

# Dosage and temporal dependent Arsenic-induced mortality in *Ceriodaphnia dubia*: An effective Biomarker for Arsenic Pollution

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## ABSTRACT

Arsenic (As) is a metalloid that causes severe water pollution due to its extravagant toxicity. *Ceriodaphnia dubia*, a freshwater crustacean, was selected as a model system to evaluate the degree of time and dosage dependent acute toxicity caused by pentavalent As [As(V)]. *C. dubia* were collected from a natural pond and treated with different concentrations of As(V) for 24 hours and 48 hours. For both 24 hours and 48 hours treatment periods, the mortality rates were increased significantly ( $P < 0.05$ ) with increase in As(V) concentrations. Simultaneously, it was also observed that As(V) - induced mortality in *C. dubia* also depended on the time of exposure to the metalloid. We propose this model as a low-cost technique towards rapid screening of water quality in relation to As contamination.

**Keywords:** Arsenic; Water pollution; *Ceriodaphnia dubia*; Biomarker

## 1. INTRODUCTION

Arsenic (As) is a metalloid of severe environmental concern due to its extravagant toxicity and abundance (Bissen and Frimmel 2003). It is known to cause deleterious effects in both plants and animals through the metabolic pathways (Liu et al. 2005; McGeachy and Dixon 1989). Inorganic as well as organic forms of As are present in the environment,

and the former seems to be more toxic and slightly more accumulated in some freshwater aquatic species than the latter (Spehar et al. 1980). Being a potent endocrine disruptor, it can alter hormone-mediated cell signaling even at extremely low concentrations in such organisms (Kaltreider et al. 2001). Water contamination by As has been extensively reported in India, China, Taiwan, Bangladesh, Vietnam, USA, Argentina, Chile and Mexico (WHO 2001).

The freshwater microcrustacean *Ceriodaphnia dubia* (common name: water flea, order: Cladocera) can be used in short-term standardized tests to estimate the acute or chronic toxicity of different metals, chemicals and effluents (Naddy et al. 1995; Peters et al. 1991). Naddy et al. (1995) examined the sublethal interactive effects of arsenic (As), molybdenum (Mo), and selenium (Se) on *Ceriodaphnia dubia* using the three-brood static renewal toxicity test. Results showed that these metals can significantly reduce *C. dubia* fecundity. According to Cooper et al. (2009), combination of copper and zinc can increase mortality rate of *C. dubia* significantly.

The use of this organism is justified because of its widespread geographic distribution and for taking an intermediate position in pelagial or planktonic food webs as it consumes algae and detritus and in turn is consumed by various predators. *Ceriodaphnia* is very sensitive to various toxic chemicals, is easily reared under laboratory conditions, and has a moderately short life cycle.

The current study was aimed at investigating the dosage and temporal dependence of As(V)-induced acute toxicity in *Ceriodaphnia dubia*. The bioassay was performed towards the development of an economic model for water quality testing in the context of As contamination.

## 2. METHODS

### 2. 1. Chemicals and Reagents

Sodium arsenate dibasic heptahydrate ( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ) procured from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). The remaining chemicals used were of standard analytical grade.

### 2. 2. Culture and maintenance of *C. dubia*

*C. dubia* were collected from a natural pond located in Kolkata (22°34'N, 88°22'E), India. They were cultured at 25 °C with a 2:1 photoperiod in 100 ml of chlorine-free water in a glass jar and were fed with yeast (Hansen et al. 2002). One day prior to performing the acute toxicity test, 30 mature females were transferred to 150 ml fresh water. The parthenogenetic females reproduced rapidly and a large number of neonates were formed. After 24 h, the neonates were collected and transferred to a new jar. These neonates (less than 24 h old) were used for the acute As(V)-induced toxicity test.

