The Effects of Varying Concentrations of Aspartame on the Survival Rate of Zebrafish Embryos by Natalie Maufort

Abstract

Over the course of five days fertilized zebrafish embryos were observed after being exposed to varying aspartame levels on the first day. The concentrations were 1x, 2.5x, and 5x of that of a human dosage. It was thought that the aspartame would increase the death toll of the embryos, however it remained unchanged. The 2.5x concentration of aspartame was found to be significant which was an oddity for the 5x showed no significance. Other than the 2.5x, the data was similar to other studies that also concluded aspartame was not harmful.

With aspartame being so common in everyday diets it is important to understand its effects on the body. The aspartame showed no negative health effects on the zebrafish embryos. With the development of the zebrafish and humans being so similar it can be inferred that the aspartame will also not have any negative effects in humans.

Introduction

In the last few years, aspartame has increasingly become more of a concern in society. It is in many foods, and while many companies are detering away from including aspartame in their products, it is still prevalent in many common foods/drinks. Aspartame is an artificial sweetener commonly found in many foods and drinks. While it is still endorsed by companies like: World Health Organization and American Heart Association, it has been linked to many health problems such as: headaches, nausea, dizziness, and even cancer and lupus (Lillis, 2018). It has also been found to cause hyperactivity and behavioral problems in children (Wolraich, 1994).

Why zebrafish? The research on zebrafish has exploded in the last few years due to the fact the fish are the perfect test subject. The fish are very easy and inexpensive to maintain (Parngwen, 2002). The gestation period of the zebrafish is significantly shorter than those of other test subjects. Due to the zebrafish's size it is easy to house. The fish develop outside of the mother, making it easy to see development and causing no hard to the mother. The development

of a zebrafish in the early stages is very similar to that of a human, thus toxicants affect them similar ways (Why use zebrafish in research?, 2014). This experiment is looking at how aspartame impacts the development of zebrafish embryos? With increasing aspartame concentrations there is reason to believe the survival rate of the zebrafish embryos will decrease.

Materials and methods

Quantity	Item
1 bottle per group	Stock solution of Aspartame (1x, 2.5x, 5x aspartame)
1 per group	Beaker for dead embryo and liquid disposal
1	Sharpie
1 bottle per group	Instant Ocean
1 per group plus extras	Disposable pipette (1.5 mm)
1 per group	Disposable pipette (1 mL)
1 per group	Plate with wells
1	28.5℃ Incubator
1 per group	Depression slide with cover slip
1 per group	Dissecting and compound microscope

Day 1

A. Obtain rinsed embryos from the teacher,
B. Label the well plate. Label the aspartame concentration of each well using the sharpie provided
C. Fill the one well of the play with 1 mL of Instant Ocean/Embryo Media solution using the disposable pipette. Fill the remaining well with the appropriate aspartame stock solutions. Divide the embryos so there are approximately 10 embryos in each well. Label the plate on the student data sheet.

D. *Record exact numbers of live embryos on student data sheet.*

   Note: Dead embryos should be discarded.

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

F. Place each plate in the 28.5°C incubator

Day 2

A. Remove plate from incubator.

B. Remove dead embryos from plate using the disposable pipette. Squirt dead embryos into waste beaker. Be careful to only remove the dead embryos

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

D. Remove aspartame stock solutions from each well of the plate.

   Note: Tilt the plate so the embryos settle and remove the liquid from the top.

E. Replace the aspartame stock solutions with the appropriate fresh aspartame stock solution using a clean pipette each time.

F. Place plate under dissecting microscope and *record observations on student data sheet. Note/describe any developmental markers and abnormalities*. **Repeat** for all aspartame concentrations.

G. Remove 1-2 embryos and place on the depression slide with the cover slip. Observe the embryos using the compound microscope. *Record observations on student data sheet*. **Repeat** for all the aspartame concentration.

