

# Assessment of drinking water quality at the cellular level

## Abstract

To assess the quality of drinking water, blood cells of test-organisms and human peripheral blood lymphocyte cultures were examined. The prospects of using hematological parameters of test organisms and humans in biotesting are shown. The method consists in determining the action of toxicants on specially selected organisms under standard conditions with the registration of changes at behavioural, physiological, cellular and sub cellular levels. As an optimal set for determining some structural and functional changes in the cell genome due to toxic effects, a micronucleus test and a quantitative indicator of blood lymphocytes were proposed as a biomarker. Particular attention is paid to assessing the risk to human health of those factors and substances whose genotoxicity and cytotoxicity are detected using blood cell biomarkers.

**Keywords:** genotoxicity, blood cells, micronucleus test, drinking water, cytotoxicity

Volume 5 Issue 1 - 2019

Maya Vergolyas

International Academy of Ecology and Medicine, Ukraine

**Correspondence:** Maya Vergolyas, PhD, Associate Professor, International Academy of Ecology and Medicine, Kiev, Ukraine, Email [vergolyas@meta.ua](mailto:vergolyas@meta.ua)

**Received:** December 17, 2018 | **Published:** March 29, 2019

## Introduction

Polluted aquatic environment has a negative impact on the environment, leads to increased environmental effects and poses a threat to all living organisms, including human health. The methodological possibilities of studying the toxicity of various substances on test organisms have significantly expanded in recent years. An intensive search for the most sensitive test objects and indicators is under way, instrumental methods of analysis are being worked out, various methods are used to assess the quality of the aquatic environment, changes in the parameters of physiological systems and the biochemical status of test organisms. In studies,<sup>1,2</sup> studies are carried out at the subcellular and molecular levels, where the experiments are time consuming and expensive, as a result of which their widespread use is practically limited.

The use of ecotoxicological bioassays (plant and animal test organisms) and their cellular biomarkers is extremely important for objective and comprehensive monitoring of the ever increasing number of aquatic polluting xenobiotics, most of which are not standardized by existing standards, but have the ability to cause a variety of toxic, cytotoxic, genotoxic or mutagenic effects. The universality of cellular organization opens up broad opportunities for toxicological studies using various groups of animals and plants and subsequent extrapolation of the obtained results to cells and the human body.<sup>3</sup>

The purpose of this work is to determine and justify the most optimal approaches for technical simplicity and versatility to the study of the quality of water samples at the organism level and especially at the cellular level. Research of this kind is necessary, since the total amount of chemical compounds in the environment reached > 80 million. They have several advantages over physical and chemical analysis, which often fails to detect unstable compounds or quantify ultra-low concentrations of toxicants. Bio testing also provides the ability to quickly obtain an integrated assessment of toxicity. To analyze the effect of toxic substances in aquatic samples on the body and its cells, the following set of biotests was selected: aquatic organisms-danio rerio fish and *Xenopus* spur frogs; warm-blooded animals - Wistar rats; culture of human peripheral blood lymphocytes. A set of cellular criteria includes the proportion of cells with micronuclei and abnormal nuclei (they register structural disorders in the hereditary apparatus of

the cell) and quantitative characteristics of leukocytes in peripheral blood (reflect functional changes). Hematological indicators of living organisms are an indicator not only of the physiological state of the organism, but also one of the main criteria for detecting contaminated drinking water.<sup>4</sup>

## Materials and methods

To compare the data obtained on hydrobionts and warm-blooded animals, as well as on human lymphocyte cell culture, a series of experiments were conducted to identify toxicants in various types of water (dechlorinated, water, packaged, artesian). In the work used artesian, packaged and tap water. The control water was prepared in the laboratory according to the recommendations of DSTU 4174: 2003 (Sovereign standard of Ukraine), which corresponded to the requirements of SANPiN 2.2.4-171-10 (Sanitary Rules and Norms). Biotesting on aquatic test organisms was performed on 40 individuals of *Danio rerio* fish and 40 species of spur-fishes *Xenopus* frogs cultured in the laboratory. Test organisms were divided into 4 groups of 10 individuals. Each group was placed in a specific aquarium: No. 1 control water, No. 2 tap water, No. 3 artesian water, and No. 4 packaged water. After exposure, after 96 hours, blood was taken from each fish from the tail vein. From each frog, blood was taken from the hindpaw after 192 hours. Preparation and analysis of cytological preparations from peripheral blood of fish and frogs was carried out according to the standard method.<sup>4</sup> In parallel, an experiment was conducted on 40 Wistar white rats. Rats were also divided into 4 groups (10 animals each). All animals were kept in a special water regime for two months, in particular, the rats of group No. 1 drank control water; Group number 2 - tap water. The rats of group No. 3 drank artesian water, and the animals of group No. 4 - packaged water. To determine hematological parameters, blood was collected from the tail vein. Complete blood count with leukocyte count was performed according to the standard method.<sup>5</sup>

