# Effects of Arsenite on the Caspase 3/7 Activity of Daphnia magna

### Introduction

Due to years of smelter pollution, arsenic is found in high levels in some lakes in the South Puget Sound. Arsenic is a known human carcinogen, pervasive in freshwater ecosystems across the globe, though its mechanisms of action are still unclear. Previous studies in mammalian cells *in vitro* revealed that chronic exposure to arsenite (As III), in low to moderate concentrations inhibits caspase

activity, highlighting a possible connection to tumorigenesis which warrants further investigation. Thus, **this study aimed to determine** if As III exposure would produce a similar inhibitory effect of caspase 3/7 activity in invertebrates using the model organism *Daphnia magna*, a freshwater planktonic crustacean.



# Methods Daphnia magna cultured in ADaM Sublethal dose determined 48 hour exposure to As III 0.2 μg/ml 0.02 μg/ml 0.002 μg/ml 0.002 μg/ml 0.002 μg/ml 0.002 μg/ml D. magna protein extractions Bicinchoninic acid (BCA) Protein Assay Caspase-Glo 3/7 assay Absorbance measured and compared to BSA standard to determine protein concentration Luminescence measured to determine caspase 3 and caspase 7 enzyme activity

### Assays

A BCA assay was performed to create a standard curve of protein concentration, which was used to standardize Caspase 3/7 activity observed through the Caspase-Glo luminescence assay.



Caspase-Glo® 3/7 Assay

## Results & Discussion

Caspase activity of the acute exposure was significantly lower than the chronic exposure. Acute exposure to As III was shown to have no significant inhibitory effects on caspase activity in *D. magna* (Figure 1). Statistical analysis using one-way ANOVA resulted in a p-value of 0.46.

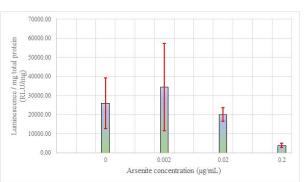


Figure 1.

Caspase 3/7 activity in D. magna protein extraction after 48-hour exposure to varying concentration of Arsenite (As III). A t-test found no significant difference between caspase 3/7 activity of exposure groups and control.

One-way ANOVA analysis found no significance between caspase 3/7 activity of groups (n= 4, p=0.46). Error bars represent standard error.

Chronic exposure of As III significantly decreased Caspase 3/7 activity in *D. magna* in the low and high As III concentrations. Caspase activity in the arsenite-exposed samples were lower than the control. Within the chronic exposures, the lower arsenite concentrations (0.002 and 0.02 mg/mL decreased caspase activity, while the highest concentration, 0.2 mg/mL increased caspase activity (Figure 2). Statistical analysis using one-way ANOVA resulted in a p-value of 0.004.

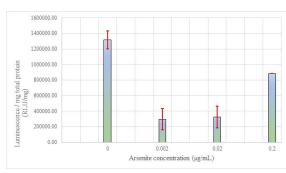


Figure 2. Caspase 3/7 activity in *D. magna* protein extraction after seven day exposure to varying concentration of Arsenite (As III).

A one-way ANOVA analysis found a significant effect of As III concentration on caspase 3/7 activity of *D. magna* under chronic conditions (n= 4, p=0.0046). Error bars represent standard error.

### References

To view the references used, please scan the following QR code:



