Effects of Acetone on Zebrafish Embryos and How it Connects to Human Pregnancies

Hannah Bott

Waukesha North High School

Abstract

Zebrafish embryos are shockingly similar to humans, with 70% of the same DNA and many similar bodily functions. Zebrafish embryos are used to test on because of this, the effects on the embryos can be evaluated and compared to humans. This experiment was meant to help women who are pregnant know if acetone is safe to use while they are carrying their baby. This experiment was conducted over the course of 5 days, the length of time it normally takes a zebrafish to hatch and grow to a healthy state. Each day the embryos were photographed, counted, and observed. It was recorded how many embryos were alive, how many were hatched, and how many were dead. The results showed some interesting data, having 95% of the embryos die after the first day. After completing the Chi Square analysis, it was proven that the data had no statistical significance. But, a conclusion can still be found based on the data, the results showed that even with some unknown variable affecting the growth and development of the embryos, the control fish still withstood the variable better than the fish that were in a concentration of acetone.

After further research or more experimentation, this could end up proving that acetone has a negative effect on a fetus' growth and development.

Introduction

This experiment was conducted to further the little amount information available to people about the effects of acetone on pregnant woman. Will it affect the development of a child in the womb? At what concentration? Many women use acetone during pregnancies in many ways, some use it during a career, but most women use it to remove nail varnish. Acetone isn't a rare substance, many people use it in their everyday life. Even so, acetone is toxic in many ways to a human. According to Bull (2010), if breathed in deeply, acetone will cause irritation in the throat and lungs, it may also cause tightening of the chest; when ingested, acetone with cause nausea, vomiting, and inflammation of the mouth; if acetone is on skin, it will cause irritation, dry skin, and dermatitis. If acetone comes in contact with eyes, it will lead to irritation and even corneal damage after prolonged contact. Even so, acetone is all around the environment. Acetone occurs naturally in the human body during the breakdown of fat. Pregnant women, diabetics, and those who drink alcohol or exercise excessively may have high levels of acetone in them that do not (usually) cause health problems. According to Medicines in Pregnancy (2016), following acetone poisoning in a pregnant patient, maternal toxicity is likely to be major determinant of risk to the fetus.

But why use zebrafish? Why would zebrafish relate to human development? Zebrafish are found to be very similar to humans, according to Burke (2016), "70% of humans genes are found in zebrafish. Furthermore, zebrafish have two eyes, a mouth, a brain, a spinal cord, intestines, a

pancreas, a liver, bile ducts, a kidney, an esophagus, a heart, ears, nose , muscle, blood, bone, cartilage, and teeth (Burke, 2016, para. 4). Humans also have all of these body parts. Not only are zebrafish very similar to humans, but they were a much easier specimen to use, rather than an animal like mice, for this experiment. The embryos are clear which gives scientists a easier way to see the development of the fish.

When it comes down to it, this experiment answered a question that could clarify the safety of acetone use when women are pregnant. Will acetone affect the hatching and development of a zebrafish embryo? When zebrafish embryos are exposed to four different dilutions of acetone (0.0%, 0.5%, 1.0%, 2.0%), then the highest dilutions (1.0% and 2.0%) will increase the mortality the majority (80-90%) of zebrafish embryos because acetone is a toxic substance and it affects adult humans when exposed in high concentrations; zebrafish embryos are smaller and more vulnerable to toxicity, therefore a smaller amount of acetone would affect the embryos.

Materials and Methods

Throughout the process of conducting this experiment, the materials needed include the following: 5 mL of Acetone, 40 mL of water, 4 100 mL beakers, a well tray with (at least) 4 holes, 5 large-bore pipettes, 4 fine-bore pipettes, a 28.5 °C Incubator, a beaker (50 mL) for dead embryos and liquid disposal, dissecting and compound microscope, and 40 zebrafish embryos.

A procedure was required to complete this experiment in order to ensure a non-biased result, four different dilutions were made to give more varied results. The dilutions were a mixture between acetone of water, the percentage of acetone varied (0.0%, 0.5%, 1.0%, 2.0%). Each dilution was made in a singular beaker, about 10 mL of each dilution was made. Each dilution

