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Assessment of the Physiological Adequacy of Drinking Water by Means of Biotesting

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Abstract

The possibilities of new approaches to assessing the physiological adequacy of drinking water using biotesting methods are disclosed. Biotesting of waters, both depleted and enriched with potassium, sodium, calcium and magnesium, was carried out according to the indicators of acute water toxicity on freshwater fish *Brachydanio rerio*, genotoxicity on its blood cells, and chronic toxicity on *Ceriodaphnia affinis*. It was found that water samples with the optimal content of K^+, Na^+, Ca^{2+}, Mg^{2+} do not exhibit toxicity, while in the samples in which the content of these elements is outside the range of optimal concentrations, chronic toxic and genotoxic effects are found.

Keywords: drinking water, physiological adequacy, biotesting

INTRODUCTION

The physiological usefulness of drinking water is determined by the adequate mineral composition meeting the needs of a human organism and relies on the reasonableness of taking into account not only maximum permissible concentrations in water but also minimal necessary levels of a number of biogenic elements.

The criterion of physiological usefulness of water was introduced for the first time in 1912 by an outstanding Russian chemist and biologist N. K. Koltsov [1]. He proposed to term the set of cations and anions physiologically adequate to human organisms as physiologically useful; he insisted that water should become a valuable source of macro- and microelements for a human organism. The fact that drinking water makes a substantial contribution to the vital activities of humans has been confirmed by means of science and recognized by the World Health Organization, in spite of the statement that water cannot be considered as the basic source of essential elements for humans [2–7].

Epidemiological studies that have been carried out in different countries during the recent 50 years showed that there is a significant correlation between an increased number of cardiovascular diseases followed by lethal outcomes and the consumption of low-hardness water [8]. This regularity can be followed in a very clear manner by comparing the physiological usefulness of low-hardness water with hard water rich in Mg^{2+}. The consumption of water with low Ca^{2+} content may lead to increased risk of bone fractures in children, neurodegenerative changes, preterm birth and too low body mass of newborn babies, some kinds of cancer [3]. According to [9], an optimal balance between Ca^{2+} and Mg^{2+} in drinking water may play a potentially protective part at the early stages of the development of endothelial dysfunction in children and teenagers.

The observed trends are explained by the fact that the income of necessary macro- and microel-

TABLE 1

Parameters of the physiological adequacy of drinking water

Parameter	Optimal content, within the range, mg/dm^3
Ca^{2+}	25–75
Mg^{2+}	10–50
Na^+	2–20
K^+	2–20

ements in sufficient amounts is not achieved through food due to various reasons (in particular, because of the imperfectness of the modern technologies of food preparation) [2, 8]. Food products cannot compensate the lack of Ca^{2+} and Mg^{2+} if drinking water is depleted of these elements.

In the case of acute deficit of micro- and macroelements, even relatively insignificant income of these elements with drinking water may play an essential protective role, especially if we take into account the fact that these elements are present in water in the form of free ions and can be more readily absorbed from water than from food, where they are bound in various compounds. Ionized minerals of drinking water are characterized by high physiological activity, biological availability and absorption [2, 8, 10, 11].

It was stressed in [2] that drinking water may compensate the lack of necessary macro- and microelements that arises as a consequence of incorrect nutrition, it may promote prophylactics of arterial hypertension, cardiomyopathy and other diseases, and promote recovering after intense physical load, after work under the conditions of high ambient temperature.

Analysis of scientific publications and safety regulations showed that the problem of the investigation of the adequacy of drinking water with respect to the concentrations of the main macro- and microelements and their effect on health is extremely relevant. However, in the international practice (for example, in the countries of the European Community) the approaches to normalization of macro- and microelements in drinking water from the viewpoint of its physiological adequacy can be traced only in scientific literature rather than in the actual regulations related to drinking water [2, 11].

The term physiological adequacy of drinking water was introduced in regulations for the first time in the Russian Federation in 2002 by adopting sanitary rules and norms for bottled water – SanPiN 2.1.4.1116–02. Sanitary norms and regulations "Requirements to the physiological adequacy of drinking water" were adopted in the Republic of Belarus in 2012 [12].

The requirement for the physiological usefulness of drinking water was introduced into the state standard related to drinking water quality for the first time in the international practice in Ukraine in DSTU 7525:2014 "Drinking water. Requirements and methods of quality control", which was developed at the Dumansky Institute of Colloid and Water Chemistry, NAS of Ukraine (ICWC) (Table 1) [13].

