Effects of Atrazine on Zebrafish Embryo Survival and Hatching Rates

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Abstract

Atrazine is a very commonly used herbicide used all around the Wisconsin area, so finding out how it affects embryonic development is important, because it could be affecting many people in the Wisconsin area, due to the heavy emphasis on farming. The study done was using zebrafish to model human embryos, due to the very similar embryonic structure the two share. The experiment was done by taking 160 embryos, and exposing 40 to each treatment: a control, 1.0 mg/L atrazine, 2.5 mg/L atrazine, and 3.5 mg/L atrazine. The zebrafish were monitored for four days, tracking the hatching and survival rates. The study revealed that atrazine seemed to accelerate the hatching rate of zebrafish, and lower the survival rate of the zebrafish. The experiment done here is significant because atrazine is so prevalent an herbicide, and seeing how atrazine affected the zebrafish after four days really raises the question of how much drinking atrazine contaminated water can affect a developing child.

Introduction

Zebrafish are often used in biomedical research studies as models for humans, including addiction and autism studies. (Suriyampola, et al., 2016) These fish are genetically similar to humans, so using zebrafish for studying human disease processes is very beneficial to the scientific community. Zebrafish can be stored and monitored in a much easier manner, because of their much smaller size and transparent color, as compared to other lab animals, making these fish ideal models for human development studies (Brennan, 2014).

Atrazine is an herbicide used by many farms around the local area. Additionally, at low ecologically relevant concentrations atrazine can be a formidable endocrine disruptor, which can cause an imbalance of hormones in the body, which comes with a multitude of problems (Hayes, et al., 2010). This chemical is not naturally occurring. Farmers can spread atrazine in liquid, powder, or granular form, and are able to do so before crops sprout above the ground, as well as after. In general, unless one lives near farms that use atrazine, people aren't usually exposed to this chemical. However, atrazine can contaminate drinking water, which is how someone could end up getting exposed to atrazine. Research has shown that this chemical affects how many organs and organ systems work. Not enough data is available for scientists to conclude the extent of the effects atrazine has, and it is the many possibilities that could be found that prompted this particular study (Public Health Statement... 2003).

The research being done in this study is based on the idea that applying atrazine solutions to zebrafish embryos will cause a lowered survival rate, as well as an accelerated hatching rate. The independent variable of this data set is the amount of atrazine the embryos are exposed to. The dependent variable is the survival rate and hatching rate of zebrafish embryos exposed to atrazine.

Materials & Methods

Materials:

- 1 bottle of each atrazine stock solution (1.0 mg/L, 2.5 mg/L, 3.5 mg/L)
- 1 bottle of Instant Ocean/Embryo Medium Solution
- 1 Beaker for dead embryos and liquid disposal
- 1 Sharpie
- 1 1 mL disposable pipette for each treatment
- 1 1.5 mm disposable pipette for transferring eggs
- 1 plate with wells
- 1 28.5℃ incubator
- 1 dissecting and compound microscope
- 1 data table to record hatching and survival information

Procedure:

<u>Day 1:</u>

A. Obtain rinsed embryos.
B. Label plate with name and class hour. Label the atrazine concentration of each well using the Sharpie provided.

C. Fill the one well of the plate with 1 mL of Instant Ocean/Embryo Media solution using the disposable pipette. Fill remaining wells with the appropriate atrazine stock solutions. Divide the embryos so there are 10 embryos in each well. Label the plate on the student data sheet.

D. Record exact numbers of live embryos on student data sheet. (*Dead embryos should be discarded*)

E. Observe embryos under the dissecting microscope. Record observations on student data sheet.

F. Place each plate in the 28.5℃ incubator overnight.

Day 2:

G. Remove plate from incubator.

H. Remove dead embryos from plate using disposable pipette. Squirt dead embryos into waste beaker. (*Be careful to only remove the dead embryos)*

I. Count remaining embryos, hatched fish, and record in data table.

J. Remove atrazine stock solutions from each well of the plate. (*Tilt the plate so the embryos settle and remove the liquid from the top)*

K. Replace the atrazine stock solutions with the appropriate fresh atrazine stock solution using a clean pipette each time.

L. Place plate under dissecting microscope and record observations on student data sheet. Note/describe any development markers and abnormalities. Repeat for all atrazine concentrations.

M. Return the plate to the appropriate 28.5℃ incubator.

Day 3:

N. Repeat Day 2 work and observations. Record all data.

Day 4:

O. Repeat Day 2 work and observations. Record all data.

P. Place all embryos and fish in waste container to be properly disposed of.

Above procedure was taken from: SEPA Program-UW Milwaukee.

Statistical unpaired t-tests were performed on data to reveal statistical significance of survival and hatching rates.

Data & Analysis

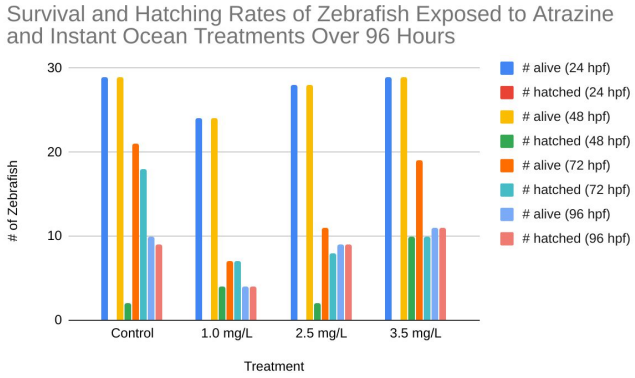

Figure 1

Figure 1 (above) shows the hatching and survival rates of zebrafish embryos in each treatment, including the control, on each day of the experiment.