was put into a well, each well had 1 mL of dilution, 4 wells were used in total. Before the dilutions were put into the well, ten zebrafish embryos were placed in each well with a large-bore pipette. The amount of embryos in each well was checked, in order to ensure the same amount of embryos in each well. This was done using the dissecting microscope. The transfer liquid was then removed with the same pipette used to transfer the embryos. Then, the dilutions we put into each well using a different large-bore pipette for each dilution. Each day, the zebrafish were observed through a microscope, the amount of fish hatched and amount of fish died was recorded. When a zebrafish embryo is black underneath the microscope it is dead, it also looks white when looking at it without a microscope. Each day, the dead zebrafish and any scraps of an embryo from hatched fish was removed using a fine bore pipette, and the dilution replaced with fresh dilution. Every night the well tray was placed in a 28.5 °C incubator for 24 hours. The experiment was conducted for a total of 5 days, with pictures taken of the image underneath the dissecting microscope and all observations (any irregular growth, such as a bent spinal cord, if there is a visible heartbeat, speed of growth, etc.) recorded. Throughout the experiment many safety precautions were taken to ensure the safety of everyone near, gloves were worn at all times that the dilutions were being worked with and, when working with substances such as acetone, protective eyewear was worn when creating the dilutions. The dilutions were not strong enough to harm a human if gotten on bare skin, but when making the dilutions 100% acetone was used. A Chi Square Analysis of the data was completed to ensure statistical significance.

Results

It was hypothesized that zebrafish embryos would show increased mortality as the concentrations of acetone increased, towards high concentrations of acetone. While the experiment was being conducted, many observations and numerical data (any noticeable heartbeat, how many fish hatched, how many fish alive/dead, ect.) was gathered to help support the claim of the hypothesis.

The experiment was conducted in order to discover how many zebrafish embryos survived (mortality rate, independent variable) three different dilutions of acetone (independent variable). All information gathered from the different dilutions of acetone was compared to a 0.0% acetone solution, which acted as the control. Many different variables could have affected the growth and development of the zebrafish embryo, these variables were controlled in order to ensure that mortality was due to the acetone. Some of these variables include: the wells in which the zebrafish were raised, the incubator used (the temperature the zebrafish were kept at), the solutions used, and the procedure followed when changing the solutions each day. Even so, the amount of acetone in each dilution increased, the amount of zebrafish that were deceased increased as well.

As can be seen in Figure 1, the acetone didn't show any immediate signs (either negative or positive) when exposed to acetone. Even so, results may have varied. A Chi Square analysis was completed in order to decipher whether two variables are independent or not, independent meaning that that the two factors are not related. After completing the Chi Square analysis, it may be considered irrelevant. The degree of freedom when completing the Chi Square analysis was three, seeing as there were three different dilutions of acetone being compared to the control. The critical value used to compare to was

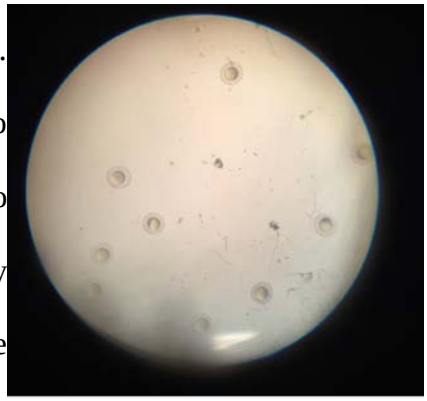

Figure 1: A image of the embryos directly after they were exposed to acetone in a small dilution (0.05% acetone).

7.82, the value that was compared to the critical value was the Chi Square value (6.33). This mean that the null hypothesis was accepted, meaning that there may have been some other variable that caused the results to vary. As you can see in Figure 2, the amount of zebrafish declined steeply in both the control and the different dilutions. It can be assumed that some unknown variable caused the rapid spike in mortality between day 1 and day 2. This can also been seen in numerical values in Table 1.

As the experiment continued, some fish were pronounced dead due to the dark, blackish color that was see within the embryo through the microscope (fig. 3). Even so, some of the control fish did hatch. There development was also slightly slower than usual (this can be see in figures 4 and 5), it took about 4 days for the zebrafish to develop into the later stages of the hatching period.

