Arsenate Toxicity and Hemoglobin Response in *Daphnia magna*

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INTRODUCTION:

Arsenate (As V): A potent pollutant in the South Puget Sound area due to the plume of the industrial ASARCO smelter which operated for nearly 100 years.[7]
Arsenate has been known in organisms to cause diabetes, cardiovascular disease, developmental effects, cancers, and problems in hematological processes.[4,5]

ASARCO Smelter Tacoma, WA.[1]

Daphnia magna: Freshwater zooplankton well researched for environmental toxicity as an indicator species.

- Entire genome sequenced
- High populations and fast reproduction cycle

Daphnia Hemoglobin:
- Produced in environments characterized by hypoxia/anoxia
- Increases aerobic activity within the organism
- Colors organism red
- Four known hemoglobin involved genes in the organism: Dhb1, Dhb2, Dhb3, and Dhb4.[3]

Daphnia hemoglobin production displayed between two daphnia.[2]

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METHODS AND DATA ANALYSIS:

Experiment Methods:

Establish sub-lethal As V Concentrations:
- Small to large concentrations: measure mortality to make experimental concentrations.

Acute Exposure:
•3 and 24-hour exposures to Arsenate at 0.00, 0.50, and 5.00 µg/ml.
•Two replicates for each condition.
•Five Daphnia for each replicate (60 Daphnia in total.)

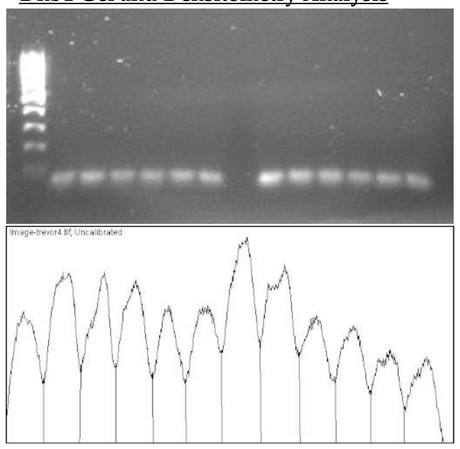
RNA Extraction and cDNA Synthesis:
•Trizol reagent procedure.[8]
•Superscript III First-Strand Synthesis.[6]

•PCR and Gel Electrophoresis:
•Target Genes: Dhb1, Dhb2, Dhb3.
•Housekeeping genes: β-Actin and GAPdh.

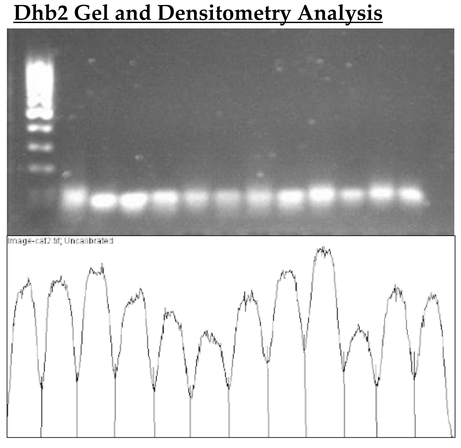
DATA ANALYSIS:

ImageJ Densitometry and Peaks Analysis:
•Integration of peaks to create quantitative data for analysis

