out to be the most sensitive (figure). The organic compounds were extracted with a 1 : 1 diethyl ether–hexane mixture upon acidification of the initial water sample to pH 2–3. When analyzing the genotoxicity of organic pollutants extracted with organic solvents, the dried extracts were dissolved in methanol and mixed with physiological saline (aqueous sodium chloride

Table 1. Comparative analysis of bioassaying methods for diluted solutions of "black liquor" of the pulp-and-paper industry

Test object	Minimum concentrations at which a toxic effect appears, % black liquor	Analysis duration
Fertility of ceriodaphniae	0.001–0.002	5 days
Level of LPO (native water samples)	0.002 (stimulation) 0.05 (suppression)	1 h
Activation of fish liver oxygenaseis	0.02	7 days
Growth rate of infusoria cell	0.05	24 h
Change in luminescence of luminous bacteria	0.5	5.5 h
Survival rate of Daphnia magna	0.5	4 days
Ames test (salmonella/microsome)	Strain T98 0.1*, 0.067** strain T100 0.1*, 0.13**	2 days

* Without metabolic activation.
** In the presence of the cytochrome P-450 system.

solution). The control contained the same amount of methanol (≤10%), but without extract additives.

The mutagenic activity of the organic extracts was assessed both with and without metabolic activation. In this connection, a fraction of enzymes capable of metabolizing xenobiotics, the cytochrome P-450 enzyme complex (S9 fraction from rat liver), was added to the water sample to be analyzed. In parallel, an experiment in the absence the metabolic activation system was carried out. The test lasted for 2 days. To assess the contribution to the cytogenetic effects of water-soluble components, the suspended particles of mineral and biological origin were removed from the water samples by filtering them through membrane filters with a pore diameter of 0.45 and 0.22 μm.

RESULTS AND ITS DISCUSSION

Comparative Analysis of Bioassaying Methods

For a comparative analysis of various methods of bioassaying, we selected dilute black liquor solutions formed during the kraft process as a model multicomponent toxicant (Table 1) [33]. As follows from the data presented in Table 1, the most short-term test objects are luminescent bacteria and the test system is LPO; the most sensitive are ceriodaphnia and LPO.

Cytogenetic Methods for Bioassaying the Quality of Natural and Waste Water

Although there is an agreed protocol of short-term tests using mammalian cells for assessing the genotoxicity of individual chemicals in vitro [34], studies on the applicability of cytogenetic methods to assessing the genotoxicity of natural and waste water samples have not been conducted. We conducted an analysis of samples of natural and waste water, as well as water extracts from soils, for genotoxicity [32]. It is shown that the proposed method can be applied to estimating the genotoxicity of native samples of natural and waste water, without their preliminary treatment or sterilization, in other words, without loss of the initial properties of the analyzed water objects.

The contribution of water-soluble ingredients to the toxicity of the natural aquatic environment is usually not regarded as significant; however, our experience in analyzing critical ecotoxicological situations in natural water bodies suggests that just water-soluble compounds can play a key role in the formation of genotoxic properties of the natural aquatic environment [32]. As follows from the data presented in Table 2, the contribution of water-soluble components of both natural water and wastewater is at least 50%.

Native samples of natural and waste water demonstrate much greater genotoxicity than organic-solvent extracts from the same water samples. In a number of cases, the contribution to the genotoxicity of water-insoluble organic compounds extracted with organic solvents was less than 2%.

The data of Table 3 suggest that only a weak qualitative correlation is observed between the mutagenic activity measured in the Ames test and the genotoxicity measured by the proposed method. Moreover, there are examples of genotoxicity not being observed for water samples with a relatively high mutagenic activity.

Note also that no correlation was revealed between the Ames test results and the genotoxicity of hexane extracts from drinking water samples taken from

Table 2. Comparison of the cytogenetic effect of water samples filtered through a membrane filter (∅ 0.45 μm) (1998)

Analyzed sample of water	Sample type	Number of treated cells	Damaged cells, %	Chromosomal aberrations per 100 cells		
				fragments	exchange	total
Protva River (spring)	Native	200	5.0 ± 1.5*	3	2	5.0 ± 1.6*
	Filtered	200	4.5 ± 1.5*	2.5	2	4.5 ± 1.5*
Protva River (summer, sample 1)	Native**	100	25 ± 4.3*	20	14	**34 ± 5.8***
	Filtered	100	13 ± 3.4*	11	8	19 ± 4.4*
Protva River (Summer, sample 2)	Native***	100	28 ± 4.5*	22	13	**35 ± 5.9***
	Filtered	100	16 ± 3.7*	10	11	21 ± 4.6*
Effluent water, sample 1 (Obninsk)	Native	100	24 ± 4.3*	16	14	**30 ± 5.5***
	Filtered	100	11 ± 3.1*	9	6	15 ± 3.9*
Spontaneous level		100	1 ± 1.0	1	0	1 ± 1.0
Physiological saline		100	1 ± 1.0	1	1	2 ± 1.4

* Significant difference from the spontaneous level at $P < 0.05$.
** Water was analyzed 1 h after sampling.
*** Samples were stored frozen and tested 4 days after collection.

Table 3. Comparative results of evaluation of mutagenic activity of natural and waste water in the Ames test and frequency of chromosome aberrations in Chinese hamster cells

Type of sample	Value of K* in the test						Cytogenetic test	
	TA97		TA98		TA100			
	(−S9)	(+S9)	(−S9)	(+S9)	(−S9)	(+S9)	Ma**	K_{Ma}***
Protva River (spring)	Unch.	Unch.	+	–	+	–	5.0 ± 1.6	+
Protva River (Summer, sample 2)	Unch.	Unch.	–	++	+	–	**34 ± 5.8**	–
Tsymlyansk reservoir	Unch.	Unch.	++	++	+	–	2 ± 1.4	++
Reservoir-cooler of Rostov NPP	Unch.	Unch.	+	++	+	–	6 ± 2.4	+
Zhizdra River	Acute toxicity	0.88	5.69	1.06	1.0	0.98	7.0 ± 1.9	5.69
Reseta River	7.69	1.17	10.8	1.85	1.0	1.34	7.3 ± 2.2	10.8
Effluent water, sample 2 (Obninsk)	Unch.	Unch.	–	+	+	+	**30 ± 5.5**	–

The "−" sign corresponds to the absence of a toxic effect in the Ames test, the "+" sign shows the presence of toxicity, the "++" sign shows increased toxicity; unch. stands for unchanged; TA97, TA98, and TA100 are strains of microorganisms used in the Ames test.
* The multiple of the control level in the Ames test.
** The number of chromosomal aberrations per 100 cells.
*** The multiple of the control level in the cytogenetic test.

underground sources of water supply in the settlements of the Kaluga region that were exposed to radioactive contamination after the Chernobyl accident. As follows from the data given in Table 4, there are examples where, in spite of the absence of Ames mutagenic activity, a high level of genotoxicity of the same sample was recorded. In samples with a high content of hydrophobic toxicants (BP and PCB), an increased level of genotoxicity was detected.

GENOTOXIC ESTIMATION OF HCs

Using the genotoxic method of bioassaying based on Chinese hamster cells as a test object, we have studied the toxic properties of a number of hydrophobic HCs.

Benzo[a]pyrene. The average daily amount of BP entering the body of a person with water is only a thousandth of the amount coming from air, food, or cigarette smoking, and is estimated at ~0.0011 μg [35].

Table 4. Results of bioassaying of hexane extracts of drinking water from underground sources taken in settlements of the Kaluga region in 1998

Place of sampling	Content of pollutants, ng/dm^3		Genotoxicity				Ames test (salmonella/microsome) in the presence of S9 fractions* (analyzed sample/control)		
			CH lines 237		CH lines 237 + S9				
	BP	Σ PCB	anomalous cells, %	chromosomal aberrations per 100 cells	anomalous cells, %	chromosomal aberrations per 100 cells	TA 97	TA 98	TA 100
Control	–	–	2	2	1	1	–	–	–
Zikeevo	0.3	23.3	4.5	8.0	22.5	38.5	1.00	1.21	2.79
Sudimir	9.4	8.0	5.0	8.0	Acute toxicity	Acute toxicity	0.92	1.26	3.40
Kollektivizator	–	–	7.5	9.0	19.0	26.0	0.90	1.13	0.89
Muzhitino	1.0	17.9	4.0	7.0	16.0	23.0	1.00	1.14	1.02

* All four extracts in the Ames test without metabolic activation (–S9) exhibited acute toxicity with respect to *Salmonella typhimurium*.

Table 5. Cytogenetic effect of different doses of BP with two hours exposure to CH 237 cells cultured in physiological solution without microsomal metabolic system (cell fixation 10 h after exposure)

Concentration of BP in the aqueous medium, μg/dm^3	Metaphases studied	Damaged cells, %	Chromosomal aberrations per 100 cells		
			fragments	exchanges	total
Spontaneous level	200	1.0 ± 0.7	0.5	0.5	1.0 ± 0.7
0.01	200	5.0 ± 1.5	6.0	2.0	8.0 ± 2.0
0.1	200	10.0 ± 2.1	9.5	3.5	13.0 ± 2.5
1.0	100	12.0 ± 3.2	12.0	1.0	13.0 ± 3.6
10	100	14.0 ± 3.5	16.0	0.0	16.0 ± 4.0
2500	100	7.0 ± 2.6	12.0	6.0	18.0 ± 4.2

Benzo[a]pyrene is considered to be exclusively a promutagenic agent, which exhibits genotoxic properties only after its metabolic activation. However, as regards Chinese hamster cells, we discovered a genotoxic effect of ultra-low concentrations of BP as a mutagenic agent of direct action (Table 5).

Polychlorinated HCs. Of the large variety of HCs, the greatest attention, as potential toxicants, is given to polychlorinated organic compounds, such as polychlorinated phenols, biphenyls, dibenzo-*p*-dioxins, and dibenzo-*p*-furans. The distinctive features of HCs are their high chemical resistance in the environment, low solubility in water and, on the contrary, high solubility in the nonpolar phase, which leads to the effects of bioaccumulation of HCs in fat of aquatic animals and fish.

