Effect of Hypoxia on Sodium and Ammonium Acetate Toxicity for Daphnia

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Exposure of *Daphnia* in degassed (boiled) culturing water (hypoxia simulation) led to solitary lethal outcomes after more than 24 h. Before this term, hypoxia had no appreciable effect on the toxicity of sodium or ammonium acetate salts. The sensitivity of daphnias to the lethal effects of the tested chemicals did not change under conditions of normal oxygenation and increased sharply (by two orders of magnitude) under conditions of hypoxia, loosing the linear relationship with toxicant concentration. Ammonium acetate toxicity more markedly increased under conditions of hypoxia than sodium acetate toxicity. These data should be taken into consideration when predicting the results of combined effects of toxicants on water ecosystems and on human organism.

Key Words: *Daphnia magna; hypoxia; ammonium acetate; sodium acetate; lethal effect*

Combined exposure to hypoxia and ammonia (ammonium ions) is associated with many human diseases and is an important problem for water ecosystems [2]. The effect of hypoxia on ammonia toxicity for hydrobionts is characterized as a synergic one [3], but was not evaluated quantitatively.

We evaluated the effects of hypoxia and ammonium on *Daphnia magna Straus* fresh water crustaceans.

MATERIALS AND METHODS

The study was carried out on female daphnia aged 7 days, grown under laboratory conditions in accordance with the requirements of the International Standard for water biotesting [1]. The toxicities of sodium (SA) or ammonium (AA) acetate (extra pure, Merck) were evaluated by the hydrobiont death during a certain period spent in solutions of these substances. Hypoxic conditions were created by thermal deaeration of culture water at atmospheric pressure (5-min boiling with subsequent cooling in a full hermetically closed vessel), which reduced oxygen concentration to 3 μM.

The tested preparations were added (1 ml) to incubation medium as fresh prepared solutions in aerated (control) or deaerated (experiment) culturing water. Deaerated culturing water (1 ml) was added to the samples in the groups which served as the preparation controls. Daphnias (n=5) were placed into completely filled flasks (24 ml) with the test solution in 5-10 logarithmically ascending concentrations. In experiments with hypoxia simulation the flasks were directly closed; in the control they were left open (the conditions of animal exposure in these samples were considered as normoxia). In order to plot the survival curves under conditions of hypoxia the survival was recorded every 0.5 h.

The mean lethal concentration (LC_{50}) of the chemicals was estimated by the probit method using a specially designed program for 24 and 30 h. Experiments were repeated 6 times for each of the studied concentrations of each toxicant; the mean LC_{50} values were estimated and the significance of differences between the groups was evaluated using Student's t test. The differences in the survival function in the groups

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were evaluated using Gehan–Wilcoxon's test. Initially each experimental group consisted of 25 daphnias.

RESULTS

Daphnia mortality over 30 h without hypoxia linearly depended on the concentrations of the test substances. Ammonium acetate was 7-fold more toxic for daphnias than SA.

Two periods were distinguished in animal reactions during incubation under hypoxic conditions. During the first period (no longer than 24 h), hypoxia had virtually no effect on the toxicity of the test substances and even slightly reduced it. During the second period (in experiments with exposure longer than 24 h), the sensitivity of daphnias to the test substances increased sharply (by more than 2 orders of magnitude). The mortality lost the linear relationship with the toxicant concentrations, which precluded estimation of LC_{50} over 30 h (Table 1). After 30-h hypoxic exposure without test substances, the mortality was no higher than 50%, while the presence of toxicants for 24 h even in so low concentrations as 0.01 LC_{50} (estimated for normoxic conditions and constituting 0.90 and 0.13 mM for SA and AA, respectively) caused death of all daphnias. The lethal effect of AA under conditions of hypoxia was more pronounced than that of SA, despite the fact that the toxicant concentrations constituted equal ($^1/_{100}$) fractures of their concentrations, which were similarly effective under conditions of normoxia. The difference in the survival functions (Fig. 1) was significant between the hypoxia group and each of two other groups ($p<0.00001$) and between hypoxia+SA group and hypoxia+AA groups ($p<0.00286$).

Hence, the effect of hypoxia on daphnias was determined by a certain threshold duration, after which toxicities of the test substances sharply increased. This sensitization phenomenon was not specific for AA, because it was also observed with the control substance (SA). However, toxicity of AA increased greater under conditions of hypoxia than that of SA. These data should be taken into consideration when predicting the result of combined effects of toxicants and hypoxia on water ecosystems and on human organism.

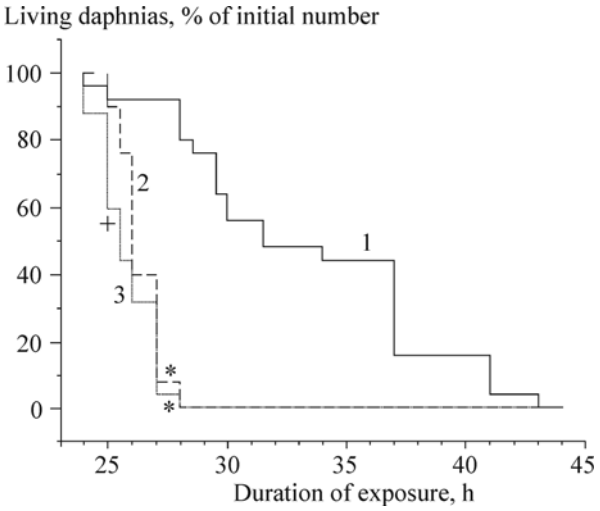

Fig. 1. Survival curve of daphnias exposed for 24 h to hypoxia alone (*1*) and its combinations with SA (*2*) or AA (*3*) in concentrations constituting 0.01 LC_{50} under normoxia conditions.*$p<0.00001$ compared to the *1*; $^+p<0.00286$ compared to the 2.

TABLE 1. Toxicities of SA and AA for Daphnias at Different Oxygenation Levels in Incubation Medium ($M\pm m$, $n=6$)

Test substance	Oxygenation conditions	LC_{50}, mM	
		24-h incubation	30-h incubation
SA	Normoxia	89.9±9.0	89.9±9.0
	Hypoxia	99.0±12.0	≤0.123*
AA	Normoxia	13.4±2.1	12.3±1.9
	Hypoxia	14.6±2.5	≤0.039*

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