The Effect of Herbicides on Zebrafish Development

Abstract:

In this lab, zebrafish were observed over the course of four days. On the first day, ten embryos were placed among two well plates and then solution was added to each well. The wells were split into four groups: one control group and three experimental groups. The wells in the control group were filled with instant ocean solution and the the wells in the experimental group were filled with a solution of 0.1, 0.2, or 0.4 mg of herbicides mixed with 100 mL of instant ocean. After observing embryonic development for four days, it was observed that there were the most embryos alive in the control group and the least amount of embryos alive in the experimental group with 0.4 mg of herbicides at day 1. Likewise, there were the most hatched zebrafish in the control group and the least amount of hatched zebrafish in the experimental group with 0.4 mg of herbicides by day 4. The results for the other two experimental groups followed this pattern. These results are significant because it shows that herbicides do have a harmful effect on embryos suggesting that women who are pregnant should not be largely exposed or able to inhale significant amounts of herbicides(while they garden, for example) because the herbicides can have a damaging effect.

Background Information:

According to the US Environmental Protection Agency (2016), pesticides are a popular substance that many farmers use to control weeds, insect infestation, and disease. Herbicides proved to be an interest to use for an independent variable in this experiment because they contain toxins that could potentially harm the development of the zebrafish. For example,

according to a study done on the effect of the pyrethroid insecticide on zebrafish embryos, it was found that zebrafish embryos can be negatively affected by insecticides. After the zebrafish were exposed to the insecticide, the findings were consistent with mammalian studies such that the same abnormalities were observed in zebrafish as the mammals(DeMicco, Cooper, Richardson, and White, 2010). Because both zebrafish and mammals experience similar abnormalities when being exposed to insecticides, zebrafish were seen as practical to experiment with when trying to conclude if herbicides would be harmful to humans, which are mammals. Another experiment was performed that showed similar results to the results of the zebrafish experiment that was conducted in class Roundup, which has been a top-selling weed killer, was found to kill human cells. Even more specifically, it was found to kill embryonic cells (Gammon, 2009). This connects to the zebrafish experiment that was conducted because the effects of herbicides were studied on zebrafish embryos as well and the results in this experiment proved that herbicides caused harm to the development of the zebrafish embryos. Lastly, another experiment conducted tested the effect of pesticides on male brown rats. It showed that their sperm count decreased, and similar to the other experiments, the male brown rats began to produce abnormal cells (Yekeen, Fawole, Bakare, and Emikpe, 2016, p.47-56).

If the herbicide concentration increased, then it was thought that the amount of alive or hatched zebrafish could be lower because the herbicides used contained chemicals such as glyphosate and isopropylamine salt, which according to the US Environmental Protection Agency, have the potential to cause harm on the development of zebrafish. The main purpose for testing the effect of herbicides on the development of zebrafish was to understand how the developmental process changes when being exposed to herbicides. With this, conclusions about

the effect of herbicides on human embryos can be made because of the conclusions drawn from the first experiment introduced in the previous paragraph which supported that mammals and zebrafish experience similar abnormalities when exposed to insecticides. This comparison can also be made because zebrafish are vertebrates and they show higher levels of thinking similar to that of humans (Petering, 2015, p. 12). It is important to use an organism that is similar to a human's level of thinking because this shows similarities of brain development between humans and zebrafish which is very important for the results of the experiment because then the effects of toxins on development should be similar. Zebrafish were used because they are cheaper to buy and they are translucent making it easier to analyze their anatomy. Finding developmental abnormalities is easier when the organism is translucent. The development period, the size, shape, and the cranial structure can be easily seen in the translucent zebrafish. After the experiment was conducted, it was concluded that women who are pregnant should not use weed killers in order to be safe from inhaling herbicides because the herbicides can lead to abnormalities in developmental processes and it can harm the developing embryo.

