Experience in the Application of Bioassays with Planarian in the Studies of Drinking Bottled Water Safety

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Doi:10.5901/mjss.2015.v6n2s4p29

Abstract

The samples of drinking water in bottles were compared by organoleptics, chemical composition and safety. To determine safety the method of biotesting with Planaria Dugesia tigrina was used. The degree of Planaria regeneration in different water samples was measured. The data obtained showed that this test object may be successfully used for assessment of safety of drinking bottled water with sufficient correlation with organoleptic properties and toxic elements content. The properties of water were shown to depend on the source and producer.

Keywords: *Dugesia tigrina, drinking water, food safety, toxic elements, organoleptic indicators*

1. Introduction

In 21st centure the level of hazard caused by anthropogenic influences achieved its maximum, and ecological prognosis shows further acceleration of such influence. Special attention has to be paid for foodstuffs because they are in the end of ecological chains: xenobiotics – soil – air – plants – animals –foodstuffs – human being. Great russian scientist V. I. Vernadsky even in 1926 proposed a term “biosphere” for a unique system of humans, animals, plants, microorganisms and their environment. Now in biosphere there are more than 60,000 different xenobiotics invasing into natural circulation of substances. These xenobiotics enter the human organism mainly from foodstuff (70%), air (20%) and water (10%). That’s why it is so important to improve new appropriate methods for assessment safety of foodstuffs and water on all the steps of technological chain: producing, storage, transportation, purchasing, with aim to provide the commonwealth with foodstuffs of high quality.

In addition to traditional physico-chemical methods it is necessary to produce and use methods of biological control – biotesting which allow getting objective integral data about food and water safety. Biotesting usually is based on the economical express methods of registration of integral reaction of alive test-organisms to different food and environmental sources. Recently different primitive organisms were used as test-objects: infusoria, bacteria, plathelminths and others. They are characterized by necessary sensitivity and stability of reactions to individual and complex toxicants. It is very important that registration of their reactions is rather simple and stable. [1, 3, 4]

One of such techniques is the use of flatworm Planaria, asexual strain of *Dugesia tigrina* for assessment of safety of liquid foodstuffs. This population exists since 1970 in the Institute of Biological Physics, Russian Academy of Sciences, in Pushchino, Moscow region. It is fully adapted to the existence in city conditions. These organisms are very sensitive to different negative influences of their environment including the xenobiotics in foodstuffs and water. Planarias of this race reproduce asexually by separation of their tail fragment. For this a worm sticks to the glass wall of aquarium and abruptly turns its back part off. Then initial individual regenerates a new tail, and removed fragment forms new miniature Planaria. [5] During all the time of observation no features of their biological degradation was noticed. Planarias not only re-establish their size but also their body proportions. As a result of this operation both parts regenerate their deficient fragments, no individual dies, but only process of reproduction takes place. The flatworms of genus *Dugesia* are covered with brown or black epithelium, and new regenerated parts for the first 4-5 days do not have pigmented cells, so they clearly differ from the old pigmented parts of regenerants. This difference may be clearly registered by special equipment including video camera and computer. Then special computer programmes allow to count and analyze the effect of environmental factors on the dynamics of regeneration. [2, 6, 7] In this study we investigate the possiblity of using bio-testing with Planaria for express assessment of safety and quality of drinking water in bottles.

Recently everythere the demand for drinking water significantly increased because consumers all over the world prefer to use water in bottles not only for drinking but also for cooking. To achieve more profits some unprincipled producers prefer to falsificate drinking water, so one often may buy infringing foodstuffs and water instead natural ones. That's why new integral express methods of foodstuff assessment are so important and actual.

2. Materials and Methods

We investigated samples of drinking water in bottles of different producers (12 trade marks), 5-7 samples of one producer. They were purchased in variable shops. It was found that more than 30% of samples of water were clearly falsified: water in these bottles did not comply with international standards of quality. A clear correlation between organoleptic, physico-chemical indicators and results of biotesting with Planaria was strictly demonstrated.

For demonstration of our results we have chosen 3 samples of drinking water in bottles of one trade mark "Aqua Minerale" purchased at different trade points. This is pure, still, drinking artesian water. Sample 1 was produced by certified producer with guaranteed level of quality, we used it as our inner standard for comparison. Sample 2 was purchased in the shop with medium level of sales volume. Sample 3 was purchased in the small shop of individual owner without certificate of production. As a standard we used artesian water which was habitual for test-objects: Planarias were adapted to it. All analyzes were performed 5 times, results were processed with use of computer program «Statistika-7» that confirmed their reliability.