To conduct an experiment on the culture of human peripheral blood lymphocytes, we used a lymphocyte culture obtained from a practically healthy donor - a 40-year-old woman who did not take medicine and was not examined radio graphically throughout the year. Blood was collected with a disposable syringe from the cubital vein, poured into 1 ml sterile heparinized (5 units in 0.1 ml of saline - 0.9% NaCl) centrifuge tubes. Lymphocytes were separated from

whole blood in the following way: layered into a test tube containing a lymphocyte extraction solution (ficoll-urografin), 1.0 ml, pre-mixed by pipetting the contents of the test tube, preventing mixing of liquids, closed the lid of the test tube. Next, the tube was centrifuged at 4000 rpm for 20 minutes, the supernatant was removed without disturbing the pellet. 500 µl of sterile saline was added, the lid of the tube was closed and the tube centrifuged for 10 minutes at 4000 rpm. Removed the supernatant without touching the precipitate. The sediment contains peripheral blood lymphocytes. The resulting lymphocyte sediment was also divided into 4 groups. Next, in the blood tubes under sterile conditions, the culture medium and native water samples (control, artesian, packaged, and tap water) were added successively in 0.1 ml each. Cytological preparations were prepared after 54 hours in order to examine the cells of the first mitosis. In vitro cytogenetic tests are aimed at demonstrating the induction of chromosomal abnormalities in cultured cells, in this case, peripheral blood lymphocytes, which are evenly distributed and are in one phase of the cell cycle ( $G_0$ ).<sup>6</sup>

## Results and its discussion

Biotesting and cytological analysis of the water samples studied was carried out. Cytogenotoxic assessment of different types of water (artesian, packaged, dechlorinated tap) on blood cells of test organisms and human lymphocyte cell cultures are shown in Table 1. The experiment took into account only lymphocyte counts for comparison with the data obtained on the blood of fish, frogs and rats. Statistical processing of the obtained results was performed using Microsoft Excel statistical analysis software. The arithmetic mean, mean deviation, arithmetic mean error, toxic effect was calculated with a statistically significant difference with the control<sup>7</sup> Table 1&2. Cytotoxic evaluation of different types of water (artesian, packaged, tap) on the blood cells of test organisms and cultures of human peripheral blood lymphocytes are shown in Table 1&2.

As a result of the study, data on the content of erythrocytes with micronuclei and double nuclei in packaged and tap water samples were obtained, Table 1. The number of micronuclei and double nuclei in red blood cells in fish reached from 1.67 to 3 in packaged water, and from 3.63 to 4 in tap water. Red blood cells in frogs reacted in a similar way, in the packaged water the number of micronuclei and double nuclei was found to be less (from 1.33 to 2.33) than in tap water 3.33 to 3.66 compared to control water. In the studied water samples, packaged and tap water significantly ( $r < 0.05$ ) increased the number of erythrocytes with micronuclei and double nuclei in the blood of experimental fish and frogs, and the data of artesian water corresponded with the control water data. The effects of toxicants on the body are accompanied by changes in the number of lymphocytes, Table 2. In the experiment, results were obtained and a decrease in the number of lymphocytes in all water samples was observed. In the blood of fish, the number of lymphocytes decreased in artesian water by 4.5%, in packaged water 5.9%. And in tap water, a decrease in the number of lymphocytes was reliable ( $r < 0.05$ ) 18.3%, compared to control water. The quantitative indicator of lymphocytes in the blood of frogs in artesian and packed water samples decreased slightly by 1.7% and 3.2%, respectively, and in the sample of tap water, the number of lymphocytes in the blood significantly ( $r < 0.05$ ) decreased by 18.2%. In the peripheral blood of warm-blooded animals, in rats, a significant ( $r < 0.05$ ) decrease in the number of lymphocytes was in artesian water samples of 19.2% and tap water 10%, and in packaged water the number of lymphocytes decreased slightly by 3.6% compared to control. In the experiment on the culture of human peripheral blood lymphocytes in a sample of artesian water, the number of lymphocytes decreased by 1.8%, and in packaged water by 4.4%. A significant ( $r < 0.05$ ) decrease in the number of lymphocytes was in tap water by 11.8% compared with control water. The data obtained may indicate the development of an inflammatory reaction in test organisms as a result of the action of toxicants in water samples.

**Table 1** The frequency of nuclear abnormalities in the erythrocytes of the blood of fish *Danio rerio*, *Xenopus* frog when exposed to the studied water samples

Cell types	Indicator	Water samples			
		Control water	Artesian water	Packaged water	Tap water
Erythrocytes of peripheral blood of fish, %	MN	0	0	1,67±0,63	3,63±0,86*
	2N	0	0	3±0,79*	4±1,24*
Red blood cells of the peripheral blood of frogs, %	MN	0	0	1,33±0,52	3,33±0,74*
	2N	0	0	2,33±0,69*	3,66±0,82*

Note: MN is a cell with micro nuclei; 2N is a cell with double nuclei,

\* -  $r < 0.05$  compared to the control group.

