

Differences Between Caffeine and 5 Hour Energy Effects on Zebrafish

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Abstract -

The purpose of our experiment was to determine how different the effects of caffeine and energy shots were from each other. By treating zebrafish embryos with these substances, we found that higher concentrated 5 hour energy treated embryos were dead within 24 hours. With the caffeine we didn't actually see a lot of deaths until the 48-72 hour mark. We determined that these results were extremely significant. Not much research has been conducted on energy shots on human pregnancy. Since zebrafish are a naturally good model of study, this is extremely important information for people to know.

Introduction -

Zebrafish are a fast developing freshwater dwelling fish. We use these fish as a model for looking at the development of embryos. Zebrafish develop extremely fast compared to other animals and are transparent.

I will be comparing the effects of caffeine compared to 5 hour energy, and why 5 hour energy is much more dangerous. Caffeine is naturally found in some plants and flowers but we mainly find it in your morning coffee. 5 hour energy is not naturally found and is synthetically made. It is a concoction of a lot of different proteins and stimulants.

Caffeine and 5 hour energy generally have negative effects on development on human and zebrafish embryos. Caffeine must be extremely regulated when pregnant and 5 hour energy is just not recommended.

This research investigates how caffeine and 5 hour energy affects the survival and hatching of zebrafish. If we increase the concentrations of 5 hour energy and caffeine, then the embryos affected with 5 hour energy will be affected a lot more. This is because it appears 5 hour has more ppm of stuff within one particle of the energy shot. Opposed to caffeine, within a ppm of caffeine, only caffeine is found and it is less concentrated.

If we increase the concentrations of 5 hour energy and caffeine, then the hatching and survival of zebrafish will not be affected.

This research is significant because there is not a lot of research done on development in relation to these chemicals. I want to show people how different these two substances are.

Materials and Methods -

- Provided solutions (0.01, 0.05, 0.1) of nicotine, caffeine, 5 hour energy and alcohol
- Zebrafish embryos stored in observing container
 - 120 per each solution
- Microscope
- White paper for seeing better

- Chromebook, laptop, or phone for recording data (google sheets)

Experiment steps:

1. Obtain labeled embryos from teacher
2. Observe each section carefully
3. Look for dead, living and hatched embryos
4. Record exact number of living and hatched embryos in provided google sheet (dead hatched don't count)
 - a. Place under white paper if you need to see better
5. Repeat the steps above for every 24 hours

We analyzed this data using google sheets. Google sheets are a great way to keep all your data in one place. You can process and observe your data here too. We processed all data using tables of averages from our raw data. We did this because it is easier to put in a graph. This also allows us to see the bigger picture for each condition. To look for significance we used t-test to compare different conditions. We did this because seeing differences was what we were really trying to get at. We were trying to determine if any of these solutions, in different concentrations, had effects on the zebrafish.

Results

In this experiment we tested the effects of caffeine compared to 5 hour energy. We were collecting data on any mutations, survival and hatching rates of the fish. We looked at 0.01, 0.05, and 0.1 concentrations.

Our independent variable in the study was the concentrations of 5 hour energy and caffeine. Our dependent variable was the hatching and survival rates of the zebrafish.

Some things that were controlled were the setting and the temperature as well pH level. We were comparing all concentrations to a control group of just water.

This table shows the raw data collected for 5 hour energy:

Sample	5 hour energy	24 hours alive	24 hours hatched	48 hours alive	48 hours hatched	72 hours alive	72 hours hatched
A	0%	8	0	8	0	3	0
A	0%	8	0	7	0	4	0

A	0%	7	0	7	0	6	0
A	0%	7	0	7	0	7	2
A	0%	9	0	8	0	8	4
A	0%	9	0	9	0	9	5
A	0%	7	0	7	0	7	4
A	0%	8	0	8	1	8	6
A	0%	9	0	9	0	8	8
A	0.10%	10	0	8	0	5	0
A	0.10%	8	0	6	0	5	0
A	0.10%	7	0	7	0	5	0
B	0.10%	6	0	6	0	6	3
B	0.10%	9	0	9	0	9	3
B	0.10%	10	0	10	0	10	4
B	0.10%	8	0	8	0	8	5
B	0.10%	8	0	8	0	8	7
B	0.10%	10	0	10	0	9	9
B	0.50%	1	0	0	0	0	0
B	0.50%	1	0	0	0	0	0
B	0.50%	0	0	0	0	0	0
B	0.50%	0	0	0	0	0	0
B	0.50%	0	0	0	0	0	0
B	0.50%	0	0	0	0	0	0
C	0.50%	1	0	0	0	0	0
C	0.50%	0	0	0	0	0	0
C	0.50%	0	0	0	0	0	0
C	1%	0	0	0	0	0	0
C	1%	0	0	0	0	0	0
C	1%	0	0	0	0	0	0
C	1%	1	0	0	0	0	0
C	1%	0	0	0	0	0	0
C	1%	0	0	0	0	0	0