The safety of water was assessed by biotesting with use of alive test-object – *Dugesia tigrina*. For this purpose in the Institute of Cell Biophysics, Russian Academy of Sciences (Pushchino, Moscow region) a special computerized system was produced, it contains video camera, videograbber and personal computer. This system allows to registrate moving Planarias and subsequently to analyze selected regenerating flatworms. Videos obtained were analyzed with use of special program Plana 4.4: the main morhological parameters of Planarias (length, broadth and square of head part and of the whole flatworm) were calculated automatically. All measurements are produced in conventional units because the main criterion of regeneration is the ratio of blastema square to the square of whole body. After that statistical analysis with use of special progarm was performed.

This biotesting of safety and quality of water consisted of the following steps:

1. Selection of 30 Planarias for each test and for standard.
2. Separation of the heads of selected Planarias by means of scalpel with control of binocular microscope.
3. Transfer of decapitated objects to the glass with 30 ml of sample water under test or of standard water.
4. Incubation of the samples in the glasses at room temperature during 2 days in darkness with access of air.
5. Transfer of the Planarias under test by means of spatula or pipette to the Petri cup with standard water.
6. Registration of the size and shape of test-objects by means of microscope connected with video camera and computer.
7. Analyzing of the data obtained by means of special computer program Plana 4.4 invented by A. Dayev.

Organoleptic assessment was performed according to the standard methods. Toxic elements were also determined by standard methods (inversive volt-amperometry).

3. Results and Discussion

The safety and quality of drinking water in bottles "Aqua minerale" were assessed by organoleptical and physico-chemical methods, and the data obtained were compared with results of biotesting with use of Planaria *Dugesia tigrina*. Four organoleptic indicators were determined: smell, taste, clarity and colour. In the tabl.1 there are medium arythmetic values, standard deviations and complex indices taking into account their weight ratios. Depending on the complex index all the samples were divided into three categories of quality: high (more than 90 balls), first (more than 80 balls) and non-standard (more than 70 balls).

Table 1. Assessment of quality indices of drinking water "Aqua minerale" in bottles taking into account their weight ratiosk

Indicator	Single index $\bar{x}_i k_i$ of the samples, balls		
	1	2	3
Taste	27,4	24,8	12,7
Smell	25,0	23,5	17,0
Clarity	23,5	25,0	22,0
Colour	19,4	18,3	17,1
Assessment of the sum $\sum_{i=1}^{n} \bar{x}_i k_i$			
Complex quality index	95,3	91,6	68,8

The samples under test showed different quality levels according to the organoleptics. Standard sample 1 was characterized by highest organoleptical indices - it corresponds to the high category of quality. Sample 2 had lower indices of taste and smell, so it was downgraded as the 1st category. Sample 3 was even worse, so it was downgraded to the non-standard category.

We determined the following physico-chemical indices of the samples – the contents of toxic metals: arsenic, lead, cadmium, mercury (table 2).

Table 2. Toxic elements in the samples of drinking water "Aqua minerale" in bottles

Toxic elements, their mass concentration, mg/kg, not more than		Number of water sample		
		1	2	3
Lead	Actual value	0,0002	0,0005	0,009
	Standard value		0,03	
	Value in SanPiN 2.1.4.1116-02		0,005-0,01	
Cadmium	Actual value	0,0000	0,0000	0,0019
	Standard value		0,001	
	Value in SanPiN 2.1.4.1116-02		0,001	
Mercury	Actual value	0,0004	0,0009	0,001
	Standard value		0,0005	
	Value in SanPiN 2.1.4.1116-02		0,0002-0,0005	
Arsenic	Actual value	0,0047	0,0033	0,0083
	Value in SanPiN 2.3.2.1078-01		0,05	
	Value in SanPiN 2.1.4.1116-02		0,006-0,01	

This table shows that sample 2 has more mercury than standard value, and cadmium and mercury contents in the sample 3 exceeds acceptable limits.

We have investigated the regeneration activity of Planarias *Dugesia tigrina* cultivated in the samples of drinking water under test and in the standard water from the artesian source (test-objects were adapted to it). The measurement of the square of the body and head of regenerating Planarias were performed automatically in fixed time periods (table 3).