Survival Rate of Zebrafish Exposed to Atrazine vs Control:
P-Values and Significance of Unpaired t-test Comparisons

Comparison	P-Value	Significance
Control vs 1.0 mg/L (24 hpf)	0.1936	Not Significant
Control vs 2.5 mg/L (24hpf)	0.8394	Not Significant
Control vs 3.5 mg/L (24 hpf)	0.6704	Not Significant
Control vs 1.0 mg/L (48 hpf)	0.1936	Not Significant
Control vs 2.5 mg/L (48 hpf)	0.8394	Not Significant
Control vs 3.5 mg/L (48 hpf)	0.6704	Not Significant
Control vs 1.0 mg/L (72 hpf)	0.1622	Not Significant
Control vs 2.5 mg/L (72 hpf)	0.6125	Not Significant
Control vs 3.5 mg/L (72 hpf)	0.8016	Not Significant
Control vs 1.0 mg/L (96 hpf)	0.2283	Not Significant
Control vs 2.5 mg/L (96 hpf)	0.8952	Not Significant
Control vs 3.5 mg/L (96 hpf)	0.8701	Not Significant

Data Table 1

Data Table 1(above) shows the p-value of each treatment against the control wells, at each day of the experiment, and whether or not that value was statistically significant or not.

Figure 2 – 48 hpf Control

Figure 2 shows a photo of a healthy zebrafish for reference to what a zebrafish should look like shortly after hatching.

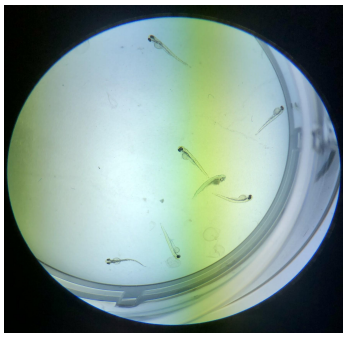

Figure 3 - 96 hpf 2.5mg/L Atrazine

Figure 3 shows a photo of 6 healthy zebrafish, and 1 fish that had died and turned blue from the methyl blue solution that prevents bacteria.

Unpaired t-tests were performed on the data, comparing the control to each respective treatment, on each day, in regards to hatching and survival rates. The t-tests were performed to find out which pieces of data were statistically significant by comparing the averages of two distinct groups. Statistical significance is the chance that a relationship between two variables is due to something other than chance, and none of the t-tests performed on the data for this study had a p-value that was less than 0.05, so none of the data was statistically significant.

Results

The experiment was set up for 40 zebrafish to be assigned to each treatment, with ten in each well of the plate. There were 40 embryos in the control, 40 embryos in the 1.0 mg/L atrazine, 40 embryos in the 2.5 mg/L atrazine, and 40 embryos in the 3.5 mg/L atrazine, with each solution being exchanged daily.

The dosages of atrazine, the independent variable, affected both the survival and hatching rate of the zebrafish embryos, being the dependent variable. The control embryos were exposed to Instant Ocean/Embryo Medium Solution, to model how a zebrafish normally develops, for comparison to the embryos exposed to atrazine. Twenty two and a half percent of the embryos exposed to instant ocean survived the experiment, while 20%, on average, survived being exposed to a toxicant.

After running statistical tests, all of our p-values were higher than 0.05, making none of them statistically significant. This means that there is a higher possibility than preferable that any effects on the embryos may have been due to a random error made while sampling. If the p-value was lower than 0.05, then the assumption could be made that all the data is very reliable and not affected by error. The p-values seen in this study make all the data very likely to be affected by random error.

Discussion

Overall, the hypothesis for the experiment was supported, however, that doesn't account for the high p-values seen. The hypothesis stated that applying atrazine solutions to zebrafish embryos will cause a lowered survival rate, as well as an accelerated hatching rate. The data showed that by the end of the experiment 22.5% of the embryos in the control set survived, while only and average of 20% of the embryos in each treatment survived, and since 20% is lower than 22.5%, atrazine must lower the survival rate of zebrafish embryos. In addition, either the same or more fish had hatched in each treatment than had hatched in the control after 48 hours, but all of the data had a very high chance of being affected by random error, so results can't be deduced that can be backed well or widely trusted, in a scientific sense.

Some things were hard to monitor in this experiment, because the technology to monitor things very closely was not available. Some things like the effects atrazine could have on the reproductive systems of the fish, were not able to be studied, because of the short timespan given to conduct the experiment. The setbacks only affected the experiment in the sense that it limited the things that could be researched.

The work done in this study did show some interesting effects on the zebrafish embryos, however it is all tainted by the fact that there is a high risk that all of it was due to error.

In general, the data showed a correlation between atrazine exposure and hatching/survival rate, but the study itself can't rely heavily on what the data implies, because the p-values were so exorbitantly high. Despite that, after observing what atrazine seemed to do to zebrafish embryos after four days, and then considering that atrazine is one of the most detected herbicides in drinkable water, the implications are bleak for how atrazine can affect children when mothers are drinking atrazine infested water (Nwani, et al., 2010).

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("Toxic Substances Portal - Atrazine", 2003)