The Lethal Effects of Caffeinated Solutions on the Development of Embryonic Stage Zebrafish

Sean Weaver
Waukesha North High School

Abstract

Caffeine is a common drug found in many things people consume every day. Some side effects like increased heart rate and jitters are known, but can it have negative results on developing organisms as well? This experiment was done to explain how developing zebrafish are affected by the caffeine, and how it could potentially affect human fetuses. Caffeine affects people in many different ways. It can potentially, if enough is consumed, be fatal. Various caffeine concentrations were used on the zebrafish eggs in the experiment, such as 0.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, 1.0 mg/mL. Then, the eggs (and eventually fish after hatching) were monitored with a dissecting microscope to see the effects of the caffeine solutions. All of the fish with the highest amounts of caffeine in their wells died. So, if they are dying from high amounts of caffeine, it can be inferred that humans can also die from the consumption of high amounts of caffeine.

Introduction

While many know that caffeine has some sort of effect on developing creatures, few really know for sure what that effect may be. One could make the assumption that it may cause harm to the organisms due to the fact that it is a drug that can cause the consumer to become dependent on it. Caffeine also has several side effects and can even cause overdose.

This experiment was done to determine if the effects caffeine has on zebrafish could possibly correlate to the effects that caffeine has on the developing human body. Caffeine has many negative effects. This is because caffeine is a substance that stimulates the central nervous system. It can make the consumer feel more awake, but it has some downsides. It can cause shakiness, jitters, and dehydration especially after a workout or physical activity. It is also possible to die from caffeine poisoning, if enough is consumed.(found in a study done by Trevor-Roper, H. (1982). The body can only take in so much caffeine. This mark is at about "118 cups of coffee each are 240 mL." Depending on the type of coffee, it could amount to "15,939 mg of caffeine", or about 3.2 teaspoons of powdered caffeine (according to compoundchem.com, and the calculator.co).

For humans, consumption of caffeine is not recommended during pregnancy as the fetus can be sensitive to the stimulant. A study done by Caffeine Intake During Pregnancy. (2016, September 01) shows that caffeine can cause miscarriages, but there are also sources stating that moderate levels of caffeine intake have no negative effects on the child. This being said, death was a possibility in the fish being experimented on. Based on this information, if the zebrafish

embryos are placed in high concentrations of caffeine, it will be fatal because it is proven that large amounts of caffeine are lethal.

Materials & Methods

Forty zebrafish eggs were used and put into a tray with four wells. Each well had ten fish eggs in it. The tray also had a cover to protect it from being spilled. Each well was filled with a different fluid concentration of caffeine. The first well was the control and contained 0.0 mg/mL of caffeine in. In the second well, there was 0.05 mg/mL of caffeine in the fluid. In the third well, there was 0.25 mg/mL of caffeine in the second solution. The last well had a concentration 1.0 mg/mL of caffeine in the solution. One small bore transfer pipette and one large bore transfer pipette were used in this experiment. These were used to change out the fluids the in wells. The fine pipette was used take the fluids out. Then, the large pipette was used to put the solutions back in the wells. A 250 mL beaker was used to put the old solutions and dead fish/eggs in it. A dissecting microscope was also used to observe the actions and appearances of the fish to see if they had any growths or deformities.

All of the materials were provided by the Wisconsin Inquiry based Scientist Teacher Education Partnership (WInSTEP) Program, which is part of the NIH Science Education Partnership Award (SEPA) Program administered by the University of Wisconsin–Milwaukee and the Children's Environmental Health Sciences Core Center.