In the zebrafish experiment, ten embryos were placed into each well of two twelve-well plates and then different concentrations of herbicides were added to each well to form one control group(no herbicides added) and three experimental groups(solutions of 0.1, 0.2, or 0.4 mg of herbicides mixed with 100 mL of instant ocean). These levels of concentrations of the herbicide were chosen according to the EPA guidelines and the average area that a crabgrass weed takes up. These values were put into proportion to find the amount of herbicide that should be used in the zebrafish experiment. The wells were cleaned out each day and the solution was put into the well again. Observations were noted and the number of dead zebrafish embryos were

recorded for the first day and then the number of hatched and healthy zebrafish were recorded for the second, third, and fourth day. This method was chosen because it easily allowed for comparison between the embryos exposed to different concentrations of herbicides through observations and data for consecutive days in order for the effects of herbicides to be determined. After conducting the experiment, the zebrafish embryos placed in the higher concentrations of the herbicide were found to have a higher number of dead embryos on the first day and more abnormalities in the hatched zebrafish throughout the other days.

Materials and Methods:

Materials used are 240 zebrafish embryos, Eliminator weed & grass killer, 2 12-cell well plates, disposable pipettes, 2-250 mL beakers, microscope, 4 small bottles with caps, and 10 mL graduated cylinder. Keep pesticides away from eyes and mouth. Pure instant ocean solution was poured into a small bottle labeled control. This bottle was set aside. 100 mL of instant ocean was poured into a 250 mL beaker along with 0.1 mg of Eliminator which was measured out by first pouring the Eliminator solution into a graduated cylinder and then pipetting 0.1 mg from the graduated cylinder. This solution was mixed and poured into a small bottle labeled 0.1. This process was repeated using 0.2 mg and 0.4 mg of Eliminator. 10 zebrafish embryos were pipetted into each well and the water was pipetted out. About 3 mL of the control solution was pipetted into all wells under column 1 in each well plate. 3 mL of the 0.1 mg solution was pipetted into all wells under column 2 in each well plate. 3 mL of the 0.2 mg solution was pipetted into all wells under column 3 in each well plate. 3 mL of the 0.4 mg solution was pipetted into all wells under

column 4 in each well plate. In 24 hours the zebrafish embryos were observed to see how many were alive and dead in each well and these values were recorded. The dead ones were removed along with the old solution in each well. The solutions were replaced with the solutions in the small bottles. In 24 hours the zebrafish were observed to see how many were hatched and not and these values were recorded. Pictures were taken of the embryos underneath the microscope and they were examined for any deformities. The dead ones were removed and the solutions were removed and replaced. In 24 hours the embryos were observed for amount hatched again and the solutions were replaced again. In 24 hours the embryos were observed again. Then the zebrafish were disposed of properly. The sample size of this experiment is the 240 zebrafish embryos that were used. This experiment should be repeatable.

Well plates:

1A	2A	3A	4A
1B	2B	3B	4B
1C	2C	3C	4C

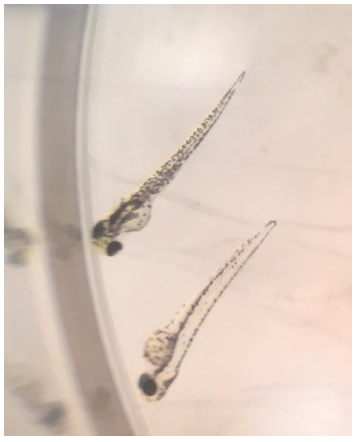
The independent variable for this experiment is the concentration of Eliminator weed and Grass killer in 100 mL of instant ocean added to the well plates. The dependent variables are the amount of dead and alive embryos, the amount hatched and unhatched embryos, and the development of the embryos over time. The control group are wells under column 1. These are the wells that have no pesticides added to them. The experimental groups for this experiment are each group with pesticides added. Wells in column 2 have 0.1 mg of Eliminator/100 mL of instant ocean. Wells in column 3 have 0.2 mg of Eliminator/100 mL of instant ocean. Wells in column 4 have 0.4 mg of Eliminator/100 mL of instant ocean. The constants are the amount of

zebrafish embryos in each well to begin with, all of the embryos came from the same place, temperature, environment(a well), type of pesticides, and the amount of time the zebrafish are subject to the pesticides.