H. Return the embryos to their wells in the plate.

I. Return the plate to the appropriate 28.5C incubator.

Day 3

A. **Repeat** day 2 work and observations. *Record all data*.

Day 4

A. **Repeat** day 2 work and observations. *Record all data.*

B. Place all embryos and fish in waste container. The teacher will properly dispose of the organisms.

Above procedure and material list were taken from: SEPA Program- UW- Milwaukee

Data

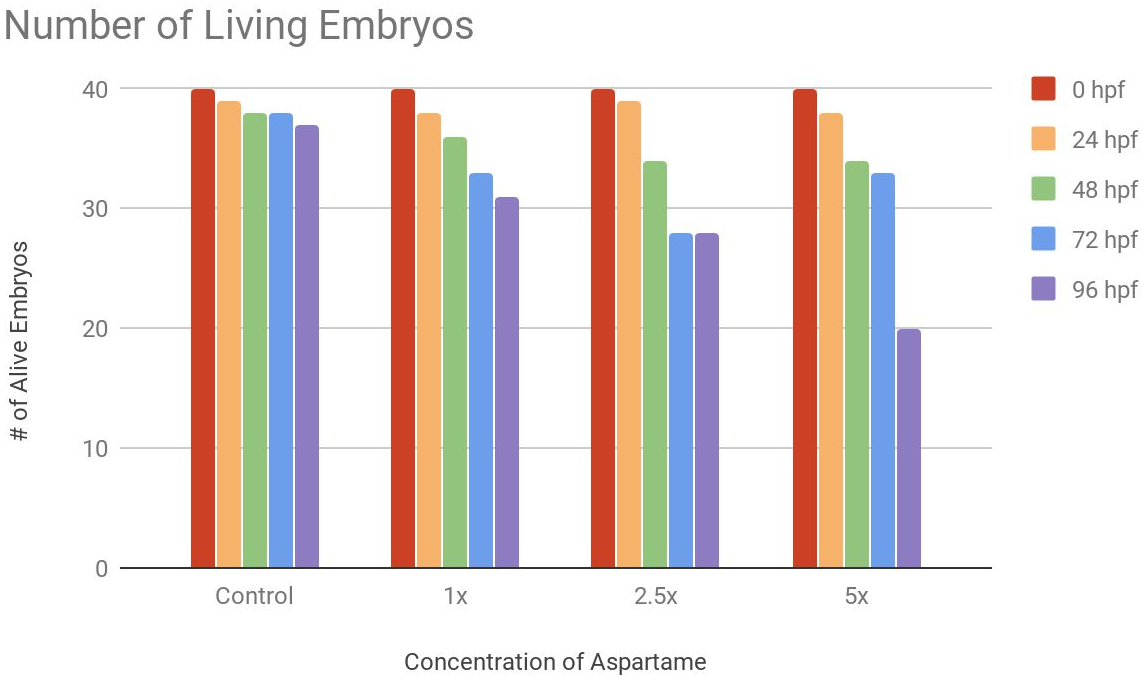
Data Table 1

Number of Living Embryos					
Concentration	0 hpf	24 hpf	48 hpf	72 hpf	96 hpf
Control	40	39	38	38	37
1x	40	38	36	33	31
2.5x	40	39	34	28	28
5x	40	38	34	33	20

Number of living embryos throughout the week with varying concentrations.

Figure 1

Number of living embryos throughout the week with varying concentrations.



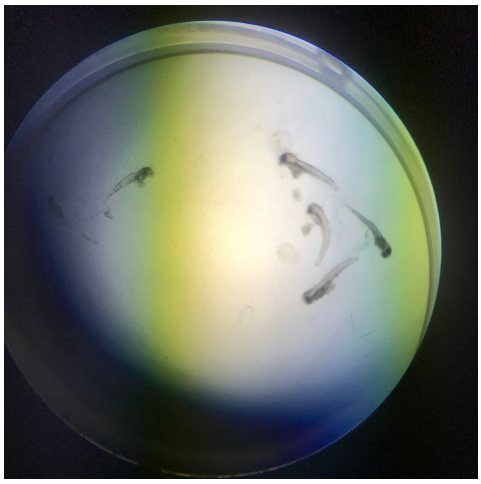


Figure 2
Collection of zebrafish in the 2.5x aspartame well on day three. No deformities or deaths visible.