C	1%	0	0	0	0	0	0
C	1%	0	0	0	0	0	0
C	1%	0	0	0	0	0	0

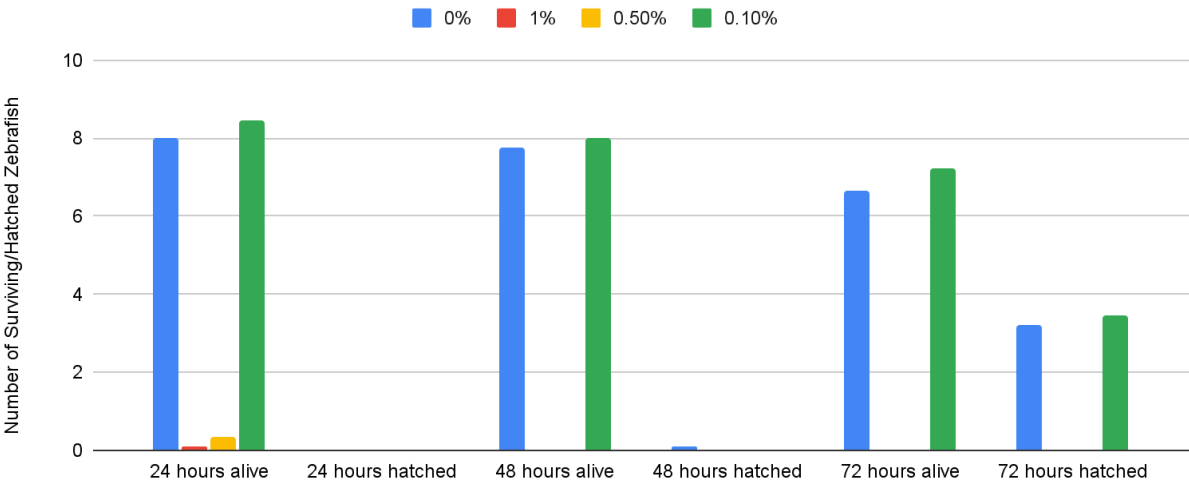
This table shows the raw data collected for Caffeine:

Sample	Caffeine mg/mL	24 hours alive	24 hours hatched	48 hours alive	48 hours hatched	72 hours alive	72 hours hatched
A	0	6	0	5	0	5	3
A	0	7	0	7	0	7	1
A	0	5	0	5	0	5	1
A	0	8	0	8	0	7	0
A	0	9	0	9	0	9	2
A	0	9	0	8	0	7	2
A	0	5	0	5	0	5	0
A	0	7	0	7	0	5	2
A	0	7	0	7	0	7	0
A	0.05	6	0	6	0	6	0
A	0.05	9	0	8	0	8	0
A	0.05	8	0	8	0	8	0
B	0.05	8	0	7	0	7	0
B	0.05	6	0	6	0	6	1
B	0.05	5	0	5	0	5	0
B	0.05	8	0	8	0	7	1
B	0.05	8	0	8	0	7	1
B	0.05	8	0	8	0	5	2
B	0.25	7	0	7	0	7	1
B	0.25	7	0	7	0	6	2

B	0.25	6	0	6	0	6	4
B	0.25	7	0	6	0	4	3
B	0.25	7	0	4	0	4	0
B	0.25	7	0	5	0	5	1
C	0.25	7	0	7	0	6	1
C	0.25	9	0	9	0	6	3
C	0.25	8	0	8	0	3	3
C	1	9	0	8	0	8	0
C	1	6	0	5	0	4	0
C	1	6	0	5	0	3	0
C	1	9	0	4	0	2	0
C	1	7	0	3	0	2	0
C	1	8	0	6	0	5	0
C	1	6	0	4	0	3	0
C	1	7	0	4	0	4	0
C	1	7	0	6	0	4	0