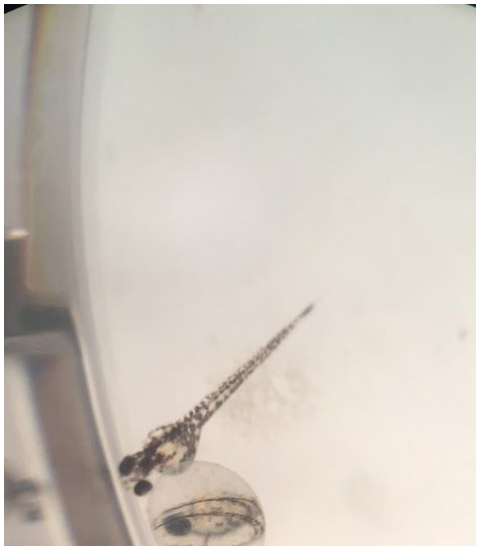
Results:

Generally the trends of our results were that as the concentrations of the Eliminator herbicides increased the amount alive and amount hatched decreased. With these results there were some supposed outliers that provided not statistically significant results. Also our picture observations showed that the greater concentrations of herbicides could have had effects on the development of the zebrafish. On the last two days of observation the control group zebrafish appeared to be normal looking with straight spines and normal looking heads. As the concentrations got greater there appeared to be some cranial abnormalities like bumps by the eyes and there also appeared to be more bent spines.

This picture was taken on day 3 of observations and was taken of a zebrafish under control conditions. It shows a straight spine and no apparent cranial abnormalities.



This picture was also taken on day 3 of observations and was taken of a zebrafish in 0.1 mg Eliminator/100 mL instant ocean. Both zebrafish in this image show slight curvature of the spine. The one on the left also shows a possible cranial abnormality. The head does not show symmetry.



This picture was also taken on day 3 of observations. This was taken of zebrafish in 0.4 mg/100 mL solution. The hatched zebrafish on the left shows possible cranial abnormalities. The sides of the head are not symmetric with the right most side protruding more.

This picture was taken on day 4 of observations and the zebrafish here was under control conditions. This zebrafish showed no abnormalities.

This picture was taken on day 4 of observations and the zebrafish was in 0.1 mg/100 mL. These zebrafish both showed slightly curved spines.

This picture was taken on day 4 of observations and the zebrafish was in 0.4 mg/100 mL. It shows large curvature of the spine.

Data Table:

*Note that IA=Instant Ocean

The Effect of the Amount of Herbicides Present on the Percent of Alive Embryos on Day 1

	Control Group	0.1 mg herbicides/100 mL IA	0.2 mg herbicides/100 mL IA	0.4 mg herbicides/100 mL IA
Trial 1	10%	80%	90%	20%
Trial 2	60%	80%	50%	80%

Trial 3	80%	50%	50%	40%
Trial 4	100%	100%	50%	60%
Trial 5	100%	80%	70%	40%
Trial 6	100%	40%	60%	20%

The Effect of the Amount of Herbicides Present on the Percent of Hatched Zebrafish on Day 2

	Control Group	0.1 mg herbicides/100 mL IA	0.2 mg herbicides/100 mL IA	0.4 mg herbicides/100 mL IA
Trial 1	0%	0%	0%	0%
Trial 2	0%	0%	0%	0%
Trial 3	0%	0%	0%	0%
Trial 4	0%	10%	20%	0%
Trial 5	23%	0%	29%	0%
Trial 6	9%	0%	0%	0%

The Effect of the Amount of Herbicides Present on the Percent of Hatched Zebrafish on Day 3

