Aspartame Effects on the Developing Zebrafish: Hatch Rate and Survival Rate

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Introduction

According to 'Zebrafish as a Developmental System', "Zebrafish are an ideal model for basic developmental research for numerous reasons: high fecundity (mature females lay several hundred eggs at weekly intervals), short generation time (3-4 months), rapid development, and translucent embryos." Aspartame is one of the most common artificial sweeteners. It is placed in both foods and beverages to lower the amount of calories in them. Some of these substances include: soft drinks, sugar-free gum, and low-sugar desserts. Equal sweetener was used as a source of aspartame. According to 'Why use the zebrafish in Research?', "70 percent of protein-coding human genes are related to genes found in zebrafish." It was hypothesized that if the amount of aspartame in the solution increases, then it will have harmful effects on the zebrafish by decreasing their survival rate and their growth and development which can lead to an increase in the death rate of the zebrafish. This is because aspartame is known to cause cancer, weight gain, decreased mental health function, seizures and birth defects in humans.

Materials

The materials included: tape, marker, stereoscopic microscope, compound microscope, parafilm to cover the beakers, tray for supplies, 12 spot well tray, five 100mL beakers, an incubator set at 28.5 degrees Celsius, two 1L Erlenmeyer flasks, five wide bore pipettes, five regular pipettes, one packet of *Equal* sweetener, depression slide, about 150 zebrafish embryos, stir stick, 10mL graduated cylinder, 250mL Erlenmeyer flask, 100mL graduated cylinder, lamp, 3000mL dechlorinated water, 15mL Instant Ocean, antifungal solution, and deionized water for cleaning.

Methods

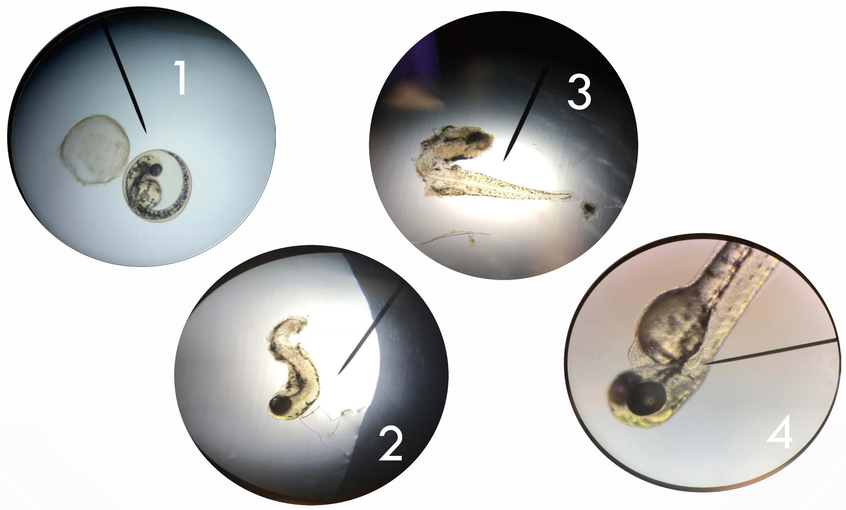
The first step in this experiment was to make the four different aspartame solutions, which were "1000x", "10x", "Normal," and "Zero". About 70 mL of each were made and stored in separate stock beakers. Next, the well plate and beaker with the zebrafish embryos were obtained from the instructor. The well tray had four rows of three wells, so each solution had three wells to be tested in. Approximately four milliliters of solution were added to each well. Approximately ten live zebrafish embryos were placed in each well, using the wide bore pipets. On each day of the experiment, the solutions in each well were exchanged for new solution from the stock beakers. Observations were done each day as well. These observations included: size, color, development stage, amount living, and amount hatched. Using the depression slide, the zebrafish were observed under the compound microscope. The stereoscope microscope was used to look at multiple zebrafish and embryos at the same time. After observing the zebrafish, all dead zebrafish and egg shells were removed and placed in a waste beaker. After each day, the well plate was placed back into the incubator at 28.5 degrees Celsius. This procedure was repeated for five days.