\*\*Cell type Indicators Samples of the investigated waters

**Table 2** Comparative assessment of the investigated waters on the lymphocytes of the blood of fish *Danio rerio*, *Xenopus* frog, Wistar rat and on the culture of human peripheral blood lymphocytes

Cell type, indicators	Water samples			
	Control water	Artesian water	Packaged water	Tap water
Peripheral blood lymphocytes fish%	86,7±2,62	82,2±2,46	80,8±2,66	68,4±1,96*
Peripheral blood lymphocytes of frogs,%	78,4±2,54	76,7±2,68	75,2±2,52	60,2±2,19*
Peripheral blood lymphocytes of rats,%	45,4±1,89	25,5±3,03*	41,8±2,82	35,4±1,63*
Culture of human peripheral blood lymphocytes,%	42,6±2,32	40,8±2,14	38,2±1,76	30,8±1,50*

Note: \* -  $r < 0,05$  in comparison with the control group.

It can be seen from the data obtained that according to the micronucleus test and on the quantitative characteristics of lymphocytes, fish react in a similar way with mammals, including humans. Therefore, fish are recommended to use for screening potentially harmful substances to humans, causing deformities and cancers, as well as “guards” of genotoxic substances entering drinking water. This is confirmed by the correlation coefficients between the rates on fish and in the culture of human peripheral blood lymphocytes. The obtained values of the linear correlation coefficients indicate the relationship of almost all of the indicators determined in fish, and the number of damaged aberrant metaphases with metabolic activation.<sup>8,9</sup> The formation of micronuclei, fragmentation of chromosomes often occur during the development of cancer, with viral infection, bacterial infection, as well as exposure to ionizing radiation and various mutagens. A strong correlation between the number of damaged aberrant metaphases and metabolic activation was found between the indices of the detected culture of human peripheral blood lymphocytes and onions.<sup>10</sup> To date, the question of whether micronucleus formation plays a special role in carcinogenesis remains open. In any case, microkernels indicate genomic instability.<sup>8,9</sup>

In determining the quality of drinking water using biotesting methods, a number of important questions arise regarding the extrapolation of the results obtained on the human body, such as whether the toxicity data of water samples obtained using animal and plant test organisms are a danger signal for humans. The works listed above enable the correct transfer of the results obtained at the cell level to higher levels of organization. The most acceptable methods for extrapolating to the human body are methods that evaluate mutagenicity, geno- and cytotoxicity, i.e. (sub) cellular effects. This conclusion is justified by the results of several international programs (Gene-Tox, International Program on Chemical Safety – IPCS), carried out in the 90s. Over 100,000 chemicals (EINECS) are registered in the European Registry. Of these, the presence and concentration of only 30–40 chemicals are regularly checked in the most important ecosystems of European countries.<sup>8,9</sup> A significant part of the substances cannot be determined in natural and waste waters due to the lack of appropriate analytical methods or the high cost of such an analysis.

## Conclusion

The prospects of using hematological parameters of fish organisms in biotesting are shown. Blood - as one of the most important systems of the body—plays a large role in its life. Due to the widely developed network of blood capillaries, it comes in contact with the cells of all tissues and organs, thus ensuring the possibility of their breathing and nutrition. Being in close contact with the tissues, the blood has all the reactive properties of the tissues, its sensitivity to pathological irritations is higher and thinner, and the reactivity is more expressive and bold. Therefore, all kinds of effects on body tissues are reflected in the composition and properties of blood. Hematological studies predict the appearance of the first, vaguely expressed clinical symptoms of the pathological process. In the peripheral blood of animals and humans, under normal physiological conditions of the

body, the formation of uniform elements and their destruction are in a state of equilibrium. Disruption of the relationship between these processes, due to the body’s response to toxic or infectious irritation, is manifested in a change in the quantitative composition of peripheral blood cells.

The methods listed in our work meet the modern requirements for the study of the quality of water samples. They determine their biological properties at the (sub) cellular level register changes in the hereditary apparatus, objectively characterize the long-term effects of their effects. Structural and quantitative changes in cells and nuclei are observed even at low concentrations of toxicants according to SanPiN 2.1.4.117575-02 (Sanitary Rules and Norms). Bio monitoring of natural and drinking waters is an urgent task at the present stage of development of society, which is carried out by research teams in many countries of the world. Chemical analyzes in determining the quality of drinking water is not entirely justified, since chemical methods cannot reveal the entire set of elements present in an aqueous solution, assess their interaction and transformation in the environment and the body. Bio testing using the optimal sets of test organisms and their cellular parameters objectively characterizes the biological component of water quality.

## Acknowledgments

None.

## Conflicts of interest

Author declares that there are no conflicts of interests.

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