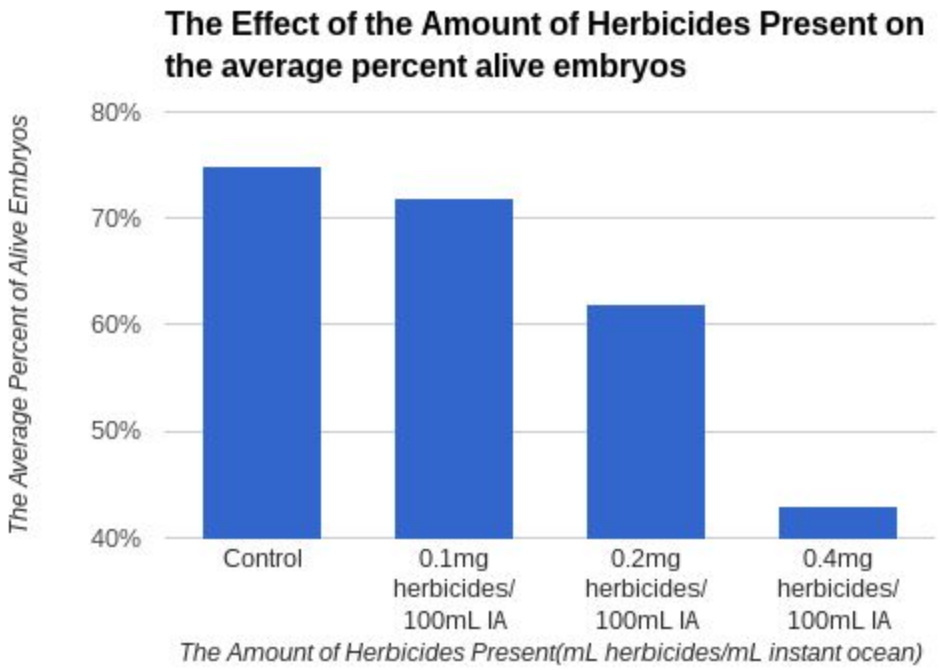
	Control Group	0.1 mg herbicides/100 mL IA	0.2 mg herbicides/100 mL IA	0.4 mg herbicides/100 mL IA
Trial 1	0%	13%	90%	0%
Trial 2	100%	63%	100%	80%
Trial 3	86%	100%	100%	50%
Trial 4	90%	80%	40%	60%
Trial 5	100%	75%	71%	100%
Trial 6	82%	75%	83%	0%

The Effect of the Amount of Herbicides Present on the Percent of Hatched Zebrafish on Day 4

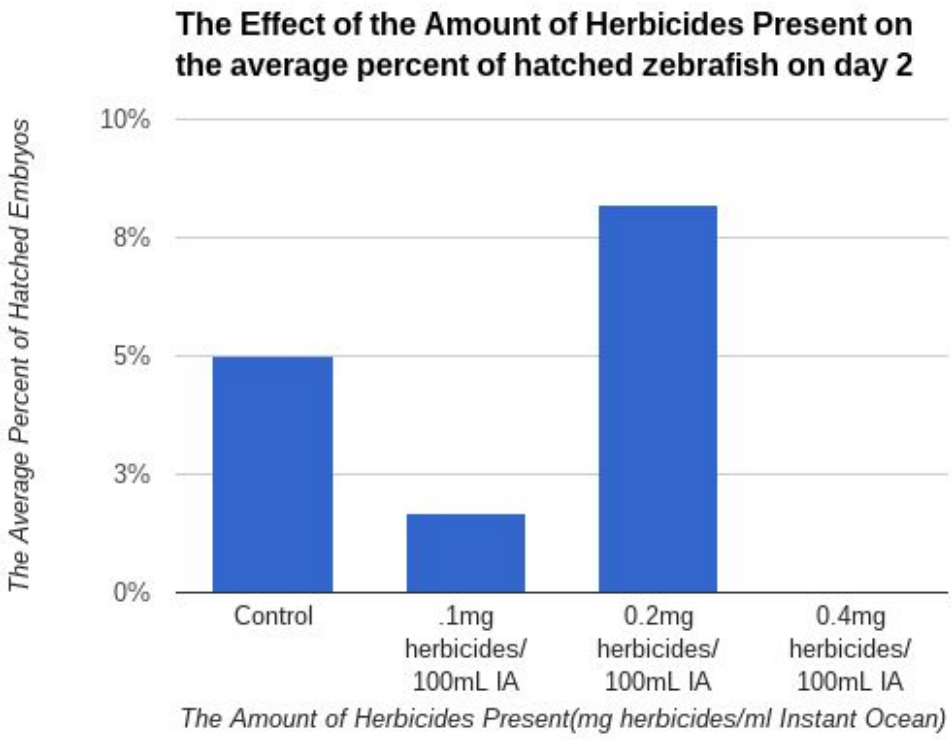
	Control Group	0.1 mg herbicides/100 mL IA	0.2 mg herbicides/100 mL IA	0.4 mg herbicides/100 mL IA
Trial 1	100%	86%	100%	0%
Trial 2	100%	100%	100%	80%
Trial 3	86%	100%	100%	50%
Trial 4	100%	90%	60%	67%
Trial 5	100%	100%	86%	100%
Trial 6	91%	100%	83%	50%

