The Effects of Aspartame on *Danio Rerio* Embryos

Kaitlin Gilbert and Shannon Kuhl
Pardeeville High School

**Abstract**

It was believed that if the zebrafish embryos were exposed to both a high and low concentration of aspartame solution, they would experience hyperactivity and an increase in overall hatch rate. Observations were used in order to see if the zebrafish would become hyperactive after hatching and if the hatch and mortality rate would be negatively affected. Aspartame was the independent variable in the experiment. Mortality and hatch rate were the dependent variables in this experiment. The control wells, A1 through A4, were filled with Instant Ocean/Embryo Media solution. The independent variable wells, B1 through B4, were filled with the high solution of aspartame which equaled 20 mg/mL. The other independent variable wells, C1 through C4, were filled with the low solution of aspartame which equaled 5 mg/mL. In conclusion, at 5 mg/mL concentration aspartame, it does not have a statistically significant effect on the survival. For the 20 mg/mL concentration, it was discovered that it had a significant result to the embryos.

**Materials & Methods**

- 20 mL, 80 mL beaker and a 12-well plate
- Compound microscope
- Zebrafish embryos
- Sharpee (for labeling names)
- Two disposable pipettes
- 20 mg/mL, 5 mg/mL of aspartame solution
- Plastic tub (used as an incubator for zebrafish embryos)
- Embryo Media Solution
- Gloves

For the experiment, embryos were delivered to the class and obtained by students from the teacher. Male and female zebrafish embryos were placed into a tank to spawn. After receiving the embryos, 10 of them were placed in each separate well for all 12 wells. Aspartame solution was added to each well. To label the solution amount, a sharpie was used to label one row 20 mg/mL, 5 mg/mL, and control. A disposable pipette was used to extract any dead embryos that were observed, and were placed into an 80 mL beaker. The remaining embryos were counted along with the number of hatched fish and these numbers were recorded into the data table. The zebrafish were observed under a microscope, and all data that was shown was recorded. The plate was in a 28.5°C incubator overnight. The aspartame solutions were removed from each well and refilled with fresh aspartame solution. At the end of examining the embryos, all remaining embryos and live fry were placed into the waste container. All of the information that was gathered was put through an online statistical t-test to find standard deviation. The zebrafish were observed under a microscope, and all data that was shown was recorded.

**Introduction**

Aspartame is an alternative to sugar and according to the International Food Information Council, it’s 200 times sweeter due to the components and chemicals used to produce it, but it is still low in calories. Aspartame is used as a sweetener in foods and beverages. Considering the amount of intake, Aspartame can be dangerous if one consumes a large amount. Currently, around 200 million people consume aspartame. It is used in over 6,000 products, like diet soda, which can cause brain tumors.

The zebrafish, also known as *Danio rerio*, is an excellent model for understanding the essential principles of vertebrate development. The point of studying zebrafish embryos are zebrafish embryos development are similar to humans. By doing this experiment researchers can see the difference in early life development, development defects, and testing human embryos.

The dependent variable in this study was the zebrafish embryos and the independent variable was the solutions of aspartame. It was hypothesized that an excessive amount of aspartame would affect early development of the embryos, and it could have major defects and cause an increase in the mortality and hatch rates.

**Results**

- Average Hatch Rate of Zebrafish on Second Day
- Average Survival Rate of Zebrafish on Third Day

**Discussion**

From the experiment conducted, there was a hypothesis created that birth defects and an increase in death would happen to the embryo. Some of the birth defects found were from Figure 1 and 2, shown in comparison, of what exactly happened to the embryos when placed in a concentration of aspartame. Figure 2, placed in 20 mg/mL of aspartame, shows an enlarged yolk sack compared to the control embryos in Figure 1. The premature development happened then the embryos quickly died off the next day. Figure 3 demonstrates the significant increase in development of the embryos. Figure 4 portrays the resulted effect showing increased mortality rate.

How the experiment was conducted to find the high concentration of aspartame and the low, was mathematically processed by figuring out that 3 cans of diet soda is equivalent to 0.5 g or in this experimental case, was a half a packet of sweetener. Then to figure out the high concentration, it was calculated to find out it was 2g or for this trial, 2 packets of sweetener.

Our conclusion was that if a pregnant woman consumed between the high and low concentration of aspartame then there would be a possible effect to the embryo. There were some aspects of the experiment that could have influenced the conclusion of the experiments data. To make the data more convincing, it would have to be continually tested with different spawning groups of embryos and by different experimenters.

It could be thought that with the low survival rate of embryos, it may be possible for pregnant women to have a miscarriage while in the womb. It could also be thought that if the fetus does not die, it will be born with birth defects. In the end, these negative effects can not be concluded as true within humans because studies have not been taken on humans. Although, zebrafish are model organisms which means the same fatal problems could apply to humans, it should not be assumed until more advanced testing has been performed.

**References**