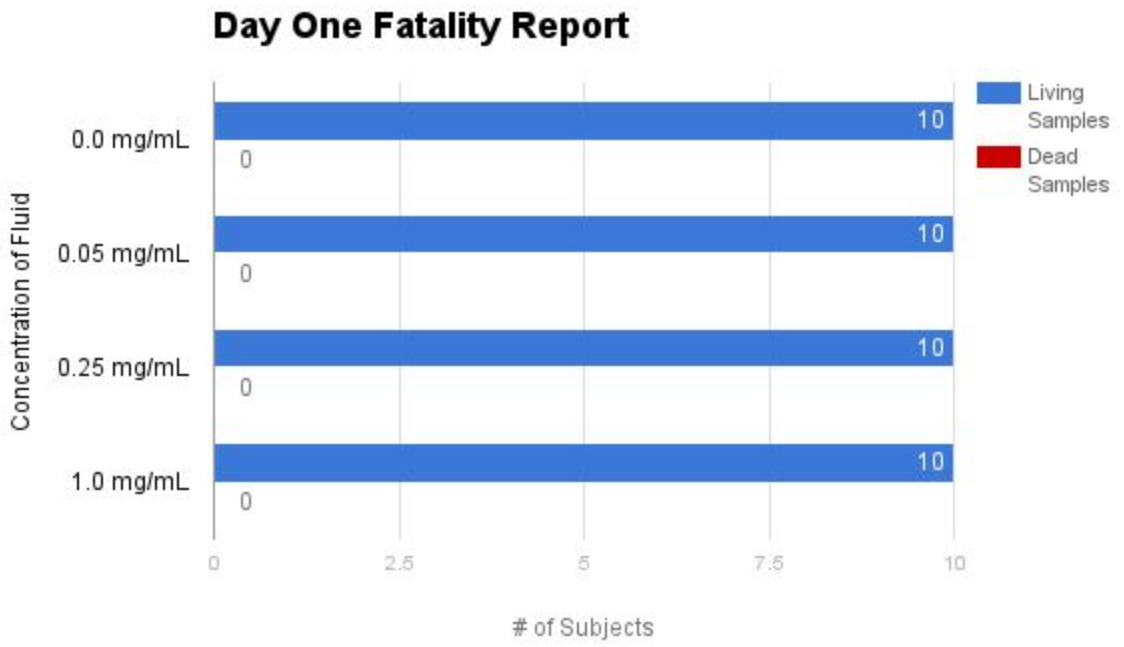
Procedure

First, all 40 of the zebrafish eggs were placed in the wells (ten in each.) Then, residual liquid from transfer was removed and the caffeine solutions were put into the wells. The solutions were placed in order from lowest to highest caffeine concentration. These concentrations were 0.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, and 1.0 mg/mL. After that, the tray was put under the dissecting microscope to observe the specimen in the wells. The embryos were photographed under the microscope through the lens for future reference of the fish. This was done for five days. Over this period they were incubated in a temperature of 28.5 degrees Celsius. Then, the next day, the fish were examined and the deceased were counted. If an egg was dead, it was gray and opaque in the middle, whereas an egg that was alive had a clear outer layer. Each day for five days, the solutions had to be changed. The fine pipette was used to remove the old solutions without taking any live eggs with it. When changing the solutions, all of the dead eggs and dead fish needed to be taken out of the wells and disposed into the beaker. Then, picture taking continued. When the fish hatched, the process was repeated. Looking for dead fish and removing them from the solutions was essential. If a fish wasn't moving at all, even with prompting, it was a possibility that it was dead. In case the fish were still alive and unmoving, they were looked at underneath the microscope for a heartbeat. If there wasn't a heartbeat then the fish was declared dead. This process was repeated and the fish were put into the larger tank. For this, the large pipette was used to suck the fish up and squeeze them into the

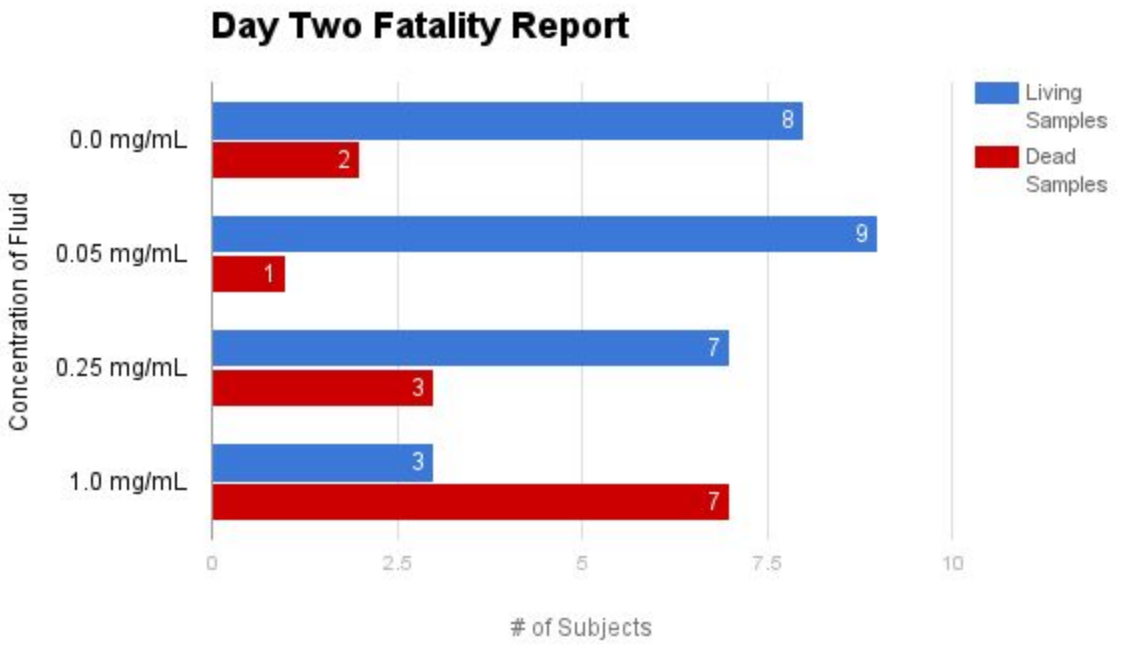
larger tank. In this report, a chi square analysis was used to test for independence between the caffeine solutions and mortality rate.

