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A Beneficiated Method for Assessment of Acute and Chronic Toxicity of Water

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Abstract

Toxicological testing is used for forecasting the influences of various pollutants on living organisms as well as for the evaluation of water toxicity. However, in many standardized techniques some seasonal and environmental factors are not taken into consideration. Here we present a new method for accurate analysis, which allows a 2-fold increase in the accuracy of toxicological analysis by means of appreciating seasonal features and the components of natural water.

Keywords: *Acute toxicity, chronic toxicity,* Daphnia magna, *pollutant, survival, reproduction.*

INTRODUCTION

Contamination negatively affects the ecosystem integrity as well as the serv-ices they provide to society (Cairns and McCormick 1992). In this connection, toxicological testing is usually used for forecasting the influences of various pollutants on living organisms as well as for the evaluation of water toxicity. With the aim of increasing reliability of the test within one laboratory, standardized techniques have been proposed (Anonymous 1985, Horning and Weber 1985, Peltier and Cornelius 1985, Biesinger et al. 1987). Bioindicators may reveal changes of ecological importance that cannot be detected by other methods (Abel 1996). Historically, cladocerans are extensively used in toxicological testing because they are readily available, easy to cultivate in the laboratory and sensitive to chemical contaminants (Mokry and Hoagland 1990). However, many of the above-mentioned techniques for acute and chronic testing do not make allowances for some factors that may influence the results of the biomonitoring procedures. Namely, the effects of the biological components of natural water are not being considered in toxicological studies. Additionally, seasonal features of *Daphnia magna* Straus resistance to pollutants are also not taken into consideration. For this reason, we present here a simple method, which increases the accuracy of toxicological analysis by means of appreciating seasonal features and the components of natural water.

MATERIALS AND METHODS

Test Organisms

Daphnia magna Straus was obtained from continuous cultures maintained in a 10 L aquaria with dechlorinated and aerated tap water at room temperature (20±2°C). Offsprings were separated at regular intervals. Test animals were 24 hr old individuals taken from 3-5 week old cultures.

Assessment of Acute Toxicity

For the assessment of the acute toxicity (96 h experiments) of water, it is necessary to use natural water (instead of laboratory water) that should be isolated from the same segment of water. A median lethal concentration (LC50) should be used for the acute toxicity test with *D. magna*.

For demonstrativeness, we show the procedure in details.

We used deltamethrin as a model pollutant. We have previously found that 0.1, 0.2 and 1 μgL^{-1} are the vital, median effective concentration and lethal concentration of pollutant for *D. magna* (at 20°C after 4 days), respectively (Ratushnyak et al. 2005). Biological surveys should be performed monthly for a year. The pollutant (deltamethrin) should be diluted by using natural water and tap water.

One-day old *D. magna* should be placed in a beaker with the test water. Each experiment should be run in triplicate with a control (without toxicant) and with the toxicant. The duration of the experiments were 96 h. Each day, a percentage of surviving females was calculated. After 96 h, a median percent of surviving females were calculated using the following formula:

$$\overline{A} = \frac{\overline{Xk} - \overline{Xi}}{\overline{Xk}} \cdot 100\%$$

where $\overline{Xk}, \overline{Xi}$ median number of surviving *D. magna* in the control and experimental variant, respectively.

Assessment of Chronic Toxicity

We suggest performing the experiments in the spring-summer and autumn-winter seasons using tap water with *Chlorella* or using natural water with live plankton. Live concentrate of plankton can be achieved by filtration of natural water keeping in the mind average daily volume of water filtered by *D. magna* (or consumed food) for the fixed time (Gutelmaher 1973). After each experiment, it is necessary to detect the presence of chronic toxic action of water using the parameters of survival and reproduction. Concentration of pollutant when compensation effects were absent owing to *D. magna* interaction with components of natural water is considered as a threshold of adaptive capabilities of the test organism or as "ecological" admissible concentration limit.

Statistically significant difference of *D. magna* survival or reproduction between control (in natural water without pollutant) and experimental condition (in natural water with pollutant) can be considered as criterion of chronic action.

To evaluate survival, we suggest using a coefficient of survival, which can be estimated as sum of percentages of 100% survival (taken for 1) for each day with subsequent cleavage on the number of experimental days. This coefficient was introduced for detection of cumulative alterations in *D. magna* survival in diurnal dynamics.

Example of the Assessment Procedure

The assessment of chronic toxicity is performed with deltamethrin-treated *D. magna* using tap water and natural water with *Chlorella* (600,000 cells per mL) or plankton concentrate feeding, respectively. Plankton concentrate should be obtained by filtration of natural water. Apshtein mesh (size of mech 0.12 mm) has to be used for sampling. Water volume (V) to be filtrated is calculated using the following formula:

$$V = v_{median} \bullet n_{initial} \bullet t, \qquad (1)$$

where:

V_{median} - median speed of filtration, mL/copy day,

$N_{initial}$ - total number of the initial *D. magna* females in variants with natural water;

t - duration of experiment, days

Values of Vmedian are equal to 100-120 mL/copies day in autumn-winter period while about 60 100-120 mL/copies in other seasons (Gutelmaher 1973, Burns and Gilbert 1986, Kruchkova 1989). Two-day old *D. magna* should be placed into 400 mL glasses; then tap water or natural water should be added. Each experiment should be in triplicate; the duration of the experiments is 14 days.

