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## Effects of Copper Sulfate on Mortality Rates of *Daphnia magna*

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EFFECTS OF COPPER SULFATE ON MORTALITY RATES OF

*DAPHNIA MAGNA*

By

Grace Vilem

## **Abstract**

Many reservoirs used for drinking water end up growing algae that needs to be removed. Copper sulfate ( $\text{CuSO}_4$ ) can be used to reduce the growth of algae, however this chemical has been shown to have negative effects on invertebrates living in aquatic environment. This experiment exposed *Daphnia magna*, an aquatic zooplankton, to various concentrations of  $\text{CuSO}_4$  for 48 hours to determine an LC50 value. Six sets of data were collected and averaged, finding an LC50 value of 36  $\mu\text{g/L}$ . Further testing is needed to determine effects of  $\text{CuSO}_4$  on fecundity between generations of *Daphnia*, as well as effects that *Daphnia* mortality may have on other organisms.

## **Introduction**

Reservoirs are an important source of drinking water throughout the world. Upground reservoirs are manmade bodies of water with water being pumped into it from a river or creek because the landscape relief is insufficient to produce an in-stream (Hale et al. 2006). These bodies of water also have an auxiliary function for sports fisheries. Management practices to assist in preserving drinking water may conflict with this function and the practices that help sustain a healthy aquatic ecosystem. (Duvall et al. 2001, Mischke et al. 2009). One of these conflicting practices is the application of copper sulfate as an algal control.

Copper sulfate ( $\text{CuSO}_4$ ) has been used as an algaecide for over 100 years and has been found to be effective in controlling algal blooms (Moore and Kellerman 1905). However, it has been shown to cause issues in aquatic environments. After treatment with  $\text{CuSO}_4$ , the amount of copper in the sediment can increase significantly and can still be

present after 30 days (Hullebusch et al. 2003). CuSO<sub>4</sub> has also been found to cause toxicity in vascular plants, (Muller et al. 2001, Mal et al. 2002), zooplankton (Havens 1994a), oligochaetes (Meller et al. 1998), and numerous macroinvertebrates (Kosalwat and Knight 1987, Warrin et al. 2009). CuSO<sub>4</sub> application in reservoirs has currently posed no threat to humans, however, with the EPA limiting copper concentrations to 1,000 µg/L (USEPA 2002).

*Daphnia magna*, a freshwater zooplankton, has been proven to be advantageous in toxicity studies. These advantages include “easy handling, sensitivity to many toxic compounds, ubiquitous occurrence, parthenogenetic reproduction, high fecundity and short life span” (Koivisto, S., Ketolla, M. 1994) *Daphnia* are also of value ecologically as they are an important food source to many freshwater fish.

The purpose of this study was to determine the effects that copper sulfate can have on the environment when used as an algaecide. Previous experiments done on *Daphnia* have shown detrimental effects from 22 to 32 µg/L and significant mortality from 18.5 µg/L in harder water (Dave, G 1983) and 60 µg/L in softer water (Beisinger, Christianson 1972). As the current EPA limit for copper concentrations is 1,000 µg/L, this experiment was designed to examine the effects copper sulfate can have on *Daphnia magna* mortality by exposing them to 0 to 100 µg/L concentrations of copper sulfate and calculating an LC50 value, or the value of concentration that the mortality rate reaches 50%. This data was then compared to the Screening Quick Reference (SQuiRT) Table values for acute and chronic copper values which are used to determine levels that will affect aquatic organisms such as mortality and deformities

## Materials and Methods

Water was collected from the tap in the BGSU Life Sciences building and left to dechlorinate for 24 hours. 14.415g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was added to one liter of deionized water, and 10 mL of this solution was added to 990 mL of deionized water. 2.22, 4.44, 6.67, 8.33, 10.00, 11.67, 13.33, 33.34, and 55.56 mL of the second solution was added to enough dechlorinated tap water to make one liter of total solution, creating concentrations of 8, 16, 24, 30, 36, 42, 48, 120, and 200  $\mu\text{g/L}$ . The solutions were poured into plastic containers. The control received no copper sulfate. Approximately 20 *Daphnia magna* retrieved from the BGSU Greenhouse were added to each container of solution. The containers were left to sit for 48 hours in an environmental chamber at 20°C. The *Daphnia* were then exposed to an iPhone 6S flashlight. This helped to determine the living organisms by stimulating them and causing them to move. The living and dead *Daphnia* were counted and the mortality rate was calculated.

$$(\text{dead})/(\text{living} + \text{dead})$$

Two data sets were run during each trial and six data sets in all were collected. The data sets were then averaged and compared to Screening Quick Reference (SQUIRT) Tables.