Na^+, K^+, Mg^{2+}, Ca^{2+} are dominant vital cations of drinking water. Their normal content in human blood plasma is: Na^+ – 136–144 mmol/L, K^+ – 3.3–5.0 mmol/L, Mg^{2+} – 0.74–1.23 mmol/L, ionized Ca^{2+} – 1.1–1.4 mmol/L, total Ca – 2.1–2.6 mmol/L. Variations of the concentrations of elements in blood plasma above or below the indicated ranges cause hypo-/hyper- natraemia, potassemia, magnesemia and calcemia [14, 15].

K^+ is the major intracellular cation (98 % of its total amount is present inside the cells, only 2 % – out of cells) playing the dominant part in many physiological processes: cell fission, synthesis of proteins, enzymatic activity, regulation of volume and acid-base equilibrium in cells, formation of the electric potential of cell membranes, *etc.* [10]. A specific feature of K^+ is its ability to stimulate enhanced excretion of water by kidneys from the organism in the case of increased ionization.

The major fraction of Na^+ in an organism (about 65 %) is present in the extracellular fluid, only about 8 % is in cells, and the rest fraction of Na^+ (about 25–30 %) is deposited in bone tissue [15].

Na^+ and K^+ play the leading physiological role in sustaining the volume of extracellular fluid, in intracellular osmolarity, regulation of acid-base balance, generation of transmembrane electrochemical gradients, active transport of various substances (in particular saccharides and amino acids) through membranes, the transmission of nervous pulses, muscular contractions and other processes providing cell vitality. In addition, sodium-potassium equilibrium provides adequate synthesis of proteins and carbohydrate exchange. In these processes, K^+ and Na^+ act simultaneously moving against the gradients of their concentrations due to the potassium-sodium pump located in the cell membranes, with its operation provided by the presence of a special enzyme – sodium-potassium adenosinetriphosphatase [16].

Mg^{2+} is the second widespread intracellular cation [3]. Its content in an adult organism is 20–28 g (60–65 % is present in the skeleton, and 1 % – in extracellular fluid). Mg^{2+} is a co-factor for more than 300 enzymatic reactions, it is an active participant of the metabolism of carbohydrates, lipids, proteins, nucleic acids, and it plays an important part in the mineralization and development of the skeleton, in cell permeability, and in neuromuscular conduction. The lack of Mg^{2+} causes an increase in neuromuscular excitability and enhances K^+ excretion with kidneys, causes an increase in the risk of cardiovascular diseases and type II diabetes.

Ca^{2+} is the most widespread alkaline earth metal [3]. Its total content in an organism of an adult person is about 1.2 kg (99 % is concentrated in bones, and 1 % is present in extracellular fluids and cell membranes). In addition to the basic structural component of the skeleton, Ca^{2+} participates in the regulation of the activity of multiple enzymes and hormonal reactions, blood clotting, the transmission of nervous impulses, traction and relaxation of muscles (including the normal rhythm of the heart), vessel traction and many other physiological processes. The lack of Ca^{2+} in an organism may provoke disorders in blood clotting and cause an increase in the risk of bone fractures. In general, the consumption of low-calcium water causes an increase in the negative effects of a series of pathological disorders accompanying the course of such nosological forms as rickets, hypertension, coronary heart disease and stroke.

Chronic intake of demineralized water containing <10 mg/dm^3 Na^+, <15 mg/dm^3 Ca^{2+}, <5 mg/dm^3 Mg^{2+}, <3 mg/dm^3 K^+ and less than 50 mg/dm^3 total hardness may change the ion and molecular composition of extra- and intracellular fluids, initiate the deficit of Na^+, K^+, Mg^{2+}, Ca^{2+} in the organism [15]. In addition, the consumption of water with low Na^+, K^+, Mg^{2+}, Ca^{2+} content may affect the tonicity, osmolality of fluids in the organism and therefore the effective cell volume, blood pressure, thus causing the osmotic stress. Investigations of several cell lines of mammals allow assuming that the osmotic stress, independently of its origin, may cause DNA damage, leading to chromosome aberrations.

According to [17], since 1998 till 2007 the number of colorectal cancer cases increased by a factor of 2 in the countries of the Persian Gulf, where the population drinks desalted water. Analysis of the data available by 2012 for nine countries of Western Asia (with three of them, namely Saudi Arabia, Kuwait, and Qatar are among the top ten countries in the world in the production of desalted water) revealed a wide occurrence of colorectal cancer, stomach, esophagus, oral cavity, gulp cancer among the men and women of that region [18, 19]. The fact that along with an increase in the consumption of desalted water in the countries of the Persian Gulf, the rate of oncological diseases affecting the organs that are most probably in direct contact with demineralized water or are involved in its metabolic transport and excretion is not chancy It is reported that in the countries of West Europe and Northern America, where the consumption of desalted water is not dominating, the most characteristic diseases are benign prostatic hyperplasia, lung cancer, and cervical cancer [20]. Another essential factor is a substantial increase in the number of neoplasms in the children living in the countries of the Persian Gulf, who drink desalted water since birth.