Table 3. Results of measurements of body sizes of Planarias regenerating in standard water sample

Number of Planaria	Standard sample		
	Body square, conventional units	Head square, conventional units	Ratio of squares (head/body)
1	7747,6	127,1	0,0164
2	7508,1	148,6	0,0198
3	9584,9	178,6	0,0186
4	7173,5	65,1	0,0091
5	3820,6	74,3	0,0194
6	5591,4	92,7	0,0166
7	6878,1	121,6	0,0177
8	5088,4	94,6	0,0186
9	5757,3	131,5	0,0229

10	2937,0	46,0	0,0157
11	5467,3	69,1	0,0126
12	4548,5	59,9	0,0132
13	4705,6	104,02	0,0221
14	7968,6	134,5	0,0169
15	7488,3	136,7	0,0183
16	6301,4	129,7	0,0206
17	5202,0	76,9	0,0148
18	4821,2	114,6	0,0238
19	5335,7	116,9	0,0219
20	6456,5	113,9	0,0176
21	4639,4	53,3	0,0115
22	5674,2	71,8	0,0127
23	8038,0	156,5	0,0195
24	4366,7	74,2	0,0170
25	4166,4	69,7	0,0167
26	5729,8	94,2	0,0164
27	3247,1	56,9	0,0175

Table 4 contains statistically manipulated data from the table 3.

Table 4. Results of statistical manipulation of the data from table 3 (regeneration in standard sample)

Name of index	Standard sample		
	Body square, conventional units	Head square, conventional units	Ratio of squares (head/body)
Medium value	5786,5407	100,4852	$1{,}73*10^{-2}$
Standard error	308,9487	6,8012	$0{,}07*10^{-2}$
Number of test-objects	27,0	27,0	27,0
Total sum	156236,6	2713,1	0,4678
Minimal value	2937,0	46,0	$0{,}91*10^{-2}$
Maximal value	9584,9	178,6	$2{,}38*10^{-2}$

If one removes minimal and maximal values, the error of measurement will be lower.
In a similar manner the measurements were performed in the case of the samples of drinking water “Aqua minerale” in bottles. The data obtained are summarized in the table 5. Ratio of squares (head/body) is the most essential criterion that characterizes the speed of Planarias regeneration which depends on the extent of toxicity of environment and correlates with inhibition of reproducing capacity by regeneration.

Table 5. Results of measurements of body sizes of Planarias regenerating in the samples under test

Sample	Body square, conventional units	Head square, conventional units	Ratio of squares (head/body)
Standard	5786,5	100,4	$1{,}73*10^{-2}$
№1	5811,2	92,1	$1{,}58*10^{-2}$
№2	6576,9	71,3	$1{,}08*10^{-2}$
№3	7287,1	65,9	$0{,}90*10^{-2}$

The ratio of squares in the case of standard sample was 0,0173, and in the case of sample 1 - 0,0158. Therefore, one can conclude that regeneration of Planarias incubating for two days in the drinking water of the sample 1 was reduced by 10%. In a similar manner the data for the samples 2 and 3 were calculated (Table 6).

Table 6. Influence of water quality on the extent of inhibition of Planaria regeneration, %

Number of the water sample		
№1	№2	№3
- 10	-37,6	-52

These data allow to reveal correlation between the quality level of drinking water and the extent of inhibition of Planaria regeneration.The sample 1 chosen as the standard and purchased from the producer, equipped with safety certificate, was shown to have organoleptic properties of high category and to fit standard according to the contents of toxic elements – it caused the reducing of regeneration by 10%. The sample 2 (1st organoleptic category) exceeding standard by the mercury contents and therefore unacceptable for consumerst caused the reducing of regeneration by 37,6%. The sample 3 purchased from the individual salesman without safety certificate did not comply with quality standards by its organoleptic properties and contents of toxic elements (cadmium and mercury) and therefore reduced the regeneration of Planarias by 52%.

4. Conclusions

Analyzing our empirical data and comparing them with organoleptic and physico-chemical results of investigation of a number of the samples of drinking water in bottles we conclude that proposed method of biotesting may be used for operative express control of drinking water sold to the consumers. We suggest dividing of drinking water into two categories: 1 – safe for consumers water that causes reducing of regeneration not more than by 30%; 2 – hazardous and unacceptable for consumers water that causes reducing of regeneration by more than 30%.

5. Acknowledgements

Authors wish to thank Pro-rector of Pushchino State University, the head of Scientific Educational Centre of the Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences – Harlampy P. Tiras.

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