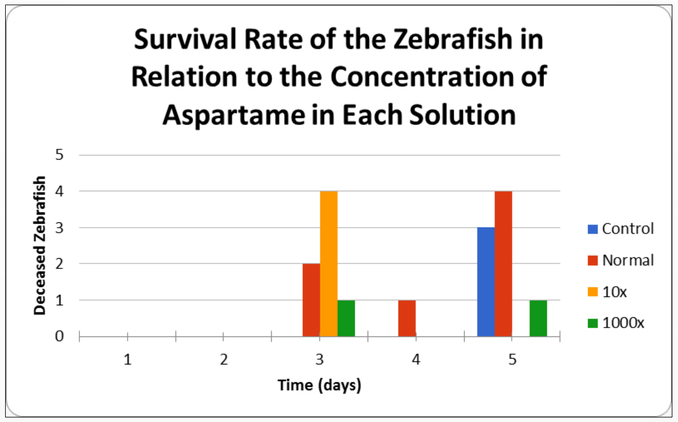
Picture Descriptions

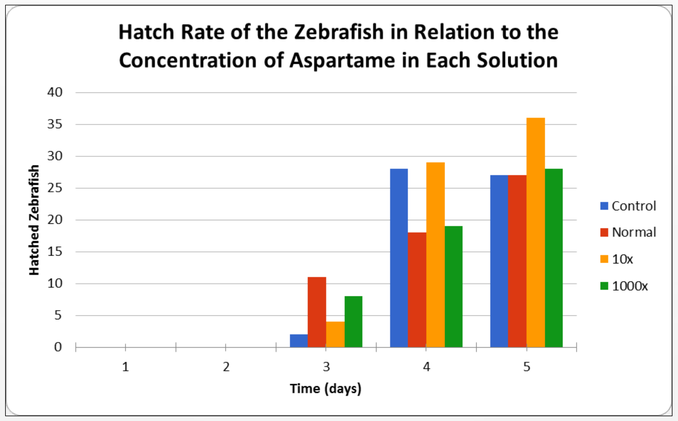
The first image shows the development of all the embryos on day three. On the left is an empty, burst open egg. On the right is an embryo in the in chorion 48hpf state of development. The second image is a dead, underdeveloped fish that had just recently hatched on day four. The next image is also a dead fish on day four. This one is a little more developed than image two. The final image is of a living fish from day five. The fish was developed enough to see the blood flow through its body, along with seeing its heart pumping.

Abstract

Zebrafish, also known as Danio rerio have become a popular model organism to conduct research to study diseases. Zebrafish share 70 percent of the same genes as humans, so the results obtained by this experiment can be related back to human health. This experiment tested the effects of aspartame on the development, hatch rate and survival rate of the zebrafish embryos. The zebrafish fish embryos hatched and developed in four different aspartame solutions for five days. Aspartame did affect the development of the zebrafish, at high amounts the zebrafish were very underdeveloped







Results

The experimental design of this experiment was to expose approximately the same number of zebrafish to four different solution concentrations of aspartame. Each solution was made using serial dilutions. Over a period of five days, the zebrafish were observed and data was gathered and recorded in the appropriate data tables.
The control group of this experiment consisted of a solution that contained no aspartame; just dechlorinated water mixed with instant ocean. This was the control because they were in a normal environment, and no variables were added or changed. The independent variable in this experiment was the amount of aspartame in each solution. The dependent variables in this experiment were the survival rate, the hatch rate, and the defects in growth and development of the zebrafish. These all depended on the amount of aspartame in each fish groups environment. Some constants in this experiment were: the number of fish in each well, the environment of the fish and the temperature at which they were kept. The results of the experiment were inconclusive. There was no pattern found in the data that could be accurately concluded to be related to the aspartame. Neither the hatch rate or the death rate varied significantly between the different groups of zebrafish. Therefore, the results were not significant enough to prove that aspartame has harmful effects on both zebrafish and the human body.

Discussion

If aspartame is added to the zebrafish embryos in a small amount, the hatch rate, survival rate, and the development rate will increase, but if the aspartame is added in a large amount, the hatch rate, survival rate, and development rate will rapidly decrease. This is because a large amount of aspartame will act as a toxin to the zebrafish embryos, but a small amount will act as a fuel source. Testing different concentrations of aspartame on the development of zebrafish gave results that did not completely support the hypothesis stated. Some of the higher concentrations of aspartame had high hatch and survival rate, while zebrafish in low concentrations and none at all (the control) showed higher death rates and lower hatch rates to begin with. This experiment was only conducted over a five day period. This is a limitation to compare it to human health because most humans live for years. The zebrafish were also raised in an unnatural environment, which may have caused them to develop differently. Only one spawn was tested in this experiment, this was a limitation because that particular spawn could have different reactions to the aspartame than other spawns. The sweetener used as the source of aspartame had other ingredients that could have interacted with the zebrafish hatch, survival, and development rate. Since the results were inconclusive there is no way of tying these experimental results to human health.

References

Aspartame. (n.d.). Retrieved February 22, 2017, from https://www.cancer.org/cancer/cancer-causes/aspartame.html

Zebrafish Genome Found Strikingly Similar to Humans | Genetics. (n.d.). Retrieved February 22, 2017, from http://www.sci-news.com/genetics/article01036.html