Variants "tap water plus *Chlorella*" and "natural water plus plankton" are as follows:

1. without pollutant (control);
2. with deltamethrin (1 μg/L).

Then, the volume of the everyday portion of concentrate (Vp), which should be added to each glass, is calculated using the following formula:

$$V_p = \frac{V_{con} \cdot v_{median} \cdot n}{V} \qquad (2)$$

where:

V -volume of the filtrated water, mL;

Vcon -volume of the obtained concentrate, mL;

Vmedian -median daily speed of filtration, mL/cell day,

n -number of *D. magna* species in glass.

Concentrate should be stored in a refrigerator for 3-5 days, and then renewed.

The number of surviving *D. magna* females and larva should be calculated every day; larva after the calculation should be removed from the glass. After termination of the experiment, the coefficient of survival and number of larva per female should be calculated using the following formulae:

$$Survivability\_coefficient = \frac{\sum a_n}{10mn} \qquad (3)$$

where:

an -number of *D. magna* for every day of experiment including each replication;

m -duration of experiment (number of days);

n -number of variant replication:

$$\text{Number of larva} = \Sigma \frac{M_i}{N_i} \qquad (4)$$

where:

M_i - total number of larva in each brood, number of copies;

Ni - number of alive initial females that produced larva.

Using the calculated data, it is possible to draw dependencies between survival as well as *D. magna* reproduction and season using tap water and natural water.

RESULTS AND DISCUSSION

Initially, laboratory LC50 and some other tests are usually used for ecological risk assessment procedures including evaluation of water quality (Chapman 1995). However, relative sensitivities of the test systems vary depending on many factors like type and age of the test organism, test duration, test endpoint, etc (Baird et al. 1989). Moreover, many factors, as it was stated above, are not permanently taken into consideration. Among these factors are seasonal features, type of feed (in chronic toxicity experiments) as well as the biotic microenvironment in the tested medium. So, we attempted to keep in mind these factors, and the essence of our methodological suggestion is given below.

Namely, Figs. 1 and 2 demonstrate some examples for the suggested method of acute toxicity assessment, whereas Figs. 3-5 clarify data on chronic toxicity analysis.

A conclusion on the presence of toxic action should be drawn from the statistically significant differences in *D. magna* survival and reproduction observed in natural water (or in tap water) with and without pollutant. Student's t-test may be used for statistical analysis. If t-test value is more or equal to predicted value, then it is reasonable to conclude that natural water (or tap water) with pollutant shows chronic toxic action on *D. magna*. In our case, experiments were made in triplicate; therefore, crucial t-value is 2.78 (Tables 1 and 2).

It is clear from the presented data that natural water with a pollutant is toxic for *D. magna* during all seasons whereas the same variant with tap water shows toxicity only in winter and autumn.

According to our results, the advantage of the presented method is an increase in the accuracy of the ecotoxicological assessment of potential pollutant hazard for aquatic organisms. Elimination

Table 1. Significance of deltamethrin chronic toxicity of tap water and natural water according to Student's t-test. Values of survival are compared.

Season (month)	*t*-value in tap water	*t*-value in natural water	Significance
February (II)	26.38	18.39	significant ($p<0.01$)
April (III)	4.87	0.73	non-significant ($p>0.05$)
June (VI)	10.71	0.17	non-significant ($p>0.05$)
July (VII)	8.06	0	non-significant ($p>0.05$)
August (VIII)	2.69	13.38	significant ($p<0.05$)
October (X)	46.42	1.11	non-significant ($p>0.05$)
November (XI)	0.39	266.74	significant ($p<0.001$)

Table 2. Significance of deltamethrin chronic toxicity in tap water and natural water according to Student's t-test. Values of reproduction are compared.

Season (month)	*t*-value in tap water	*t*-value in natural water	Significance
February (II)	18.53	- *	
April (III)	1.30	18.00	Significant ($p<0.01$)
June (VI)	3.18	101.37	Significant ($p<0.001$)
July (VII)	0.24	519.62	Significant ($p<0.001$)
August (VIII)	1.14	36.13	Significant ($p<0.01$)
October (X)	10.30	7.78	Significant ($p<0.05$)
November (XI)	4.84	112.10	Significant ($p<0.001$)

*- larva were absent

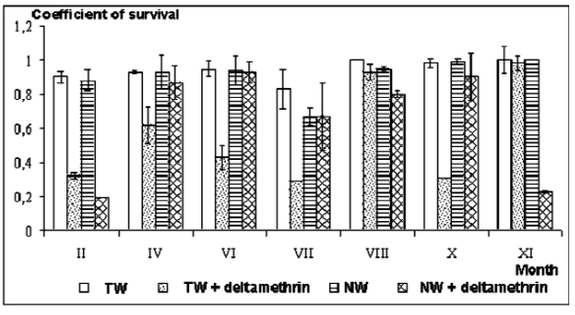

Fig. 1. Seasonal dynamics of acute toxicity of deltamethrin (1 μg/L) after 96 h. Axis Y -survival of *D. magna* (in %), axis X -months. TW: tap water, NW: natural water.
Note: in tap water, *D. magna* survival was equal to zero in February, July, August, October and November. Data are presented as mean ± standard deviation.

