# Physical and Developmental Effects of Caffeine on Zebrafish Embryos

Samantha Cook
Waukesha North High School

**Abstract**

This experiment was conducted to show how caffeine affects the human body using zebrafish as the test subject due to similar genetic structure. Caffeine is a product produced from plants and is found in soda, coffee, and tea. Caffeine causes alertness by stimulating the nervous system; due to its nature, caffeine is classified as a drug. Similar studies have been conducted on rats referenced in the study by Chen (2008) where Wilkinson and Pollard “fed pregnant rats on days 8–9 of gestation with 25 mg/kg of caffeine, causing the embryos to have reduced development of the heart, eyes and limbs.” Fertilized zebrafish embryos develop outside the mother in a clear embryonic eggs, making it easy to study their development under a microscope and making them the ideal specimen for this model. The embryos were deposited in various caffeine solutions with differing concentrations 0.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, 1.0 mg/mL. These solutions of caffeine were used to affect the zebrafish development in different ways as they grew in their experimental environment. Through data recorded daily, it was found that high amounts of caffeine had negative effects to the health of the zebrafish raising their mortality rate and as such, should be avoided by humans.

**Introduction**

Caffeine is abundant in today's society from coffee to tea and even soft drinks. Many people rely on caffeine in the form of coffee to wake up in the morning and develop a dependence upon it, but is it actually safe? Some believe yes, but studies have used zebrafish to see the possible harmful effects of caffeine on the body. Zebrafish were used as the test specimen because they possess similar genetic structure with humans, have a quick reproduction/growth rate and have mostly transparent embryonic sacs making observations under a microscope easy. An experiment conducted by Yau-Hung Chen (2008), explained that caffeine may not be as safe as some think. The results of Yau-Hung Chen’s experiment showed muscle deterioration and stunted physical attributes to the embryo's body when exposed to high dosages of caffeine (Chen, 2008). Embryos in high concentration solutions showed higher death rates and lower average body length overall.

The hypothesis was if caffeine is harmful to the zebrafish embryos and the ones placed in higher concentrations would exhibit higher mortality rates, it would be potentially harmful to humans in physical and developmental ways also. This theory is supported by the fact that humans and zebrafish both share around 70 percent of similar genes. A study done by Howe, K. (2013, April 17) states, “Zebrafish research has already led to biological advances in cancer and heart disease research, and is advancing our understanding of muscle and organ development”. Senior Prof Jane Rogers (2013) explains the human and zebrafish genome relationship, “The vast majority of human genes have counterparts in the zebrafish, especially genes related to human disease. This high quality genome is testament to the many scientists who worked on this project and will spur biological research for years to come. By modeling these human disease genes in zebrafish, we hope that resources worldwide will produce important biological information regarding the function of these genes and possibly find new targets for drug development”.

**Materials**

The 40 total zebrafish eggs were the basis of the experiment. The eggs were used as our dependent variable, while the caffeine solutions were used as the independent. The caffeine solutions (0.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, 1.0 mg/mL) were put inside the container wells using pipettes to transfer the solutions to the beaker. Liquid from other wells should not be transferred to other environments. The wells were then numbered with the chemical labeling tape and a sharpie. A dissecting microscope was used to observe the embryos through day two to five. This was used to observe the effects of the different concentrations of caffeine have on the zebrafish developmental rate. The pipette was used to collect the eggs, remove deceased fish and replace solutions within the zebrafish environment daily. Wells in the dish provide an environment for the zebrafish to occupy. Beakers are used to transport dead fish and embryos from the environment to be disposed, as well as used solutions. Fish and embryos are to be placed under an incubator overnight every night after they are fertilized. An iPad or other device should be used to document the observations and take photographs to optimize results.

Figure one: Dead Embryos (dark insides, almost “fuzzy”)

*Safety Precaution: Gloves should be worn during this experiment as a safety as potentially hazardous chemicals are being used. Pipettes are to be thrown away after usage to avoid cross-contamination.*

**Methods**

Day one of the experiment is the collection of the zebrafish embryos. Chemical labeling tape should be pulled over the lid underneath where the wells are. Write from left to right on the chemical labeling tape underneath the wells:0.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, 1.0 mg/mL. Underneath the tape, make another tape strip, about the same length horizontally across the lid. Write the experimental solution chosen on the labeling tape, with your name(s). Each well in the top of the dish should be filled with ten eggs each using a pipette, a total of 40 eggs. When followed through, place the container on the cart to be sent off to the incubator; A supervisor should do this.