Graphs:

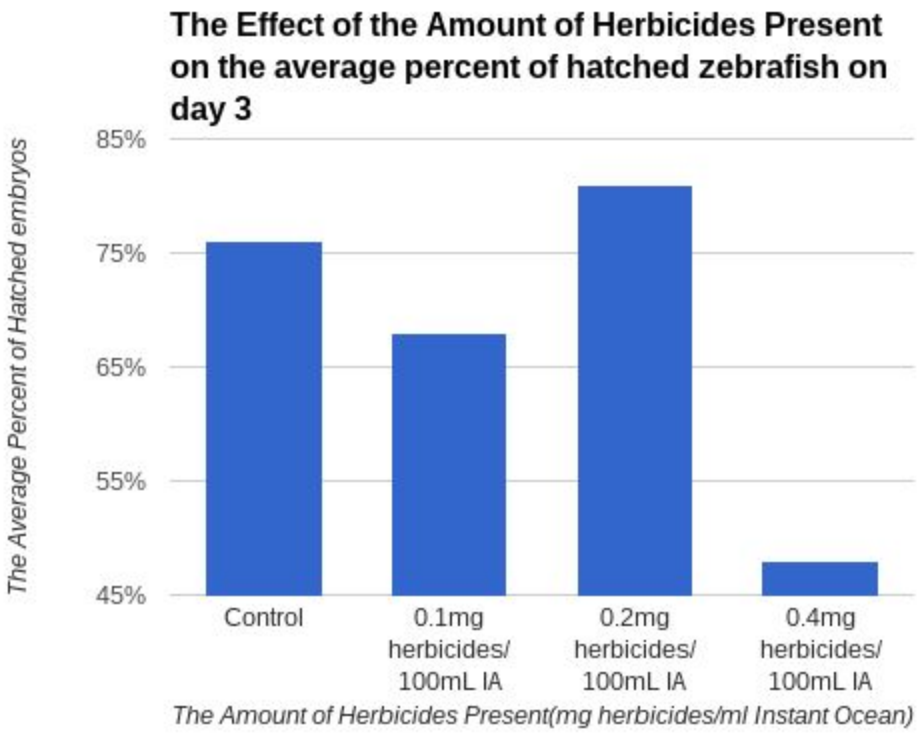
*Note that IA=Instant Ocean



As the amount of herbicides present increased, the average percent of alive embryos decreased.



Although these results were inconsistent, generally as the amount of herbicides increased, the amount of hatched zebrafish decreased.



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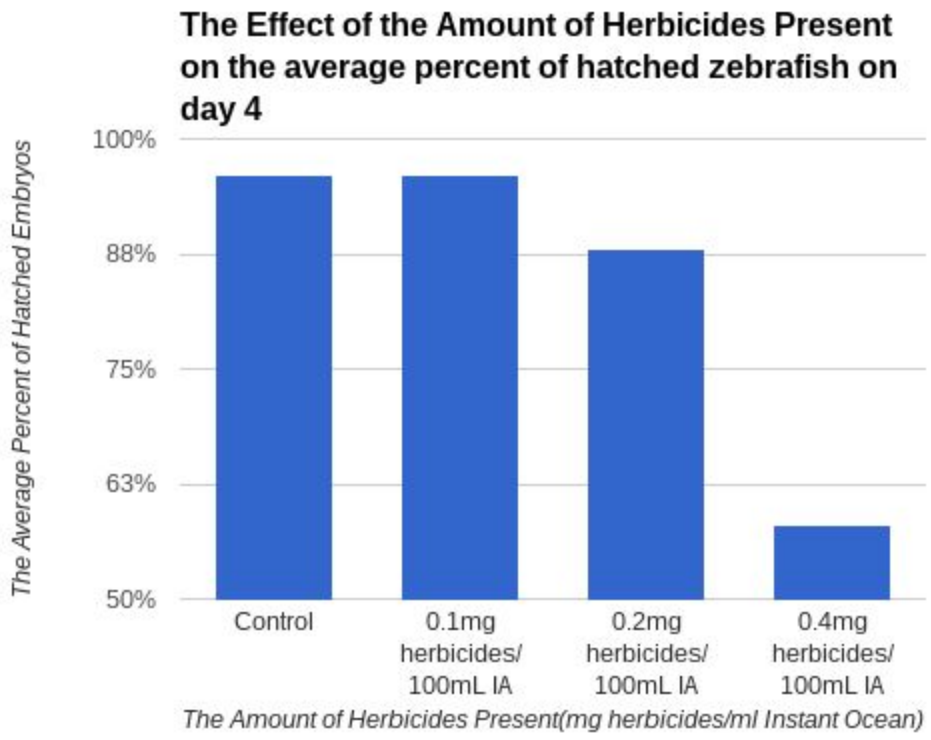