We have studied the genotoxic properties of polychlorinated biphenyls (PCBs), the MPC of which according to the hygienic standards in household-and-drinking and cultural-and-community water is 1 μg/dm^3. As in the case of dioxins, PCBs enter the body of humans and animals mainly with food; with drinking water, only 0.001% of PCBs enter the human body [35].

When Chinese hamster cells are used as test-objects for tetrachlorobiphenyl (PCB-77), usually considered as a direct toxicant starting at a concentration of 10 ng/dm^3, a clear dose–effect relationship is observed (Table 6).

WATER-SOLUBLE HCs: REDUCED SULFUR CONPOUNDS

Numerous data testify that the natural aquatic environment is contaminated by municipal and industrial wastewaters after their biological purification [36]. At the same time, no correlation between the toxicity level and the content of the pollutants under regulatory requirements was detected [37].

Along with volatile sulfur compounds, effluent waters contain water–soluble reduced sulfur compounds, which have no pronounced analytical properties and remain unoxidized during biological treatment

Table 6. Cytogenetic effects of tetrachlorobiphenyl PCB 77 on CH 237 cells

Test solution, concentration of PCB-77 in μg/dm^3	Damaged cells, %	Chromosomal aberrations per 100 cells		
		fragments	exchanges	total
Spontaneous level	2 ± 1.4	2	0	2 ± 1.4
Physiological saline (0.9% NaCl)	5 ± 2.2	4	1	5 ± 2.2
0.01	13 ± 3.4	11	6	17 ± 4.1
0.03	17 ± 3.8	17	7	24 ± 4.9
0.1	28 ± 4.5	34	10	44 ± 6.6
0.3	31 ± 4.6	38	14	52 ± 7.2
1.0	38 ± 4.9	48	22	70 ± 8.4
3.0	45 ± 5.0	62	34	96 ± 9.8

of wastewater. These compounds are inert with respect to O_2, but effectively react with H_2O_2 [38]. When entering the natural waters, these substances affect the oxidation–reduction processes involving natural hydrogen peroxide, which leads to the effect of "redox-toxification" of the natural aquatic environment [39].

Analytical control of water-soluble reduced sulfur compounds that give rise to the toxic properties of the natural aquatic environment is complicated by the fact that their concentrations in water are too low, 10^{-7}–10^{-6} M. The most accessible method of analyzing reduced sulfur compounds is to titrate with small additives of hydrogen peroxide [38].

The presence of reducing substances effectively interacting with hydrogen peroxide in the aquatic environment leads to the death of aquatic organisms with intensive water exchange, in particular, fish larvae in the early stages of their development [40]. By the example of wastewater of pulp and paper plants containing a large number of known (controlled) toxic substances (including CHs) and showing both acute and chronic toxicity with respect to different test organisms [41], we have shown that the main contribution to the toxicity of effluent water comes from water-soluble reduced sulfur compounds capable of effectively interacting with H_2O_2.

Treatment of pulp-and-paper industry wastewater or black liquor solutions, as well as treatment of communal and industrial effluents, with hydrogen peroxide in stoichiometric amounts in relation to the content of H_2O_2-titrable chemicals in them, leads to their almost complete detoxification, including the MFO-induction effect [38]. The mechanism of the toxic action of reduced sulfur compounds can be associated both with the destruction of hydrogen peroxide, which plays an important role in intracellular oxygen-dependent processes [42], and with the conversion of trace elements into a biologically inaccessible form, in particular copper ions, which play a key role in the formation of respiratory enzymes [43].

CONCLUSIONS

(1) The problem of the chemical safety of surface water bodies as sources of drinking water supply is closely related to the problem of the biological full-value of the aquatic environment as the habitat of aquatic organisms.

(2) The current water quality control system, based on measuring the contents of a limited number of pollutants and comparing them with the MPC, does not ensure the chemical safety of the natural aquatic environment.

(3) The most appropriate methods of biological control of the chemical safety of the natural aquatic environment are methods of bioassaying by means of test organisms of different trophic levels and model test systems.

(4) Test-organisms providing the most informative, rapid, and sensitive in analysis bioassaying methods are luminescent bacteria, microalgae, protozoa, and ceriodaphaniae, whereas the most effective test system is the peroxidation of liposome lipids.

(5) Application of bioassaying methods has demonstrated that the discharge of wastewater containing reducing chemicals capable of effectively interacting with hydrogen peroxide but resistant to the oxidative action of molecular oxygen results in the toxification of the natural aquatic environment.

(6) Cytogenetic methods based on the evaluation of chromosomal changes in mammalian somatic cells are most promising for the early recognition of toxic effects arising from the chemical contamination of the aquatic environment and for the prediction of early and late effects.

(7) With the use of transplantable cultures of Chinese hamster cells, it has been established that the main contribution to the genotoxicity of natural and waste water comes from uncontrolled water-soluble compounds, whereas the contribution from low-water-soluble organic compounds extractable with organic solvents is relatively small.

(8) Among the water-soluble compounds, reduced sulfur compounds play a key role in the formation of the toxic properties of natural and waste water.

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