On day two of the experiment, embryos should be observed using a dissecting microscope and any movement, death, development or physical changes should be meticulously recorded in the document. Heart beat rate may also be recorded if wished, but it is not mandatory. Take pictures of the embryos for record and closer inspection. After the day’s observations remove any dead using a pipette and dissecting scope, dead embryos will turn a darker shade and will begin to appear cloudy. Change out solutions to fresh ones in the wells accordingly and have supervisor place embryos back in incubator

On day three, change out the solutions in the corresponding wells; make sure to not cross contaminate the solutions. Record observations and the mortality rate for which will be used to compile in chi-charts for comparisons. Remove any dead embryos using the previous process.

On day four of the experiment, continue to change out the solution and remove dead daily and update information.

Observe the embryos on day five, any changes in appearance and behavior should be recorded. Dispose of gloves and any chemical ridden pipettes and make sure to not to let it touch your skin. Without harming the embryos, and place the living control fish into a tank after removing the caffeine solution. Embryos may die from shock due to the extreme change in environment when placed in a tank of water; it is not uncommon for them to go in shock.

**Results**

The point of the experiment was to show the effects that caffeine has on the developing human embryos, by observing zebrafish, which are genetically similar to humans. The experiment was used to collect data about the hatch rate, appearance and death rate of the embryos using charts and tables to organize the data. The independent variable in this experiment was the concentration of caffeine of each solution placed in the wells and the dependent variable was the mortality rate of the zebrafish embryos. These variables correlate with each other; as the amount of caffeine increased, mortality rate observed also increased. Well one, containing the 0.0 mg/mL controlled solution, had second to least deaths. The embryos in well one were not malformed upon development and appeared to react normally to environmental stimulation. The control showed healthier development overall than the embryos exposed to caffeine.

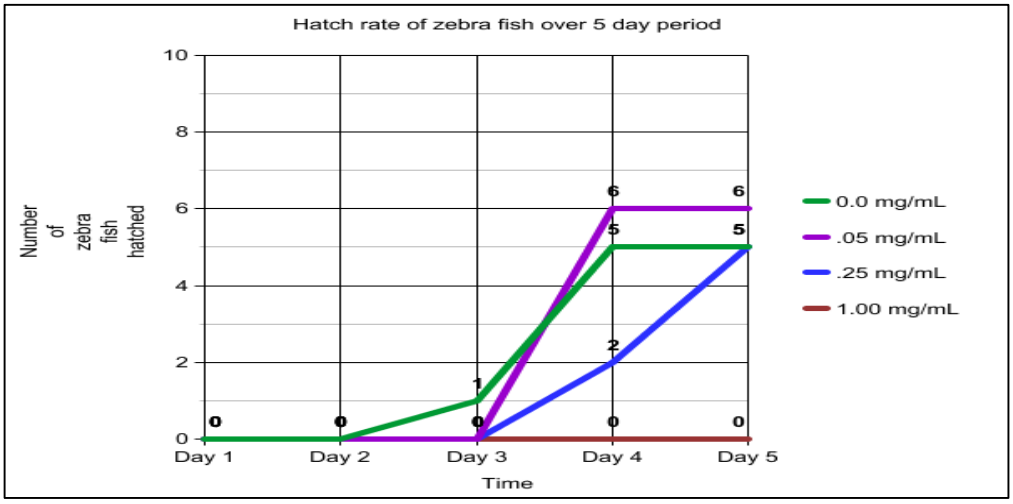


Figure three: Graph illustrating the delayed developmental rate high caffeine doses, causing embryos to hatch slower or not all.

Where as the zebrafish in figure two shows multiple deformities of the spinal region, as well as the inability to swim. The contrast between the two solutions results is easily distinguishable. The graph in figure three shows the life and death accounts of the zebrafish embryos on day five. The life and death rate can be compared to the caffeine solutions to see the relationship between the caffeine dosages and death.

Figure one: Showing the zebrafish in 0.25 mg/mL Solution day 5 with

Chi-Square Live and Dead Zebrafish Data

| **Caffeine** | **Live** | **Dead** | **Total for Rows** |
|---|---|---|---|
| **Control (0.0 mg/mL)** | 5 | 5 | 10 |
| **0.05 mg/mL** | 5 | 5 | 10 |
| **0.25 mg/mL** | 6 | 4 | 10 |
| **1.0 mg/mL** | 0 | 10 | 10 |
| **Total for Columns** | 16 | 24 | Total for Table 80 |

Figure 4: Chi-squared charts depicting life and death rates for day five. The live and death charts show the observed (O) variable and the expected (E) variable.