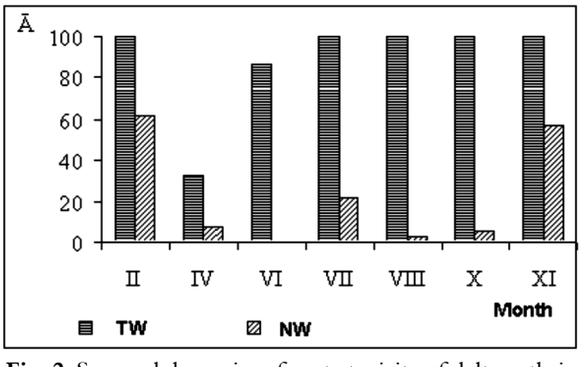

Fig. 2. Seasonal dynamics of acute toxicity of deltamethrin (1 μg/L) after 96 h. Axis Y - median percentage of survived (in the water under test) *D. magna* in relation to control (A - criterion of toxicity, %), axis X - months. TW: tap water, NW: natural water.

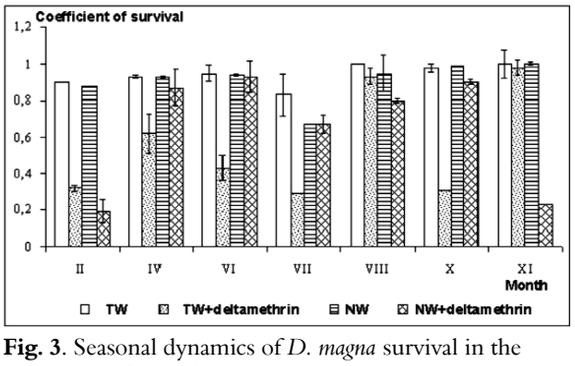

Fig. 3. Seasonal dynamics of *D. magna* survival in the experimental natural water and tap water. TW: tap water, NW: natural water.

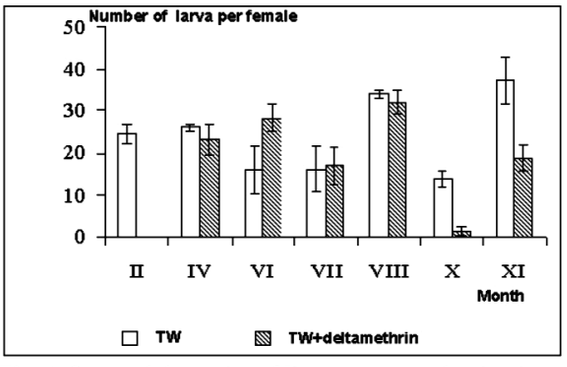

Fig. 4. Seasonal dynamics of D. magna reproduction in tap water. TW: tap water, NW: natural water.

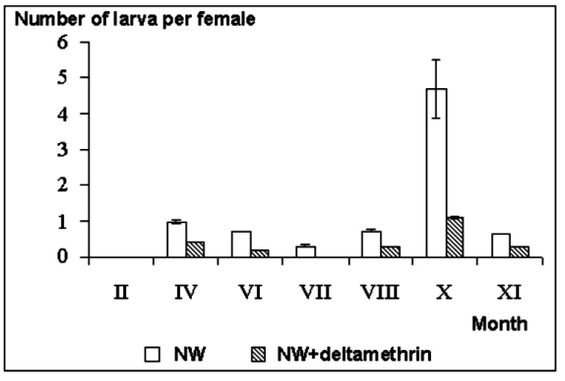

Fig. 5. Seasonal dynamics of *D. magna* reproduction in natural water. NW: natural water.

of some shortcomings related to experimental conditions are provided by the following factors:

1. Testing medium is compared with natural environment conditions;

2. Interrelations between toxicological resistance of *D. magna* and seasonal features of its development and reproduction as well as seasonal interaction with biological and abiotic components of the environment were taken into consideration;

3. *Chlorella* was eliminated from the chronic toxicity experiments since it may influence *D. magna* survival and reproduction in a pollutant and thereby decreasing accuracy of toxicological studies (Ratushnyak 2002, 2003).

From our research on results, the presented method may be applied amplitudinously by ecological and toxicological services and is marked by increased accuracy. This is especially important because inaccurate testing may cause serious imbalances in the environment (Forbes and Forbes 1994).

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