Effects of Different Cobalt (II) Sulfate Concentrations on Developing Zebrafish Embryos

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Abstract:

This experiment was conducted to test the effects of different concentrations of cobalt (II) sulfate on developing zebrafish embryos. This was done by exposing fertilized zebrafish embryos to different concentrations of the toxicant over a 72 hour period. The concentrations tested were; a control group, 0.2 mg/L concentration solution, and 0.8 mg/L concentration solution. As the embryos grew, the number of living fish and the number of hatched fish were recorded every 24 hours to observe the effects the toxicant had on survival and hatching rates of the embryos. Any anatomical abnormalities were also observed and recorded. After 72 hours, the numbers of living and hatched embryos in each testing group were compared and the results suggested that at these concentrations, cobalt (II) sulfate had no effect on either the survival or hatching rates of embryos. There were also no anatomical differences between the control group and the tested groups that were observed. This suggests that at these concentrations, cobalt (II) sulfate would have no harmful effects on human development, as well.

Introduction: Cobalt (II) Sulfate is found in many naturally occurring rocks and minerals (CDC, 2015). It is essential to human life in small doses, specifically in the vitamin B12 (CDC, 2015). Although Cobalt (II) sulfate is needed for human survival, it has also been shown to have damaging properties if consumed or inhaled at great levels (Szakmáry et. al, 2001). It has been known to cause cardiomyopathy as well as lung diseases in people that have inhaled large quantities (NIH, 2003). It has also been shown that large-scale consumption can have damaging effect on the heart and thyroid, as well as cause cancer in animals, but this has not been seen in humans (Szakmáry et. al, 2001). If a pregnant mother is exposed to cobalt (II) sulfate, the fetus will also be exposed, but it has not been proven that negative effects on the fetus occur (Cai et. al, 2012).

This experiment was performed to see the effects of large quantities of cobalt (II) sulfate on the survival and hatching rate of zebrafish embryos and whether different concentrations will have different effects on these rates. The question posed for this experiment asked, “how will different concentrations of cobalt (II) sulfate affect the survival and development of zebrafish embryos?” Zebrafish embryos were used because the development is similar to that of a human, but is much faster, as well as being external to the mother (Burke, 2016). Zebrafish embryos also are transparent and mature at the same rate, so it is easy to see developmental delays or problems (Burke, 2016). The hypothesis for this experiment states that cobalt (II) sulfate will cause delayed embryonic development, as well as lower survival rates.

Materials:

- 120 fertilized zebrafish embryos
- Stock solutions of Cobalt (II) Sulfate (0.2 mg/L, 0.8 mg/L)
- Beaker for waste disposal
- Clean well plate and cover
- 1 bottle Instant Ocean Solution
- 4 disposable pipettes
- 28.5 C incubator

- Labelling tape
- Labelling marker
- Microscope slide with depression
- Compound microscope
- Dissecting microscope
- Picture-taking device

Methods:

Prior to beginning lab, certain safety precautions must be taken. Gloves should be worn when handling cobalt solutions due to possibility of contamination or consumption. Any materials that come in contact with solutions should be kept away from face and mouth. Although these concentrations are not considered dangerous for human consumption, safety measures should be taken to avoid consumption or contamination.

The procedure used for this lab was based on the suggested procedure created by SEPA through UW Milwaukee. This procedure loosely followed that procedure with a few changes made to adjust the procedure to the chemicals used.

Data outcomes and significance were measured and compared using an unpaired t-test. Only p-values less than 0.05 were considered significant, all other values were considered not significant.

Procedure:

Before Day One:

1) Obtain 3x4 well plate and spray and wipe down with Simple Green cleaning solution. Rinse with water and dry thoroughly with paper towel. Repeat rinsing and drying procedure 5 times.
2) Mix stock solutions of Cobalt (II) Sulfate and instant ocean. Label solutions with appropriate concentrations.

Day One:

1) Obtain rinsed embryos from teacher.
2) Label plate with name/hour, label bottles and wells with correct Cobalt (II) Sulfate concentrations.
3) Place 10 zebrafish embryos in each well, use microscope to verify that they are fertilized. If unfertilized, remove the embryo using a pipette and dispose of it in specified waste disposal site.
4) Prepare slide to view under microscope and record observations of embryo development.
5) Fill 4 control wells with 2 mL clean Instant Ocean using disposable pipette.
6) Fill 8 remaining wells with 2 mL appropriate Cobalt (II) Sulfate stock solutions, 4 wells with 0.2 mg/L solution and 4 wells with 0.8 mg/L solution. Use a different disposable pipette for each solution.
7) Add 5 drops methylene blue to all 12 wells to prevent bacteria from growing.
8) Prepare microscope and slide to view under microscope.
9) Use pipette to place zebrafish embryo on slide and view under microscope. Record initial observations and take pictures to show development.
10) Cover well plates with clean well cover and place in 28.5 C incubator overnight.

