Arsenate exposure and hemoglobin gene expression *in D. magna*

Cat Bond, Trevor Cook, Cynthia Chand, Dr. Alison Gardell

University of Washington Tacoma

Abstract

The metal-refining smelters that were present in the Tacoma area are the main cause of arsenic toxicity in both the soil and water around the Puget Sound region. Arsenic is a chemical element that is known for its negative health effects in both wildlife and humans, as it is associated with several different types of cancer. The negative health effects caused by arsenic poisoning can be correlated to its ability to bind to hemoglobin, a protein in the blood containing iron that binds to oxygen in order to provide nutrients to cells in the body. This led us to propose that arsenic exposure could potentially affect the hemoglobin levels in *Daphnia magna* by altering gene expression. We hypothesized that DNA analysis would point towards a significant change in the level of hemoglobin gene expression in the Daphnia. Six groups of adult *D. magna* were exposed to 0 µg/mL, 0.02 µg/mL and 0.2 µg/mL of arsenate and were exposed for three hours to measure the effect of acute toxicity. The Daphnia from each of the groups were then crushed and RNA was extracted using a reagent called Trizol to produce complementary DNA via PCR. This process was then repeated with a different population of Daphnia to measure the effect of chronic exposure to arsenate. The DNA samples were then analyzed using gel electrophoresis, where we observed the genes *Dhb1*, *Dhb2*, and *Dhb3*. The hypothesis proposed was supported in this study, as the gene expression of the hemoglobin gene *Dhb2* was significantly affected in both exposure groups in that the acute exposure group exhibited significantly lower gene expression while the chronic exposure group exhibited significantly higher gene expression.

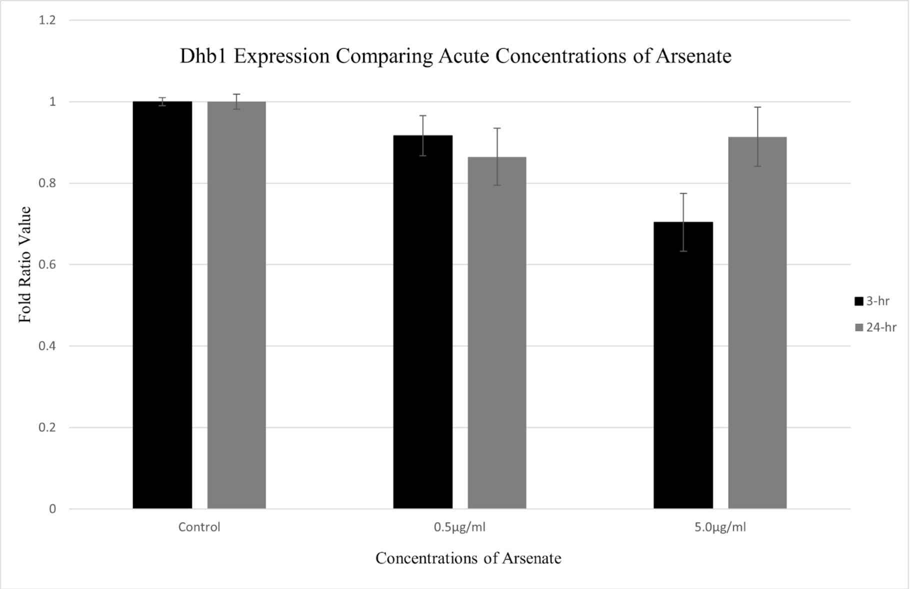
Methods

Six groups of five adult *D. magna* were exposed to concentrations of 0 $\mu g/mL$, 0.5 $\mu g/mL$, and 5 $\mu g/mL$ of arsenate. These Daphnia were left for three hours, and then frozen with nitrogen. The Daphnia were crushed, and RNA was extracted using the reagent Trizol. The RNA collected was then used to create complementary DNA using PCR. We then used gel electrophoresis to examine the expression of the genes *Dhb1*, *Dhb2*, and *Dhb3*. This procedure was then repeated with an exposure time of 24 hours.