Results

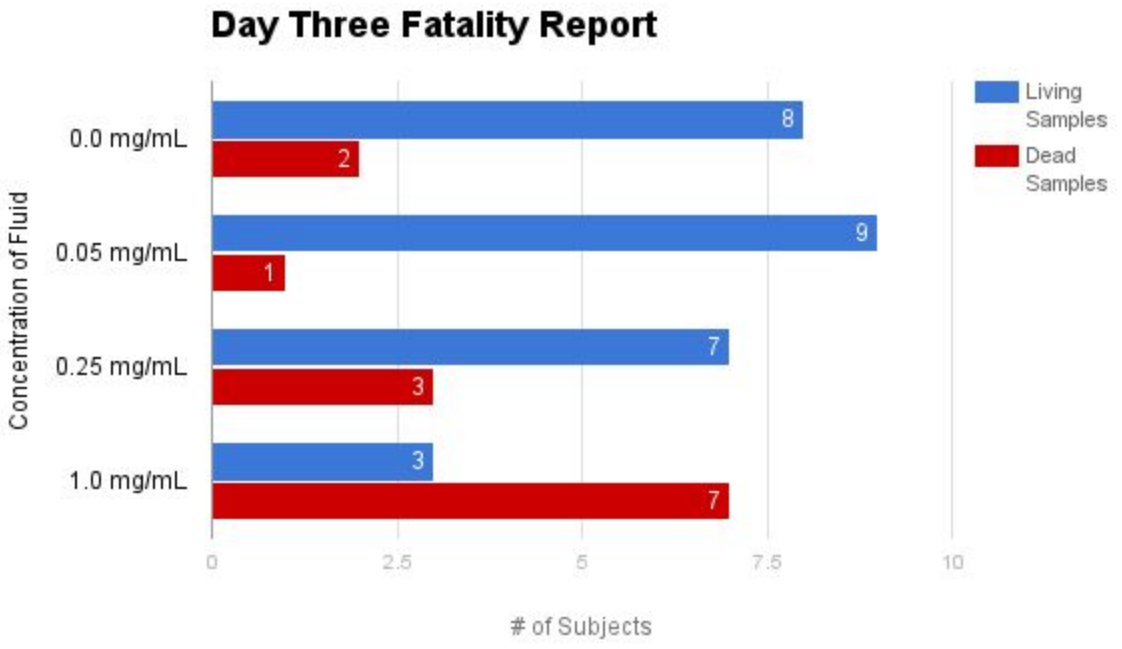
The experiment proved that caffeine is lethal to the zebrafish embryos. They prove our hypothesis correct, which is that high amounts of caffeine can have lethal effects. It especially shows with the insanely high mortality rate in the 0.25 mg/mL and 1 mg/mL wells, but also with other problems in the 0.05 mg/mL solution. These are our results from this experiment. In the five days the experiment was held the caffeine had a negative effect on most of the fish. All of the solutions were held at 28.5 degrees Celsius for the five days. Each well had of one mL of the four solutions that were designated to all the wells. The first well with 0.0 mg/mL solution had no caffeine in it making it the control. This was to show us how the fish would normally look and act in uncontaminated water. The other three wells had increasing increments of caffeine in their solution. The second had 0.05 mg/mL, the third had 0.25 mg/mL, and the fourth and last well had 1 mg/mL of caffeine in its solution. During these five days the solutions with higher concentrations of caffeine were 0.25 and 1 mg./1 mL, lead to dramatically increased mortality rates among the sample sizes. In both of those solutions, all of the fish in them died. Generally, high doses of caffeine as seen in the 0.25 and 1 mg/mL batches of developing embryos did not survive very long. The well with 0.05 mg/mL of caffeine with the lowest concentrations leading to some premature births and eventual deaths within the sample size. Losses greatly increased in the wells with 0.25 and 1 mg/mL 100% of the fish died within 3 days. One interesting development of a fish in the well with 0.05 mg/mL of caffeine was a fish that wasn't completely out of its shell. Part of its head was still stuck inside of the shell. This occurrence could have been due to its exposure to the caffeine.