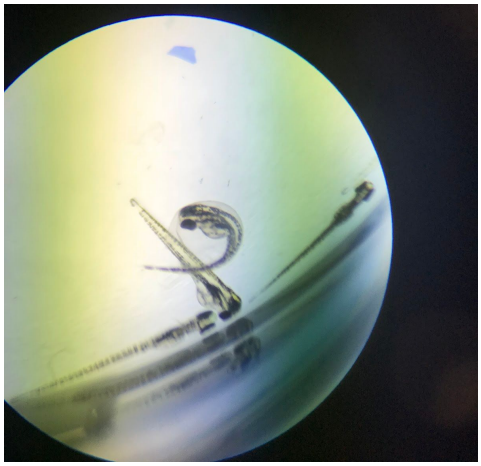


Figure 3
Zebrafish with curved spine, a defect found in the 2.5x aspartame well on day four.

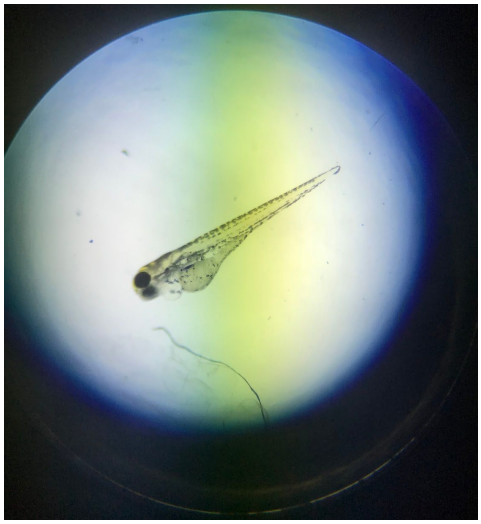


Figure 4
A zebrafish with a distended abdomen, a defect found in the 5x aspartame well on day four.

Data Analysis

A t-test was used with a p-value set at 0.05 to test statistical significance. An unpaired t-test is used to determine if two groups have different average values. This analysis that allows predictions to be made from the data set.

Figure 5

Significance of Living Embryos			
hpf	Comparison	P-Value	Significance
24	Control vs 1x	0.6704	Not significant
	Control vs 2.5x	1.0000	Not significant
	Control vs 5x	0.5373	Not significant
48	Control vs 1x	0.4682	Not significant
	Control vs 2.5x	0.0972	Not significant
	Control vs 5x	0.2071	Not significant
72	Control vs 1x	0.2872	Not significant
	Control vs 2.5x	0.0025	Very significant
	Control vs 5x	0.0667	Not significant
96	Control vs 1x	0.2350	Not significant
	Control vs 2.5x	0.0117	Significant
	Control vs 5x	0.0653	Not significant

The P-values and significance of survival rate data.

Much of the data was found to be not significant, however the 2.5x concentration of aspartame at 72 and 96 hpf were calculated to be significant and very significant.

Results

In this experiment zebrafish embryos were exposed to the independent variable, various levels of aspartame to see if there was a change in the dependent variable, the survival rate compared to that of zebrafish in the control. In the experiment 1x, 2.5x, and 5x concentration of aspartame were used. Over five days the embryos environment whether it be the instant ocean or the solutions, remained unchanged. The goal of this experiment was to see the effects, if any of the aspartame on the survival rate of the zebrafish embryos. Much of the data was found to be not significant.

Discussion

The data found partially supported the hypothesis. Early on in the experiment the aspartame did not impact the survival rate. As the concentration increased, and the experiment went on the death rate increased. The single dosage of aspartame was found to insignificant throughout the experiment. The 2.5x dosage was significant from 72 hpf on, though the 5x aspartame was not found to be significant. The 5x dosage did trend closer to significant (72 hpf - (0.0667)) (96 hpf - (.0653)) in comparison to the 1x group (96 hpf - (.2350)).

Although not quantified, the number of defects in the fish in the aspartame solutions appeared to be greater than that in the controls (see figures 3 and 4). Another study should be setup to test the number of abnormalities, if any, caused by aspartame.

There have been few studies actually showing the negative effects of aspartame. In an experiment done by S. Schiffman, it was found that aspartame was no more headache inducing than a placebo (1987). In addition to that J. Gurney and team came to the conclusion that aspartame does not increase the risk of childhood brain tumors (1997).

There is very little current research that suggests that aspartame does have a negative impact on both zebrafish and humans, however the results of this study suggest that further research should be conducted.

References

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