Dhb1 Gel and Densitometry Analysis

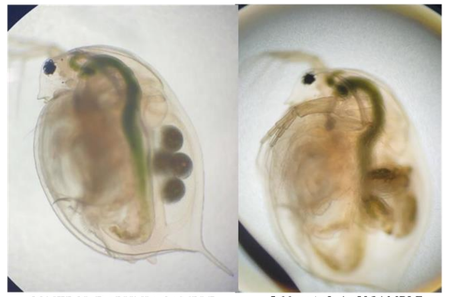


Dhb2 Gel and Densitometry Analysis

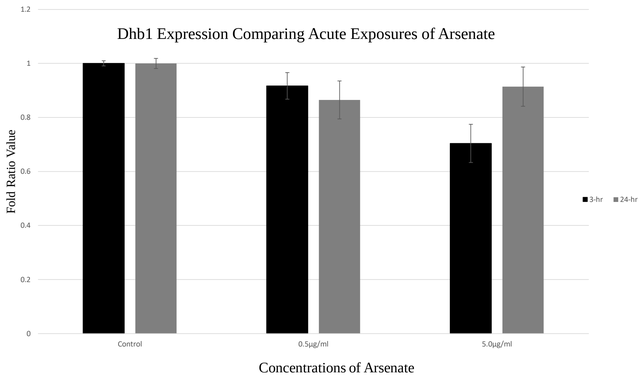


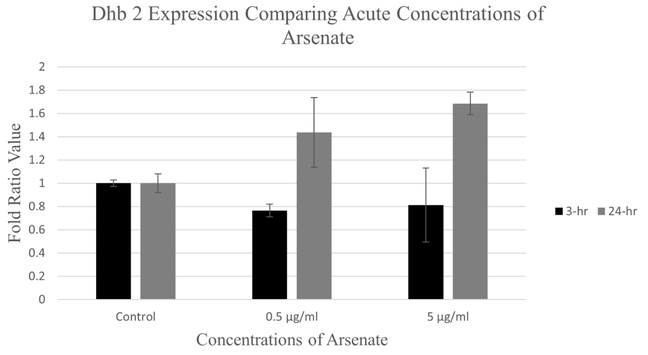
Statistical Significance:
•Normalized band area (target/housekeeping)
•Fold ratio (treatment area/control area)
•T-test
•Peaks represent the intensity of expression of genes shown by the fluorescence of gels in gel electrophoresis image

RESULTS



CONTROL DAPHNIA SAMPLE 5.00 µg/mL As V SAMPLE





Note: Dhb3 gene Gel electrophoresis was not successful and given time of failure during quarter, revisitation was not possible. Dhb3 is not included within the results.

Observational:
- Samples viewed under light microscope between control and most notable physical change.
- Left: Sample Daphnia from control population (no arsenate).
- Right: Experiment results sampling most notable changes in hemoglobin production.
- Very minimal phenotypical changes between control and treatment

Dhb1 Statistics:
3-hr Exposure:
•P-Values for both concentrations are greater than 0.05
- (Not statistically significant)
24-hr Exposure:
•P-Values for both concentrations are greater than 0.05
-(Not statistically significant)

Dhb2 Statistics:
3-hr Exposure:
- P-value for 0.5µg/ml As V = 0.049762494
- P-value for 5µg/ml As V = 0.569667125
- 0.5µg/ml value less than 0.05
-(Statistically significant)
- 5µg/ml values greater than 0.05
-(Not statistically significant)

24-hr Exposure:
- P-value for 0.5µg/ml As V = 0.36204332
- P-value for 5µg/ml As V = 0.033263683
- 0.5µg/ml values greater than 0.05
-(Not statistically significant)
- 5µg/ml value less than 0.05
-(Statistically significant)

Discussion and Conclusion:

Hemoglobin Genes:
•Dhb1 gene was not significantly expressed different than control in all conditions.
•Dhb2 gene was significantly expressed different than control under two conditions.
•With genes monitored only two cases had significance (3-hr exposure: 0.5µg/ml to control, 24-hr exposure: 5µg/ml to control)

Observational:
•Expression results combined with observed changes in coloration conclude no significant effect on hemoglobin occurred in As V exposures.

General Understanding:
- Arsenate toxicity seen in this experiment has no clear effect on the hemoglobin production of *D. magna.* Findings were unclear.

Future Experiments:
- If experimentation were to continue, having daphnia produce hemoglobin prior to exposure using anoxic/hypoxic environments would allow for further findings on effects of Arsenate on this process.
- Revisiting Dhb3 and analyzing Dhb4 as well as 1 and 2.