

### Abstract

The increased use of fracking and coal mining methods to excavate natural resources has, in effect, increased the amounts of total dissolved solids (TDS) found in freshwater streams and rivers. Higher concentrations of TDS indicate a higher trace of conductivity, which depicts the water's ability to conduct electricity. The main objective of this study is to perform a chronic toxicity test in order to determine the highest "safe" or "no effect" concentration of conductivity in the water and determine the concentration with the biggest mortality rate. The test organisms used for this experiment were Daphnia magna, a common water flea used in chronic tox tests due to their quick maturity and sensitivity to water quality. Three experiments were performed each over a four day period. In that time water chemistry was recorded daily and each concentration was replaced with a fresh solution. Results concluded that once concentrations reached 60% they became unviable for majority survival. More toxicity studies should be done to further verify the results given.

### Introduction

Various means of extracting natural resources such as coal mining and fracking has resulted in massive increases of total dissolved solids in lakes and rivers. This increases salinity as well as conductivity levels. One such effect caused by fracking excavations was seen throughout a 37-mile-long stream running through Pennsylvania and West Virginia. Dunkard Creek (Figure 1 and Figure 2), as it was called, developed brackish conditions, and cultivated saltwater algae. The freshwater ecosystem quickly transitioned into a harsh environment for native species. The Zimmer power plant residing on the Ohio river has caused an increase in total dissolved solids on the water due to coal burning. Concentrations of magnesium sulfate (MgSO<sub>4</sub>) were used in this experiment to imitate coal mining water effects and calcium chloride (CaCl<sub>2</sub>) was used to replicate fracking water conditions; with each salt being the most common one found in their respective methods.



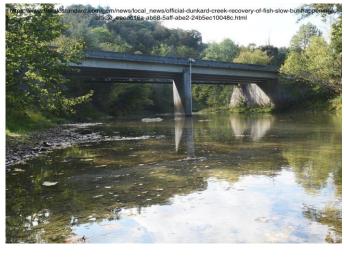
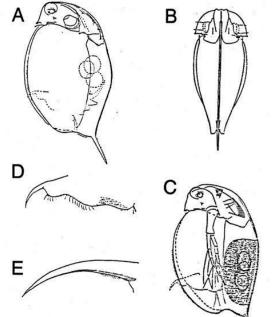


Figure 1. Fish killed by affected area of Dunkard Creek 2009

Figure 2. Recovery zone of Dunkard Creek 2009 harmful algal bloom

Daphnia magna, a popular toxicity test organism, is commonly found in freshwater. They belong to phylum arthropoda, subphylum crustacea. Due to their condensed life cycles, cultures are quickly grown and utilized in chronic tests for their rapid maturity.



# **Determination of Potential Impact of Fracking and Coal-Mining Contaminants on Daphnia magna** Luis E. Tron Esqueda<sup>1</sup>, Sarah R. Gibson<sup>2</sup> and Chris Lorentz<sup>2</sup>, Ph.D

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## Methodology

Daphnia magna less than eight hours old were collected from the United States Environmental Protection Agency at Cincinnati, Ohio. Base solution made from average toxicant concentration were made. A base solution for CaCl<sub>2</sub> was originally made with 14.7g in 1L of water for the first test, for the second and third test a base solution was made with 0.9231g in 1L of water. The base solution for MgSO<sub>4</sub> was made using 1.2243g in 1L of water. For our experiment we made 50 mL porcentage solutions starting at 20% up to 80% in increments of 20. After dilutions were made, we pipetted five Daphnia magna into each cup. Four sets of five animals each were separated, dry, and weighed to establish initial weight.

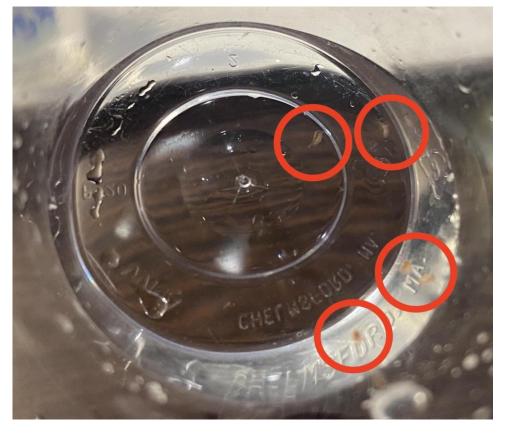


Figure 4. Experiment III test organisms D. magna

The animals were fed 0.3 mL algae and 0.2 mL alfalfa. Then the cups were covered with plastic wrap at the top. They were then placed in a 25°C incubator. After a 24-hour period, the animals were transferred to a fresh solution. During transferring animals were counted and deaths were recorded. Routine chemical measurements were taken on both old (final) and renewal (initial) solutions. Routine chemical measurements include temperature, dissolved Oxygen, conductivity, and pH. Onces animals were in a fresh solution, they were covered and fed. This was done over the course of four days. Once the four days passed, we tared identified aluminum drying pans and we placed the corresponding cups Daphnia in them. They were left to dry in a a 60°C oven for 24 hours. After the 24 hour period, the pans were let to dry for two hours before weighing them.