Day Two:

1) Remove plate from incubator.
2) Using different pipettes for each solution, remove all waste and dirty solution and place it in the appropriate waste disposal.
3) View all wells under microscope, remove any dead embryos and place them in waste disposal.

4) Record number of live embryos in each well in data table as well as the number of hatched embryos in data table.
5) Prepare slide and view development of 1-2 living control, 0.2 mg/L solution and 0.8 mg/L solution embryos under microscope. Record any observations in development of each. Take pictures of each to show any delayed development between control and other groups.
6) Repeat steps 5-7 with stock solutions, Instant Ocean, and methylene blue.
7) Place well cover back on plate and place back in incubator overnight.

Day Three:

1) Repeat steps 1-7 from day two and record observations.

Day Four:

1) Repeat steps 1-5 from day two and record observations.
2) Place all fish and embryos in waste disposal along with dirty solutions to be properly disposed of.

Data:

Data Table 1:

Number of Living Embryos

	24 hpf	48 hpf	72 hpf
Control	20	18	17
0.2 mg/L	26	25	25
0.8 mg/L	24	21	21

Data Table 1 shows the total number of living embryos between all 4 wells of each solution at 24 hpf, 48 hpf, and 72 hpf.

Figure 1:

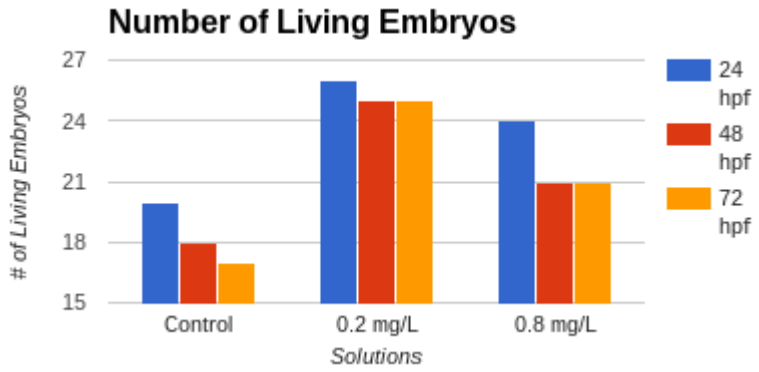

Figure 1 shows the total number of living embryos between all 4 wells of each solution at 24 hpf, 48 hpf, and 72 hpf.

Data Table 2:
Number of Hatched Embryos

	24 hpf	48 hpf	72 hpf
Control	1	2	7
0.2 mg/L	0	0	7
0.8 mg/L	0	0	9

Data table 2 shows the total number of hatched embryos between all 4 wells of each solution at 24 hpf, 48 hpf, and 72 hpf.

Figure 2:

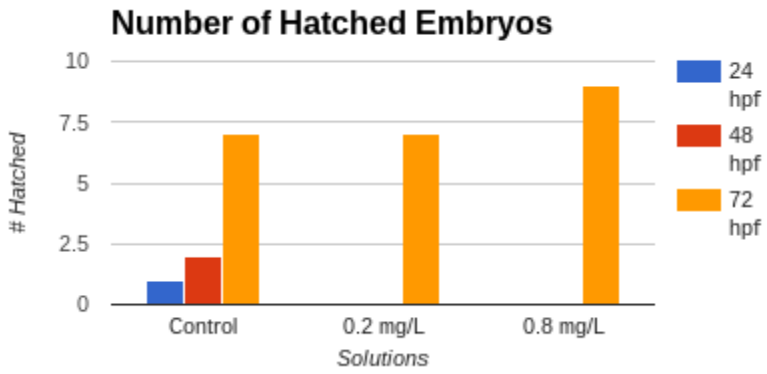

Figure 2 shows the total number of hatched embryos between all 4 wells of each solution at 24 hpf, 48 hpf, and 72 hpf.

Data Table 3:

P-Values and Significance of Unpaired t-test Comparisons

Comparison	P-Value	Significance
Control vs 0.2 mg/L 24 hpf Living	0.3001	Not Significant
Control vs 0.2 mg/L 48 hpf Living	0.3089	Not Significant
Control vs 0.2 mg/L 72 hpf Living	0.2346	Not Significant
Control vs 0.2 mg/L 24 hpf Hatched	0.3559	Not Significant
Control vs 0.2 mg/L 48 hpf Hatched	0.1340	Not Significant
Control vs 0.2 mg/L 72 hpf Hatched	1.0000	Not Significant
Control vs 0.8 mg/L 24 hpf Living	0.3559	Not Significant
Control vs 0.8 mg/L 48 hpf Living	0.4881	Not Significant
Control vs 0.8 mg/L 72 hpf Living	0.3675	Not Significant
Control vs 0.8 mg/L 24 hpf Hatched	0.3559	Not Significant
Control vs 0.8 mg/L 48 hpf Hatched	0.1340	Not Significant
Control vs 0.8 mg/L 72 hpf Hatched	0.6754	Not Significant
0.2 mg/L vs 0.8 mg/L 24 hpf Living	0.7586	Not Significant
0.2 mg/L vs 0.8 mg/L 48 hpf Living	0.6704	Not Significant
0.2 mg/L vs 0.8 mg/L 72 hpf Living	0.6704	Not Significant