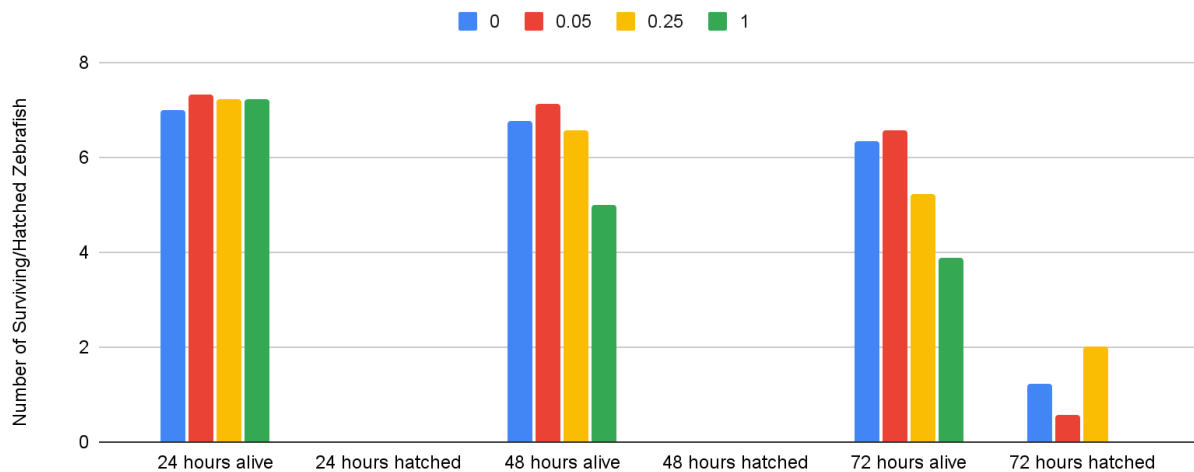
This graph shows the processed data for 5 hour energy data collection:

Hatching and Survival Rates of Zebrafish Treated with 5 Hour Energy



This graph shows the processed data for caffeine data collection

Hatching and Survival Rates of Zebrafish Treated with Caffeine



This chart shows the insignificant differences and significant differences between concentrations of caffeine. (significant differences highlighted)

TTEST CHART						
	24 hours alive	24 hours hatched	48 hours alive	48 hours hatched	72 hours alive	72 hours hatched
0 vs 0.05	0.623881555 3	1	0.603166272 4	1	0.717528260 5	0.147028776 8
0 vs 0.25	0.702756499 6	1	0.756650570 1	1	0.102100010 9	0.192748035 4
0 vs 1.0	0.733228334	1	0.022293586 6	1	0.005975670 944	0.004024899 347

This chart shows the insignificant differences and significant differences between concentrations of 5 hour energy. (significant differences highlighted)

TTEST CHART						
	24 hours alive	24 hours hatched	48 hours alive	48 hours hatched	72 hours alive	72 hours hatched
0 vs 1	0	1	0	0.3321949847	0.00000002746857471	0.004273608706
0 vs 0.5	0	1	0	0.3321949847	0.00000002746857471	0.004273608706
0 vs 0.1	0	1	0	0.3321949847	0	0

We used a t-test because we wanted to see if there were significant differences between the concentrations. A lot of our significant data was found in the data of 5 hour energy. Nearly every other comparison was significant. The t-test results with 1 shows that the fish in each trial were not significant at all. The t-test results with 0 shows a complete significant difference. This is a very strong representation of how strong of a difference the concentrations have between one another.

Discussion:

We wanted to figure out to what extent the different concentrations of caffeine and 5 hour energy differ. If we increase these concentrations the more severe the effects of these two chemicals will be. We will also see extreme effects in the 5 hour energy concentrations. This is because there is a concoction of different vitamins and chemicals in 5 hour energy opposed to just caffeine. That concoction might have just been too much for the fish to handle at that stage of their lives and could be why we saw an extreme cut off to survival and hatching rate.

The p-values of 0s all show extremely significant comparisons and differences. This also goes for the p-values of 0.00000002746857471, 0.004273608706, 0.0222935866, 0.004024899347, and 0.0222935866.

Looking at these values, it is safe to say that we reject our null hypothesis that these concentrations will have no effect on the zebrafish. We can clearly see in the graphs and the data tables that these two chemicals seriously affected the fish.

Some limitations to this study was that the fish weren't put in natural conditions. As always, we can always increase the size of our study to get more reliable and precise results. Finally, if there could have been more precise ways to measure heart rate and blood circulation I feel this would have been significant data to collect.

The whole reason we conducted this research was to come to a conclusion on these chemicals affecting child development in humans. I think this is important knowledge to consider because people should know what to put and not put in their bodies while going through pregnancy. Something new I learned in this study was mainly regarding 5 hour energy. Still to this day, little is known about the effects of the supplement, though we can come to a conclusion with this study that it is not safe for child development.

Works Cited:

Wolfenden, Elizabeth. "Can You Drink a 5 Hour Energy Drink While You Are Pregnant?" *Healthfully*, 11 Jan. 2021, <https://healthfully.com/402381-can-you-drink-a-5-hour-energy-drink-while-you-are-pregnant.html>.

"Caffeine in Pregnancy." *Home*, <https://www.marchofdimes.org/pregnancy/caffeine-in-pregnancy.aspx>.