Figure 5. Initial *D. magna* separation and pipetting



Figure 6. Water renewal within D. magna cups

Our experimental procedure was replicated a total of three times with the purpose of getting reliable and comparable data. The three replications were done during the weekdays of three consecutive weeks.

Our first replication was voided due to our survival rate being less than the permissible amount of 90%. Even though it was voided we were able to noticed that the higher concentration of CaCl<sub>2</sub> had a 100% mortality rate in the first day indicating that fracking water is not viable for survival. Our second replication was voided due to the growth rate not being viable, as it was less than 10x the initial weight. Finally our third replication was successful with a survival rate of 90% and a growth more than 10x bigger than our initial weight.

On Figure 8 we demonstrate the percentage of deaths based of the different concentrations of each toxicant. In this figure we can notice that the highest mortality ratio was at 60% CaCl<sub>2</sub> with a 45% death rate. Both toxicants have have comparable conductivity at 20,40, and 60% but had differentiable conductivity at 80% with  $CaCl_2$  having the highest conductivity as demonstrated by Figure 9.

### Results

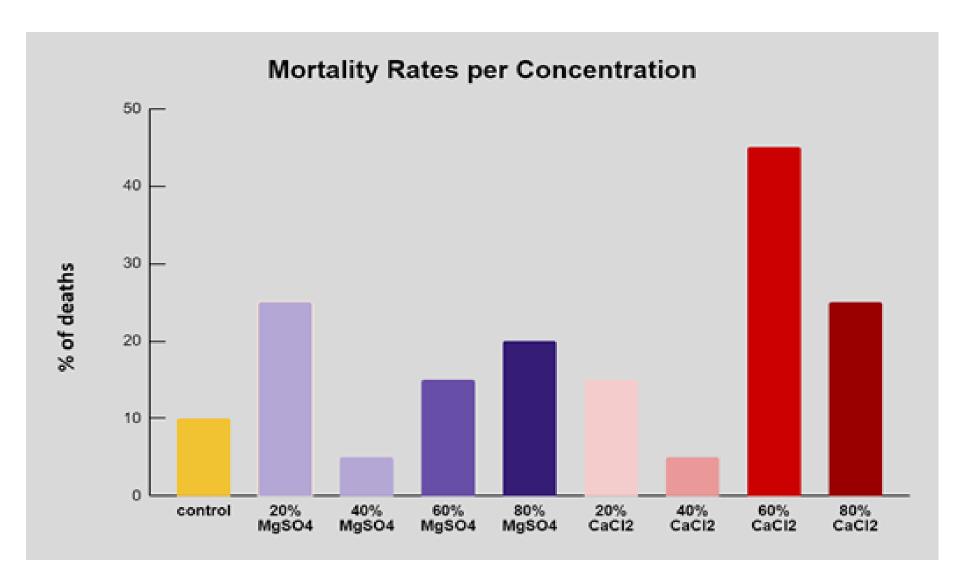


Figure 8. The percentage of deaths per concentration.

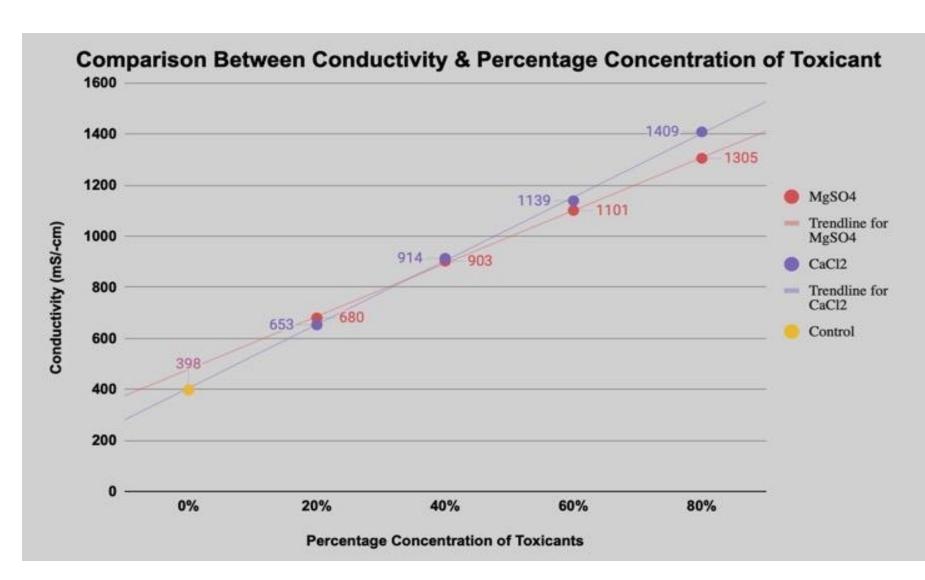


Figure 9. The percentage concentration of each toxicant vs. their respective conductivity.