Data table 3 shows the unpaired t-test results and p-values of compared solutions, as well as the significance of each value. An unpaired t-test was used to compare the results of each test to determine if results are significant or if they are due to random chance. Only p-values of 0.05 or below were considered significant. All data presented in this experiment proved to be not significant.

Figure 3:

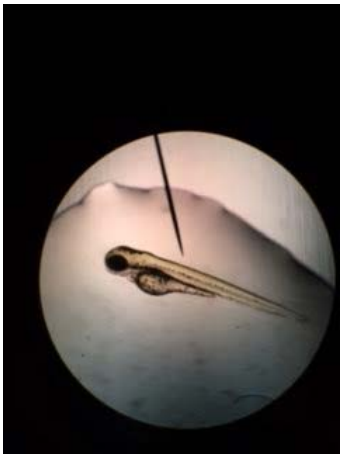

Figure 3 shows a fish treated with the control solution on day 3 of the experiment.

Figure 4:

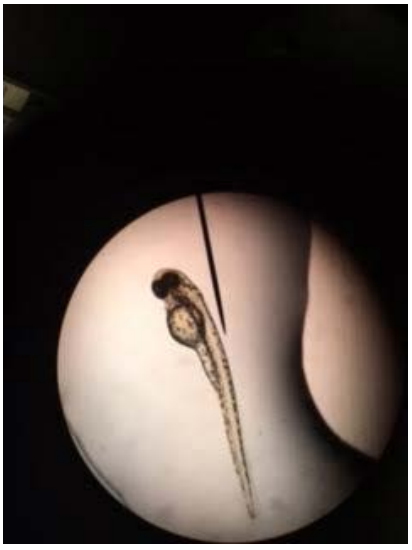

Figure 4 shows a fish treated with 0.8 mg/L solution on day 3 of the experiment.

Results:

This experiment was designed to observe the effects that cobalt (II) sulfate had on the survival and hatching rates of the embryos, as well as to observe any anatomical anomalies that occurred due to the toxicant. This was done by recording the number of hatched and living embryos over the course of three days, as well as taking pictures and writing down observations of developmental abnormalities.

The control group that was tested was grown in instant ocean solution and incubated at 28.5 C. The other groups of embryos that were tested were exposed to 0.8 mg/L and 0.2 mg/L solutions of cobalt (II) sulfate. The dependent variable was the survival of the zebrafish embryos. This variable relied on the independent variable of the solution the embryos were grown in. The independent variables were tested to see if they had an effect on the dependent variables, however in this experiment, the solutions and concentrations had no effect on the survival of zebrafish embryos.

This experiment was completed to determine whether cobalt (II) sulfate had any effects on the survival rates of zebrafish embryos. The data collected in this experiment draws the conclusion that at these concentrations, cobalt (II) sulfate has no effect on the development of zebrafish.

Discussion:

All data found in this experiment was considered not significant. All findings, when compared with an unpaired t-test, had p-values of greater than 0.05. These results suggest that cobalt (II) sulfate had no effect on the survival or developmental rates of zebrafish embryos. The data presented failed to support the hypothesis, due to the nonsignificance of the p-value comparisons. The toxicant had no effect on the survival rate of zebrafish. The 0.8 mg/L concentrated solution had a 52.5% survival rate by the end of the experiment compared to the 42.5% survival rate of the control group of embryos this had no significance when compared using a t-test.

Error and limitations may have had effects on the results of the experiment. It was impossible to see whether the toxicant had any physiological effects such as cardio or neuro malfunctions. It was only possible to see physical effects of the toxicant due to the equipment available. As with any experiment, error could have occurred, therefore, it is suggested that this experiment be repeated to confirm results.

Although the data presented in this experiment was considered not significant, data gathered in other experiments has suggested that cobalt (II) sulfate has negative effects on embryonic development of zebrafish (Cai et. al, 2012). This could simply be caused by a difference in solution concentration or experiment setup and equipment. This experiment was completed to view the effects that cobalt (II) sulfate could have on embryonic development of zebrafish, and how this could possibly compare to human fetal development. According to the results of this experiment, cobalt (II) sulfate has no negative effects on either the survival or hatching rates of zebrafish embryos, and would therefore support the idea that cobalt (II) sulfate at similar concentrations, taking body mass into account would have no effect on human development.

Works Cited:

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