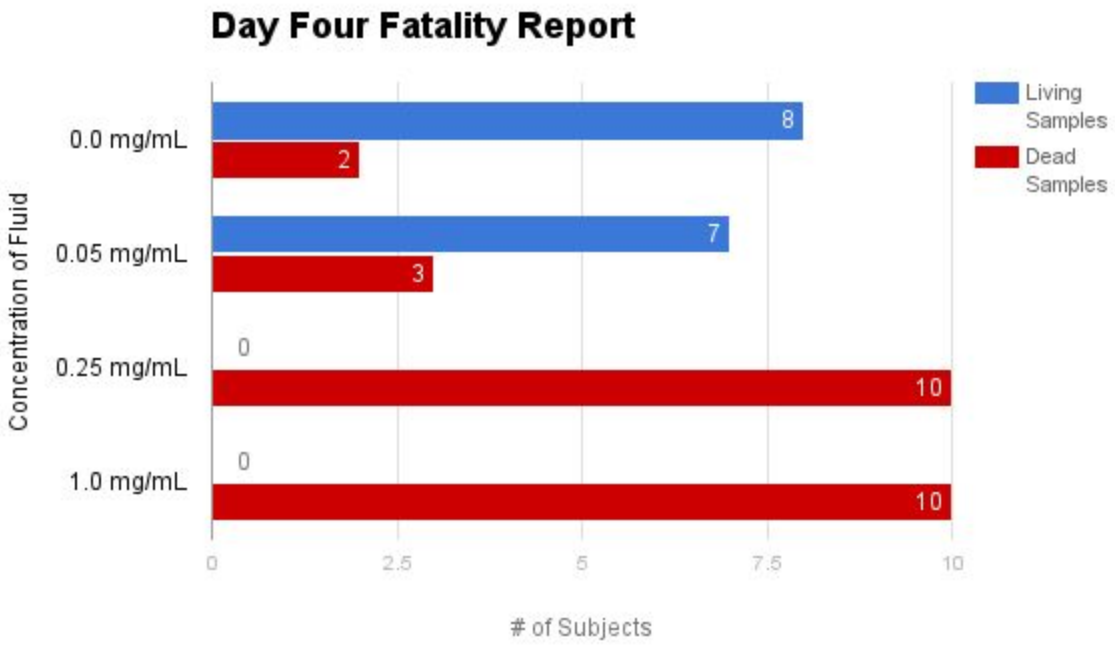
At the end of day one, it shows in the days fatality graph that all of the fish were still alive in their embryos. They seemed to be fine with no deformities or problems.



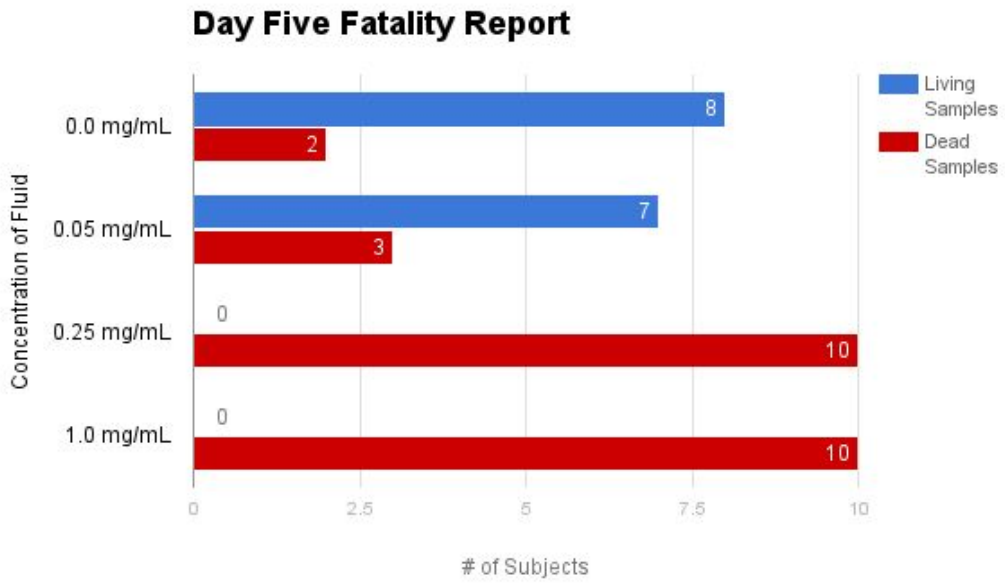
At the end of the second day, it shows in this second fatality graph that two of the embryos died in the control. One died in the 0.05 mg/mL solution. And three died in the 0.25 mg/mL, and there were six deaths 1 mg/mL solutions. Other than the deaths there are no deformities in the embryos.