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Statistics:

Day 1

For day one, when comparing the control group with the 0.1 mg herbicides/100 mL IA experimental group, the 0.1 mg herbicides/100 mL IA group with the 0.2 mg herbicides/100 mL IA group, the 0.2 mg herbicides/100 mL IA with the 0.2 mg herbicides/100 mL IA group, and the control group with the 0.4 mg herbicides/100 mL IA group, we found the first three differences to not be statistically different and the last comparison to be not quite statistically different. Our p-values came out to be 0.8499, 0.3931, 0.1442, and 0.0988, respectively.

Day 2

For day two, when comparing the control group with the 0.1 mg herbicides/100 mL IA experimental group, the 0.1 mg herbicides/100 mL IA group with the 0.2 mg herbicides/100 mL IA group, the 0.2 mg herbicides/100 mL IA with the 0.2 mg herbicides/100 mL IA group, and the control group with the 0.4 mg herbicides/100 mL IA group, we found all the differences to be not statistically different. Our p-values came out to be 0.4003, 0.2687, 0.1540, and 0.1936, respectively.

Day 3

For day three, when comparing the control group with the 0.1 mg herbicides/100 mL IA experimental group, the 0.1 mg herbicides/100 mL IA group with the 0.2 mg herbicides/100 mL IA group, the 0.2 mg herbicides/100 mL IA with the 0.2 mg herbicides/100 mL IA group, and the control group with the 0.4 mg herbicides/100 mL IA group, we found the all of the differences to be not statistically different and the last comparison to be not quite statistically different. Our p-values came out to be 0.6684, 0.4115, 0.1233, and 0.2496, respectively.

Day 4

For day four, when comparing the control group with the 0.1 mg herbicides/100 mL IA experimental group, the 0.1 mg herbicides/100 mL IA group with the 0.2 mg herbicides/100 mL IA group, the 0.2 mg herbicides/100 mL IA with the 0.2 mg herbicides/100 mL IA group, and the control group with the 0.4 mg herbicides/100 mL IA group, we found the first two differences to be not statistically different and the third comparison to be not quite statistically different. Our p-values came out to be 0.9640, 0.2854, 0.0763, and 0.0220, respectively.

Comparing the Control Group(1) and 0.4 mg herbicides/100 mL IA (2)

P-value: 0.0220

This difference is considered to be statistically different.

Group	Group One	Group Two
Mean	96.17	57.83
SD	6.15	34.12
SEM	2.51	13.93
N	6	6

Discussion:

It was expected that as the amount of herbicide concentration increased, the number of dead embryos and abnormalities in the developmental process of the zebrafish embryos would increase as well. This was reflected in the results because there was a higher percentage of dead embryos and a lower percentage of hatched and alive zebrafish in the higher concentration of herbicides than there was in the lower concentration of herbicides. Additionally, the pictures show that there were more abnormalities in the higher concentrations. Many of the zebrafish in the control wells had straight spines and normal looking heads. As the concentrations of weed killer got larger there appeared to be more spine curvature and cranial abnormalities. The cranial deformities observed were slight protruding of one side of the head by the eyes. These results were more clearly present in the highest concentration of 0.4 mg/100 mL instant ocean.