Thus, the available information points to the fact that permanent consumption of artificially desalted water with low Na^+, K^+, Mg^{2+}, Ca^{2+} content brings potential risk for the health of the population.

The above-described review of literature sources provides evidence that large-scale studies are carried out into the physiological adequacy of drinking water by comparing the data on water composition and the rate of diseases of the population. Evaluation of the theoretical and experimental studies of homeostasis of elements in the organisms of higher animals and humans based on the analysis of physiological responses under the conditions of long-term dose-dependent actions (loads) by the above-listed elements is extremely important in this respect.

In the present work, the physiological adequacy of drinking water is studied by means of biotesting.

EXPERIMENTAL

Artesian water from the pump room of Kiev (sample 1), the same water treated with the ion-exchange resin in Ca^{2+}-form (sample 2) and in Na^+-form (samples 3, 4) were used in the investigation. The parameters of the chemical composition of waters under investigation were determined using standard methods [13]. Biotesting for acute lethality, gene- and chronic toxicity

was carried out according to procedures [21–23] described in detail below. Test organisms were kept, reproduced, grown and fed under the conditions of the Laboratory of Biomarkers and Water Biotesting at ICWC according to standards DSTU 4075-2001, DSTU 4174:2003.

Determination of acute lethal toxicity

The procedure for the determination of acute lethal toxicity for fresh-water fish *Brachydanio rerio* [21] is based on the comparison of fish survival in water under test and in reference water. The survival parameter is the average number of test organisms that survived in the water under test within a definite time. Test organisms were adult *Brachydanio rerio* individuals; this species is widely used in international and national standard water biotesting procedures. Biotesting was carried out under illumination with scattered light with natural day and night periods, and O_2 concentration in water was not less than 4 mg/dm^3 at a temperature of 23±1 °C.

Glass vessels were filled with 2–3 dm^3 of reference and test water samples; then 10 fish individuals were placed in each vessel. Water was not aerated during tests; fish feeding was not carried out. Every day (for 96 h) the number of surviving fish was determined in each vessel. Dead individuals with no signs of moving or breathing for 5 min after touching them with a glass stick were removed. If 50 % of fish or more die within any time interval taken into account (acute lethal toxicity), biotesting is stopped. If the number of dead fish (n) is within the range of 10 % < n < 50 %, the water sample under test has a chronic toxic effect on test organisms. The percentage of dead fish in the reference sample should not exceed 10 % within 96 h

Procedure for the determination of water genotoxicity

The procedure for the determination of water genotoxicity for the blood cells of fresh-water Brachydanio rerio fish [22] is based on blood sampling from the individuals surviving for 96 h in the above-described experiments aimed at the studies of acute lethal toxicity. The preparations of fish blood were analyzed using an Axio Scope optical microscope (Carl Zeiss, Germany) with a ×1000 magnification. To determine genotoxicity, the number of erythrocytes with disorders (micro-nuclei and double nuclei) per 3000 cells was counted in four selected regions of blood smears. The genetic nuclear reaction for drinking water should not exceed 0.33 ‰.

Procedure for the determination of chronic toxicity of water

For biotesting aimed at the determination of chronic toxicity for *Ceriodaphnia affinis* [23], we used 20 vessels (10 + 10) with 15 cm^3 of reference and test water samples, respectively. The concentration of O_2 dissolved in water should be not less than 5.0 mg/dm^3. If this parameter was lower, water was aerated preliminarily with the help of a microcompressor. Water was not aerated during biotesting. One young ceriodaphnia was put into each vessel with the help of a plastic pipette 2.0 mm in diameter. Survived individuals were counted after 1, 6, 24 and 48 h. Ceriodaphnia were not fed during this time. The individuals were considered surviving if they were freely moving in water thickness or up from the vessel bottom not later than within 15 s after slight rocking. If 50 % or more of the individuals die in test water during any time interval (acute toxicity), biotesting is stopped. If the number of dead ceriodaphnia (n) is within the range of 10 % < n < 50 %, test water has a chronic toxic action on test organisms.

Biotesting is carried out in a climatostat or box where optimal temperature and light modes are provided. Biotesting results are considered significant if the percentage of dead ceriodaphnia in the reference does not exceed 10 % during the whole observation time, and the concentration of O_2 dissolved in water is not less than 2 mg/dm^3 after biotesting is over.

RESULTS AND DISCUSSION

The data on the chemical composition of the studied water samples are presented in Table 2, and the results of biotesting are shown in Table 3.