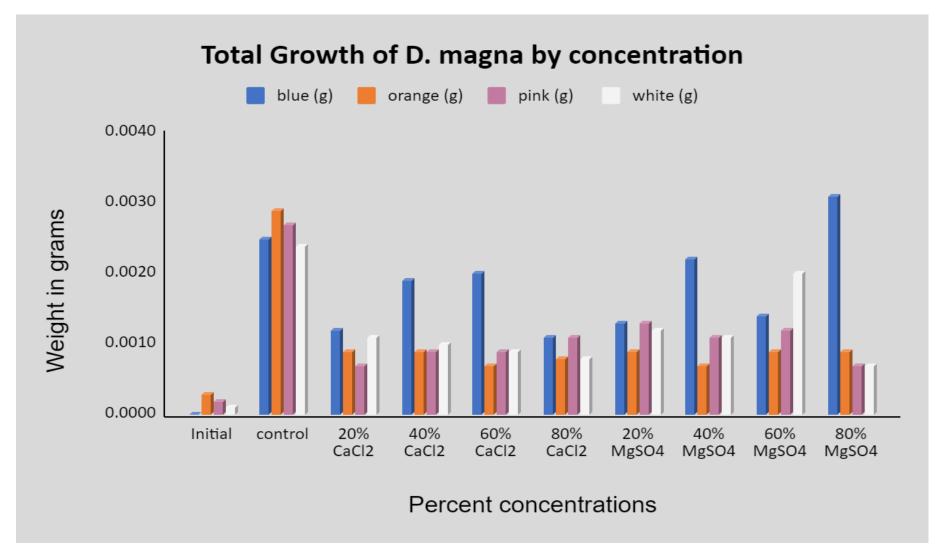


Figure 10. The percentage concentration of each toxicant and their growth to the initial weight.

**Results Cont.** During our third trial, the *Daphnia* in the control group grew more than 10x our initial weight. The biggest growth in our CaCl<sub>2</sub> was the 40% concentration and for MgSO<sub>4</sub> it was 60% concentration as demonstrated by Figure 10. On the same figure we can also notice a bigger growth rate in blue cups at all concentrations with control group being an exception.

The results indicate that the most lethal concentration is at 60% in both toxicants used. The conductivity range for this concentration is 1100-1150 mS/-cm, suggesting conductivity over 1100 mS/-cm to be toxic according to the final data. Specifically, 60% CaCl<sub>2</sub> produced the highest mortality ratio in comparison to the other concentrations, followed by 80% CaCl<sub>2</sub> and 80% MgSO<sub>4</sub> as seen in figure 8. This correlates with the data seen in figure 9 relating conductivity levels exponentially by concentration. 80% CaCl<sub>2</sub> similarly had the lowest growth rate furthermore suggesting an unviable concentration. The high mortality rate in 20% MgSO<sub>4</sub> was caused by poor handling of the animals during the transferring process, as two of them died. It was observed that the blue experimental samples, excluding the control, all had a higher growth rate when compared to the identical concentration samples. It can be speculated the increased growth was caused by the position effect altering the amount of light received. Therefore concluding that highly contaminated water caused by fracking and coal mining indicates to be an unviable habitat for animals that are as sensitive as *Daphnia magna*.

. Armstead MY, Bitzer-Creathers L, Wilson M. The effects of elevated specific conductivity on the chronic toxicity of mining influenced streams using Ceriodaphnia dubia. PloS one. 2016;11(11):e0165683.

. Steve Ferris, for the Greene County Messenger. Official: Dunkard Creek recovery of fish "slow, but happening." Heraldstandard.com. 2016 Oct 7 [accessed 2021 Jul 23].

https://www.heraldstandard.com/gcm/news/local\_news/official-dunkard-creekrecovery-of-fish-slow-but-happening/article\_eeeac16a-ab68-5aff-abe2-24b5ec10048c.html

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. U.S. Environmental Protection Agency, Office of Water, Washington, DC. (Fifth Ed.) EPA-821-R-02-



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### Conclusions

### **Literature Cited**

### Acknowledgements

# **For Further Information**



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