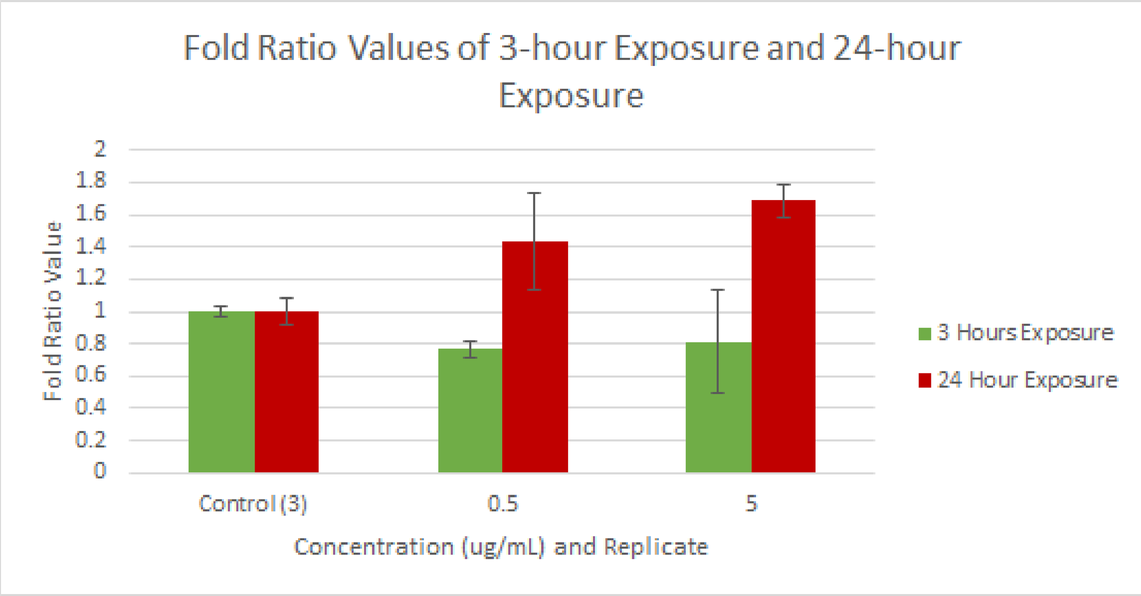
Discussion

The results determined that there was a fluctuation in gene expression between the acute and chronic exposure groups. A physical change within the Daphnia was observed in the high toxicity chronic exposure group. The Daphnia were colored pink, which is a physical indication of increased hemoglobin concentrations. Statistically significant data in the *Dhb2* gene found that gene was downregulated in the acute exposure group, while the chronic exposure group showed an upregulation. This could potentially mean that hemoglobin expression affected by arsenic toxicity could differ based on the length of exposure, possibly due to a feedback mechanism that responds to the amount of hemoglobin bound to arsenic. As cells start to experience a lack of nutrients due to hemoglobin inhibition by arsenic, gene expression of hemoglobin may increase as arsenic toxicity becomes chronic by a positive feedback mechanism. More research is needed to determine why exactly gene expression decreases because of acute toxicity.

Dhb1 Gene Analysis



Dhb2 Gene Analysis



Dhb3 Gene Analysis: Inconclusive

References

Eytcheson, S. A., & LeBlanc, G. A. (2018). Hemoglobin Levels Modulate Nitrite Toxicity to Daphnia magna. Scientific Reports, 8(1). https://scholar.google.com/scholar?hl=en&as_sdt=0%2C48&q=daphnia+hemoglobin+and+environmental+factors&btnG=#d=gs_qabs&u=%23p%3D-QL51bgixeQJ

Ha, M., & Choi, J. Effects of environmental contaminants on hemoglobin gene expression in Daphnia magna: a potential biomarker for freshwater quality monitoring. Archives of environmental contamination and toxicology. https://pubmed.ncbi.nlm.nih.gov/19471991/

Hull, E. A., Barajas, M., Burkart, K. A., Fung, S. R., Jackson, B. P., Barrett, P. M., Neumann, R. B., Olden, J. D., & Gawel, J. E. (2021). Human health risk from consumption of aquatic species in arsenic-contaminated shallow urban lakes. Science of The Total Environment, 770, 145318. https://doi.org/10.1016/j.scitotenv.2021.145318

Moos, M., Nizar, M., & Givens, L. (2021, August 18). Arsenite toxicity and Caspase Enzyme Production in Daphnia Magan [Slides]. PowerPoint.

Theegala, C. S., Suleiman, A. A., & Carriere, P. A. (2007). Toxicity and biouptake of lead and arsenic by Daphnia pulex. Journal of Environmental Science and Health, Part A, 42(1), 27–31.https://scholar.google.com/scholar?hl=en&as_sdt=0%2C48&q=daphnia+and+arsenic&oq=daphnia+and+ar#d=gs_qabs&u=%23p%3D8Xt9QJ53rxAJ

Wang, N., Liu, Y., Wei, Z., Yang, L., & Miao, A. (2018). Waterborne and Dietborne Toxicity of Inorganic Arsenic to the Freshwater Zooplankton Daphnia magna. Environmental science & technology. 52(15):8912–8919. https://pubs-acs-org.offcampus.lib.washington.edu/doi/abs/10.1021/acs.est.8b02600

Wang, X., Qu, R., Allam, A.A., Ajarem, J., Wei, Z., Wang, Z. (2016). Impact of carbon nanotubes on the toxicity of inorganic arsenic [AS(III) and AS(V)] to Daphnia magna: The role of certain arsenic species. Environmental toxicology and chemistry. 35(7):1852–1859. https://setac-onlinelibrary-wiley-com.offcampus.lib.washington.edu/doi/full/10.1002/etc.3340