## Results

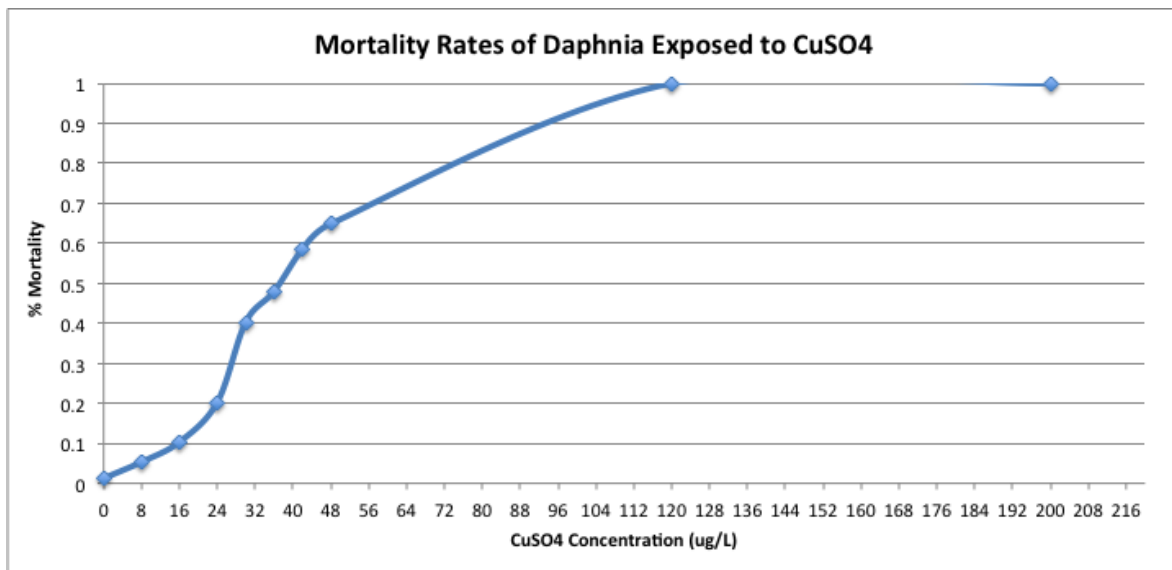
The acute effects are shown in Table 1. The data showed a higher standard deviation, which is most likely due to the higher number of floaters and the higher amount of disturbance in the earlier trials leading to slightly different data.

The graph was calculated by averaging each of the six mortality rates per concentration to create a mortality curve and find the LC50 value which occurs when mortality reaches fifty percent.

The LC50 rate was 36 ug/L with 100% mortality occurring at 120 ug/L. The SQuiRT table shows a chronic toxicity level of 9ug/L and acute toxicity level of 13 ug/L, which aligns with the data collected, as the mortality rate begins to spike at approximately 16 ug/L

**Table 1:** Mortality rate of CuSO<sub>4</sub> concentrations for each trial compared to the calculated average mortality rate with the standard deviation.

Concentration	Trial 1 Mortality	Trial 2 Mortality	Trial 3 Mortality	Trial 4 Mortality	Trial 5 Mortality	Trial 6 Mortality	AVG Mortality	Standard Deviation
0	0	0	0.076923077	0	0	0	0.012820513	0.031403715
8	0.166666667	0	0.090909091	0.0625	0	0	0.05334596	0.067636887
16	0.230769231	0.166666667	0.055555556	0	0	0.166666667	0.103276353	0.097877865
24	0.227272727	0.25	0.285714286	0.142857143	0.166666667	0.142857143	0.202561328	0.060320977
30	0.4375	0.333333333	0.363636364	0.352941176	0.428571429	0.5	0.402663717	0.063500072
36	0.5	0.333333333	0.428571429	0.416666667	0.6	0.6	0.479761905	0.107113753
42	0.571428571	0.476190476	0.75	0.615384615	0.5	0.6	0.585500611	0.097591411
48	0.7	0.666666667	0.636363636	0.642857143	0.583333333	0.666666667	0.649314574	0.039329765
120	N/A	N/A	1	1	1	1	1	0
200	N/A	N/A	1	1	1	1	1	0



**Figure 1:** The averaged mortality rates of CuSO<sub>4</sub> exposure. The LC50 value is at approximately 36 ug/L and the mortality rate reaches a consistent 100% at 120 ug/L.

## Discussion

The tap water hardness value for Ohio is approximately 119 mg/L. This value is between the hardness values reported by Dave, 1983 and Beisinger and Christianson, 1972. The LC50 rate, or the concentration value of the concentration which mortality reaches fifty percent for this experiment reflect this with a value of approximately 36 µg/L. As these values reflect environmentally relevant levels, this experiment shows that killing algae with CuSO<sub>4</sub> can have negative effects on the *Daphnia* in reservoirs. *Daphnia* are hold a central role in the food web where they both consume producers and are consumed by many predators (Lampert, W. 2006) so a decrease in these zooplankton could lead to a disruption of the food web.

This data also aligns with the SQuiRT table values as these tables show that one might see effects around 9-13  $\mu\text{g/L}$ , with the experimental data showing effects around this period.

Results may have been affected by the small space being used to work in, as well as agitation of the containers holding the *Daphnia*. More  $\text{CuSO}_4$  concentrations between 48 and 120  $\mu\text{g/L}$  would have also created a better representation of the data as there would be a smaller data gap between these values as there were between the earlier ones.

Further research is needed to better understand the effects that  $\text{CuSO}_4$  can have on future generations of *Daphnia*, as well as the effects of a *Daphnia* population decline on their predators.



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