### 2. 3. Acute As(V) toxicity bioassay

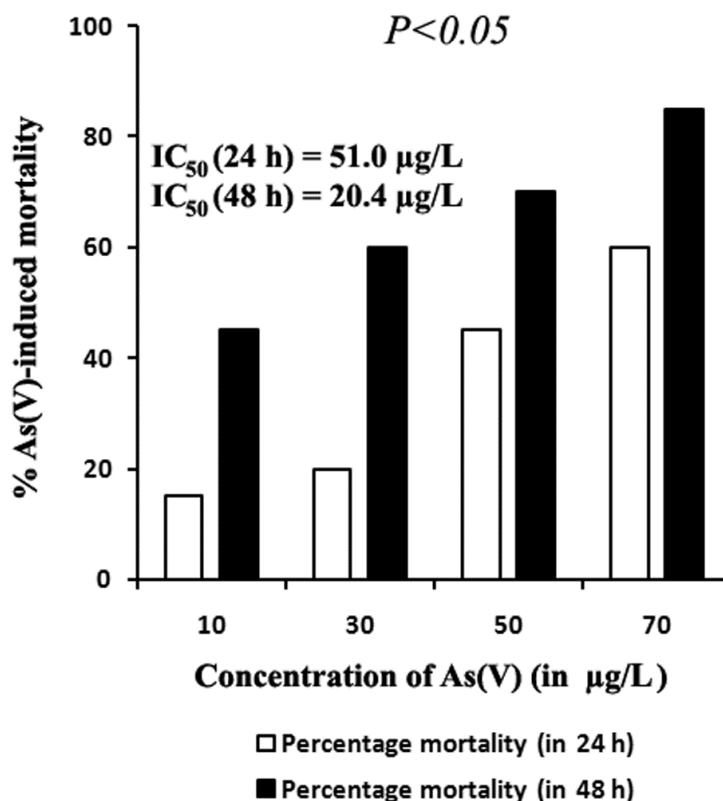
$\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$  was dissolved in chlorine-free water in four concentrations (10, 30, 50 and 70 µg/L) to prepare the As(V)-containing test solution. 10 neonates were transferred to 150 ml of each concentration of the test solution and maintained without disturbance for 24 h. Another set of 10 neonates were similarly transferred to 150 ml of each of the four concentrations of the test solution and kept undisturbed for 48 h. These were the two test

groups (24 h and 48 h test groups, respectively). Additionally, two sets of neonates (each containing 10 individuals) were also maintained in chlorine-free water for 24 and 48 h as control groups for the respective 24 h and 48 h test groups. No feeding and aeration were provided to the neonates of these four sets during the test (Eaton et al. 1995).

The 24 h test and control groups were examined under 10x magnification after 24 h to determine percentage mortality in all concentrations of As(V) in the test group. Similarly, percentage mortality with varying As(V) concentrations were also evaluated in the 48 h test group with respect to the 48 h control. An organism was pronounced dead when no heartbeat was detected for 15 sec. The  $LC_{50}$  values for all test samples for 24 h and 48 h duration of treatment were also calculated by probit analysis (Fisher and Yates, 1974). Significant differences between the groups were determined by one-way ANOVA at  $P < 0.05$ . MATLAB ver. 7.0 (Natick, MA, USA) and Microsoft Excel 2007 (Roselle, IL, USA) were used for the statistical and graphical evaluations.

### 3. RESULTS

Fig. 1 demonstrates that As(V) caused extensive mortality in *C. dubia*. For both 24 hours and 48 hours treatment periods, the mortality rates were increased significantly ( $P < 0.05$ ) with increase in As(V) concentrations. Additionally, As(V)-induced mortality in *C. dubia* was increased with the time of exposure to the metalloid.



**Figure 1.** Percentage mortality in *C. dubia* due to As(V) exposure (to diverse dosages) for 24 and 48 h. Data were tested for significant difference between groups at  $P < 0.05$ .  $IC_{50}$  values for the treatment groups were evaluated by probit analysis.

Significantly ( $P < 0.05$ ) higher percentages of mortality were observed for all concentrations in the 48 hours exposure group in comparison to the 24 hours exposure group. As(V) LC<sub>50</sub> values for the 24 h and 48 h test groups were respectively 51 and 20.4 µg/L. Since a smaller LC<sub>50</sub> value denotes higher lethality, it was confirmed that a longer exposure to As(V) caused more mortality in *C. dubia*. Time of exposure and the concentration of the toxicant, both affect the experimental organisms significantly.

The results of the current study clearly revealed that *C. dubia* is highly sensitive towards As(V)-induced toxicity. Hence, *C. daphnia* can be used as a biomarker for As pollution of aquatic ecosystems. The permissible limit for As in water is 10 ppb (10 µg/L), as recommended by the World Health Organization (WHO 2001). *C. daphnia* was found to show up to 15 % and 45 % mortality at this threshold concentration for 24 and 48 h exposure periods respectively. Thus, the species was observed to be considerably sensitive to As in the form of arsenate As(V).

#### 4. CONCLUSION

The current study reports for the first time the toxicity of As(V) in *C. dubia* with respect to varying dosages and exposure periods. In this context, we propose that the crustacean might be used as a model biomonitoring system for detecting As contamination in water. Such biomonitoring might not only be limited in testing samples from aquatic ecosystems, but may also be extended towards screening groundwater and/or drinking water samples in As-prone areas. However, further research is in prospect to determine of As susceptibility of *C. dubia* when in combination with other aquatic toxicants.

#### Abbreviations

As – arsenic; As(V) – pentavalent arsenic; LC<sub>50</sub> – lethal concentration 50.

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