Even though there was a trend in our data to form a conclusion, the experiment did not show much statistical significance because many of the results from the t-tests done showed that the results of the experiment were not quite statistically significant or not statistically significant as the p-values ranged anywhere between 0.0220 and 0.9640. Errors that could have resulted in the data to not be statistically different was that it was hard to pick up the solutions without

removing the zebrafish as well. For this reason, many embryos and hatched zebrafish were, in fact, sucked up in the pipette which could have harmed them. This has potential to cause abnormalities not caused by the herbicides, but by the pipette which could have obscured our results, especially when dealing with the control group.

Another source of error is that something like bacteria or fungus could have entered the wells. This could cause zebrafish to die, not due to the concentration of weed killer, but instead, due to the bacteria. This might have happened in one of the control wells where many of the zebrafish died after the first day. One benefit of the way this experiment was designed is that there were a total of 6 wells for each different group: control, 0.1, 0.2, and 0.4. This allowed for more control over variables like bacteria or fungus in case they infected a well. If a well were to be infected there would still be 5 other wells to draw results from. After the experiment was conducted, the data was graphed to see the results more clearly. Minus a few outliers, the results show a clear trend that as the amount of herbicides increased, the percentage of alive embryos or hatched zebrafish decreased. Statistical analyses, on the other hand, did not help to confirm this trend.

The t-tests were calculated between the consecutive increasing concentration of herbicides and also between the control group and 0.4 mg of herbicides/100 mL. The T-tests were ran this way in order to tell if the increments of increasing concentrations were significant enough and also if the exposure of herbicides at the highest concentration had any significance when compared to the control. As a result, the T-tests helped to calculate high P-values all around. These high P-values suggest that the differences were considered to be not statistically different. The only T-test conducted that computed a low enough P-value, 0.0220, to make the

results statistically different was the T-test conducted between the control group and the 0.4 mg of herbicides/100 mL on day 4. This shows that with more time and with a higher amount of herbicide concentration, this could have more of a harmful effect on the health of the developing zebrafish and cause more abnormalities.

Additionally, our standard deviation values were generally high all around which implies that while there were some zebrafish that followed the trend, there were also other zebrafish in the control group that did suffer abnormalities and deaths and there were likewise other zebrafish that were exposed to pesticides that remained perfectly healthy. Overall, the best statistical analysis that represents the data best would be the mean. The mean takes a large amount of data about the embryonic development and turns it into one easily comparable value. This value accounts for the high and low numbers from our data table giving us a more reliable value rather than just a single value from our data table that doesn't account for a large amount of the data. Understanding the meaning of the data, it can be concluded that herbicides do indeed have a harmful effect on the health of zebrafish. It can then be inferenced that this experiment can also apply to human life in the sense that it can connect to human embryos in expecting mothers. If mothers to-be do some gardening and use some weed killer, they risk the danger of inhaling the toxins in the weed killer.

This experiment shows that these toxins may have harmful effects on the growing and developing embryos inside of her as they cause death in embryos and abnormalities in developing zebrafish. In reflection of the results of the experiment, it is suggested that expecting mothers do not do gardening with weed killers in order to eliminate this risk. Some developmental issues can be produced in the human embryos which the zebrafish embryos show

in their curved spines and cranial abnormalities. This predicted outcome was seen in the Roundup study when the weed killer killed embryonic cells (Gammon, 2009). To further this work, behavior of the alive and developing zebrafish in the control group and in the groups exposed to herbicides should be studied. In this way, it can be studied how being exposed to herbicides affects the behavioral action of the zebrafish, if it would in any way. Additionally, the physiological differences should be examined between each group of zebrafish being exposed to a different amount of herbicides after a certain amount of time of exposure. With this, the differences can also be used as evidence to further the argument whether herbicides do have a harmful effect on the health of developing zebrafish or not. Also, another experiment should be conducted so that a higher amount of herbicide concentration is used and the experiment is done over a longer time period so that the effects of the herbicide on the developmental process can be more largely studied. This can help to show how time plays a factor because it is possible that more health issues could occur as time progresses.

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