On day three, there were no deaths at all. It shows this in the day three fatality graph But in the 0.25 mg/mL one of the embryos hatched which would be a premature birth, because none of the other fish have hatched



Day four is when most of the fish hatched. It shows this on the graph of day four's fatality graph. They only hatched and lived in the control and 0.05 mg/mL. But two fish did die in the the 0.05 mg/mL solution. In the 0.25 and the 1 mg/mL solutions, all of the rest of the fish that were alive in those two wells died.



On day five the fatality graph for the day shows that eight fish remained alive in the control and were all health with no deformities. In the 0.05 mg/mL solution the seven fish were still alive and hatched. But one of the fish in that solution was only partially hatched and had part of the shell on his head, but it seemed fine. This is a deformity to a fish in a caffeinated solution and may have been because of the caffeine.

Living Samples Square (Table 1)

Living Samples	Observed	Expected	O-E	(O-E)2	(O-E)2/E
Control	8	3	5	25	8.3
0.05mg/mL	7	3	4	16	5.3
0.25 mg/ml	0	3	-3	9	3
1 mg/ml	0	3	-3	9	3

Dead Samples Square (Table 2)

Dead Samples	Observed	Expected	O-E	(O-E)2	(O2/E
Control	2	2	0	0	0
0.05 mg/ml	3	2	1	1	0.333
0.25 mg/ml	10	2	8	64	32
1 mg/ml	10	2	8	64	32

Total: 19.66

Total: 64.5

A chi square analysis was conducted to determine to determine if caffeine and mortality rate are independent of each other. The chi square value was 64.33 using 3 degrees of freedom and a critical value of 7.82. This did not support the null hypothesis. Therefore, there is a strong correlation that caffeine causes mortality.

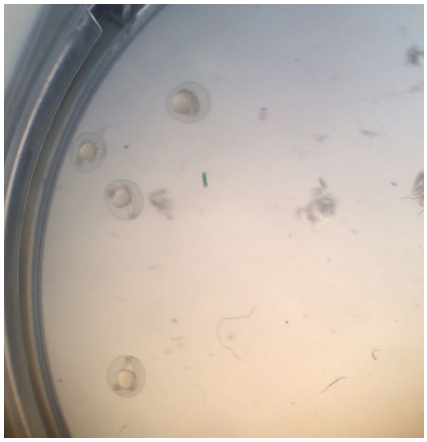
(Figure 1)

This is figure one. This picture is of the embryos on the first day of the fish in the 0.0 mg/mL solution. There seem to be no deformities or anything else wrong with these fish in the well with 0.0 mg/mL.

(Figure two)

This is figure two it is of of some of the embryos in the 0.05 mg/mL group. This picture was also taken on the first day. It also shows no deformities or other problems with the fish.

(Figure three)



This is a figure three of the well with 0.25 mg/mL on the first day. Again this well has no problems with the embryos.

(Figure 4)

This is figure four, it is a picture of the well with 0.05 mg/mL on the fourth day.This fish still has part of a shell on its head. This could have been due to the caffeine.

Discussion

Putting together the research and results of this experiment, it is clear to see that caffeine was a primary contributor to the death of the infant fish. It was found that in the 0.25 mg/mL and 1 mg/mL concentrations of caffeine, that none of our zebrafish embryos survived. The Chi square results showed an extremely high over 99% percent chance that the fish died from caffeine. Tying this into the research done beforehand, it became clear as to why it is recommended by professionals that women should not consume excessive amounts of caffeine while pregnant for the safety of their unborn child. This is because it is very likely that the caffeine could affect the unborn child. By being born prematurely or troubles with the birth. The child could also die or have a shortened life due to the caffeine. In our observations of the experiment it shows that there is clearly an amount of caffeine that is lethal. The amount may increase or decrease by animal and weight, but there is clearly an amount. We can prove this because of how many fish died in the higher amounts. We believe that our hypothesis is right because it showed that the high amounts of caffeine were lethal. We still don't know exactly how much caffeine is lethal or how much it would take to kill a human infant. But looking at the

results it is clear that caffeine is dangerous. If the experiment were to be conducted today we would take more precautions and time to check samples and measure fatalities. We would have also really understood how fast the fish mature and take more observation in the limited amount of time we had.

References

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