Analysis of the obtained data allows us to conclude that according to DSTU 4075-2001, water samples 1, 2 and 3 did not exhibit chronic and acute toxicity, while sample 4 showed chronic toxicity – 20 % (see Table 3).

According to DSTU 7387:2013, genotoxic effect for erythrocytes was not observed for fish blood cells in water samples 1 and 2, while samples 3 and 4 revealed genotoxic effect (0.66 ‰).

With ceriodaphnia, no acute and chronic toxicity was established for water samples 1 and 2 (according to DSTU 7525:2014), while chronic toxicity was revealed for water samples 3 and 4 – 30 %.

So, it follows from the data presented (see Tables 2, 3) that water samples 1 and 2 demonstrated a negative result in the test for biotoxicity. In these samples, the concentrations of K^+ (3.1–3.3 mg/dm^3), Na^+ (11.2–11.9 mg/dm^3), Mg^{2+} (22.8–27.6 mg/dm^3) and Ca^{2+} (38.0–50.0 mg/dm^3) are within the optimal range of the concentrations of these elements in physiologically useful water (see Table 1). Water samples 3 and 4 in which the concentrations of the indicated elements are outside the optimal concentration ranges exhibited chronic toxicity and genotoxic effect. Further experiments are necessary for detailed investigation of the observed effects of the indicated ions.

CONCLUSION

Drinking water does not serve as the basic source of vitally important elements for humans, however, a substantial contribution from water

TABLE 2

Chemical composition of water samples under biotesting

Parameter	Sample				DSTU 7525:2014 Requirements [13]
	1	2	3	4	
Turbidity, mg/dm^3	0.5	0.3	0.3	0.3	0.5
pH	7.3	7.4	7.6	7.6	6.5–8.5
Dry residue, mg/dm^3	319	307	316	290	1000
Hardness, mg-equiv/dm^3	4.2	4.4	0.75	0.65	7.0
Permanganate oxidizability, mgO_2/dm^3	4.5	4.5	3.2	2.2	5.0
Total alkalinity, mg-equiv/dm^3	2.9	3.1	2.6	2.5	6.5
Content, mg/dm^3:					
Aluminium	0.01	0.01	0.012	0.010	0.2
Hydrocarbonates	177	189	159	153	–[a]
Iron (total)	0.45	0.035	0.094	0.079	0.2
Calcium	**38.0**	**50.0**	**10.0**	**9.0**	130 (25–75)
Magnesium	**27.6**	**22.8**	**3.0**	**2.4**	80 (10–50)
Potassium	**3.1**	**3.3**	**1.5**	**1.84**	20 (2–20)
Sodium	**11.9**	**11.2**	**110.0**	**110.0**	200 (2–20)
Manganese	0.012	0.01	0.007	0.002	0.05
Zinc	0.73	<0.08	<0.08	<0.08	1
Fluorides	0.055	0.055	<0.05	<0.05	0.7–1.5
Sulphates	60.0	60.0	72.0	62.4	250
Chlorides	23.0	17.8	39.0	39.0	250

Note. The optimal content of some inorganic components in additionally purified drinking water is shown in parentheses.

[a] absent.

TABLE 3

Results of water biotesting

Parameter	Water sample				DSTU 7525:2014 requirements [13]	Regulations
	1	2	3	4		
Acute lethal toxicity of water for fresh-water fish *Brachydanio rerio* (*Hamilton-Buchanan*), %	0	0	10	20	10	DSTU 4075-2001
Genotoxicity for blood cells of *B. rerio* fish, ‰	0	0	0.66	0.66	0.33	DSTU 7387:2013
Chronic toxicity for *Ceriodaphnia affinis*, %	10	10	30	30	10	DSTU 4174:2003

into the intake of these elements has been confirmed. The consumption of drinking water depleted in Ca^{2+} and Mg^{2+} undoubtedly causes an increase in the mortality of the population from cardiovascular diseases. At present, there is no proof confirming that low-mineralized water itself can cause oncological diseases directly from the viewpoint of induction of genetic mutations. However, the consumption of demineralized drinking water causes changes in vessel tonus and osmolality promoting compensation of osmoregulatory adaptive protection mechanisms intended for maintaining cell volume, ion transport, protein structure and other cell parameters within the established ranges. Modern epidemiological studies show that chronic consumption of demineralized water with a very low content of mineral substances may create osmotic imbalance because of the loss of Na^+, K^+, Mg^{2+}, Ca^{2+}, in particular with urine and sweat. It was reported many times that electrolyte imbalance marked by hypoelementoses is the most frequent accompanying pathology in oncological patients. Children are especially prone to the health risk from the consumption of demineralized water.

Experimental studies provided convincing confirmation of the possibility to use the expanded biotesting procedure to determine the physiological adequacy of drinking water.

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