Dead

| **Caffeine** | **O** | **E** | **(O-E)** | **(O-E)^2** | **(O-E)^2/E** |
|---|---|---|---|---|---|
| **Control (0.0 mg/mL)** | 5 | 3 | 2 | 4 | 1.3 |
| **0.05 mg/mL** | 5 | 3 | 2 | 4 | 1.3 |
| **0.25 mg/mL** | 4 | 3 | 1 | 1 | 0.3 |
| **1.0 mg/mL** | 10 | 3 | 7 | 49 | 2.3 |

Live

| Caffeine | O | E | (O-E) | (O-E)^2 | (O-E)^2/E |
|---|---|---|---|---|---|
| **Control (0.0 mg/mL)** | 5 | 2 | 3 | 9 | 4.5 |
| **0.05 mg/mL** | 5 | 2 | 3 | 9 | 4.5 |
| **0.25 mg/mL** | 6 | 2 | 4 | 16 | 8 |
| **1.0 mg/mL** | 0 | 2 | -2 | 4 | 2 |

## Discussion

"Caffeine has been known to have effects on humans such as raising heart rate and birth complications" (Fenster, 1991). The higher death count among the embryos placed in the 1.0 and 0.25 mg/mL solutions supported not only this quote, but also the original hypothesis of the zebrafish experiment by showing that zebrafish placed in higher concentrations displayed higher mortality rates and an increase in developmental/ birth delays. Similar results were seen elsewhere in related studies. These results that were shared was the fact the muscles were deteriorating and the lack of power to swim.

From data collected from a researched experiment, movement disorder and neuromuscular change in zebrafish embryos after exposure to caffeine, it can be learned that caffeine affects zebrafish in a negative manner and should be avoided by growing human infants due to a close gene structure shared between the two species (Yeh, C., 2012).

Some errors were found in the experiment due to the stress of changing environments killing many of the 0.0 control embryos within the first day. Also when a pipette was mistakenly used to perform the transportation of different solutions into different wells of two zebrafish embryos of the 0.25 and 0.0 mg/mL. This caused a negative flux of some of the data. Cross contamination also might have played a part of the data results, causing the 0.0 control solution to have unexpected death rates, higher than the 0.05 solution of caffeine.

The experiment data has shown high amounts of caffeine concentration negatively affect the overall health of zebrafish. The results of the experiment can be observed that it would also have negative effects on human health. Caffeine should be avoided in large concentrations and for women in pregnancy to avoid birth defects in offspring.

## References

Brent, R. L., Christian, M. S., & Diener, R. M. (2011). Evaluation of the Reproductive and Developmental Risks of Caffeine. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology*, *92*(2), 152–187. http://doi.org/10.1002/bdrb.20288

Chen, Y.H., Huang, Y.H., Wen, C.C., Wang, Y.H., Chen, W.L., Chen, L.C., and Tsay, H.J. (2008) Movement disorder and neuromuscular change in zebrafish embryos after exposure to caffeine. Neurotoxicol. Teratol.. 30(5):440-447

Fenster L, Eskenazi B, Windham G, Swan S. Caffeine Consumption During Pregnancy and Fetal Growth. *American Journal Of Public Health* [serial online]. April 1991;81(4):458. Available from: MasterFILE Premier, Ipswich, MA. Accessed December 14, 2016.

Hoecker C, Nelle M, Poeschl J, Beedgen B, Linderkamp O. Caffeine Impairs Cerebral and Intestinal Blood Flow Velocity in Preterm Infants. *Pediatrics* [serial online]. May 2002;109 (5):784. Available from: MasterFILE Premier, Ipswich, MA. Accessed December 14, 2016

Howe, K. (2013, April 17). The zebrafish reference genome sequence and its relationship to the human genome. Retrieved January 05, 2017, from http://www.nature.com/nature/journal/v496/n7446/full/nature12111.html

Yeh, C., Liao, Y., Chang, C., Tsai, J., Wang, Y., Cheng, C., & ... Chen, Y. (2012). Caffeine treatment disturbs the angiogenesis of zebrafish embryos. *Drug & Chemical Toxicology*, *35*(4), 361-365. doi:10.3109/01480545.2011.627864