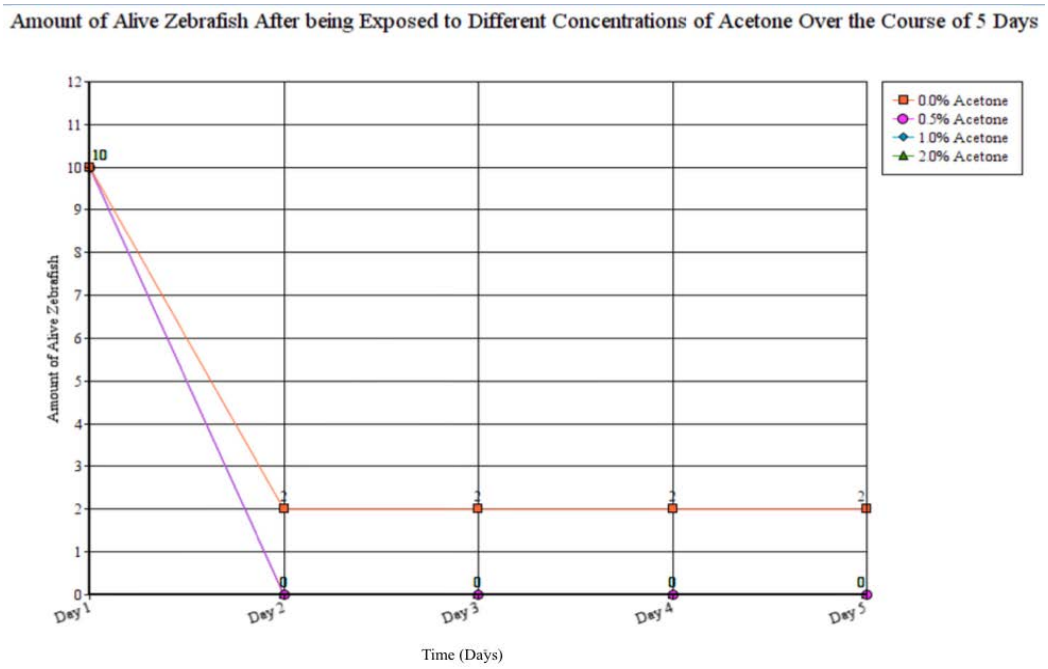

Figure 2: The amount of live zebrafish decreased the second day, but stayed the same for the rest of the experiment.

Amount of Live and Dead Zebrafish Embryos After Distinct Amounts of Time

		After 24 Hours		After 48 Hours		After 72 Hours		After 96 Hours	
Percentage of Acetone	Original # of Fish	Live	Dead	Live	Dead	Live	Dead	Live	Dead
0.0%	10	2	8	2	8	2	8	2	8
0.5%	10	0	10	0	10	0	10	0	10
1.0%	10	0	10	0	10	0	10	0	10
2.0%	10	0	10	0	10	0	10	0	10

Table 1: Numerical data over the course of 5 days, can be seen visually in the graph (fig. 1)

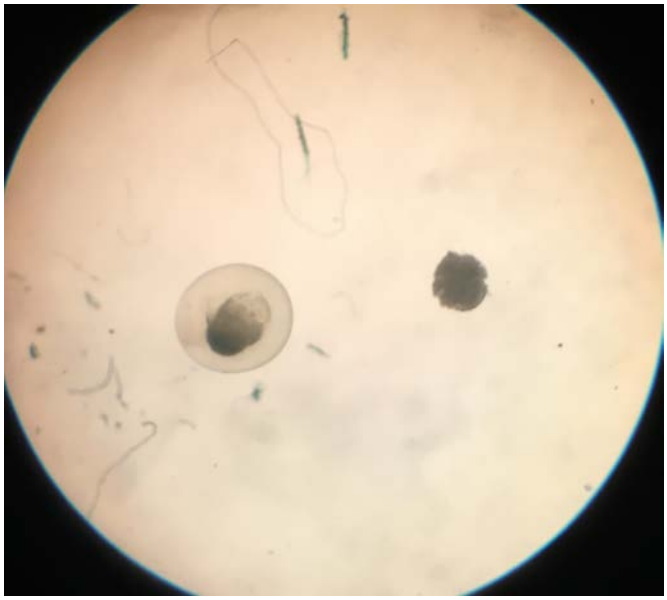

Figure 3: A dead embryo (2.0% Acetone) on day 3, note the dark color appearing in the center of the embryo

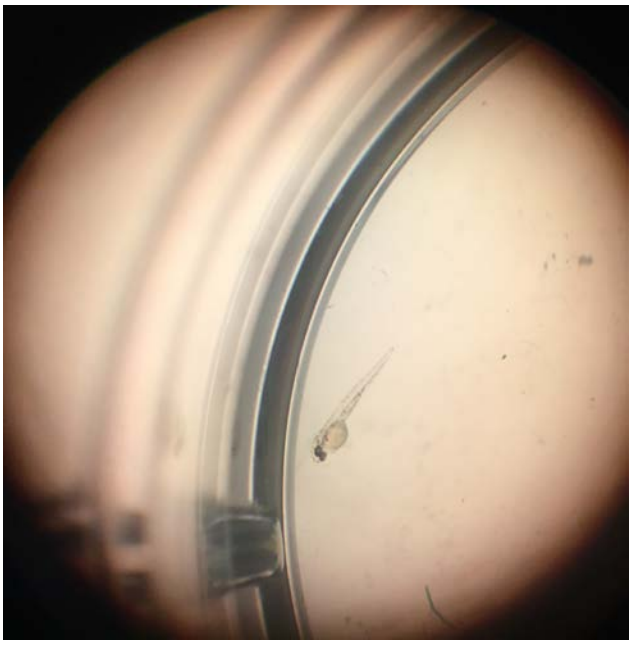

Figure 4: One of the two surviving fish on day 2, this is a control fish (0.0% acetone)

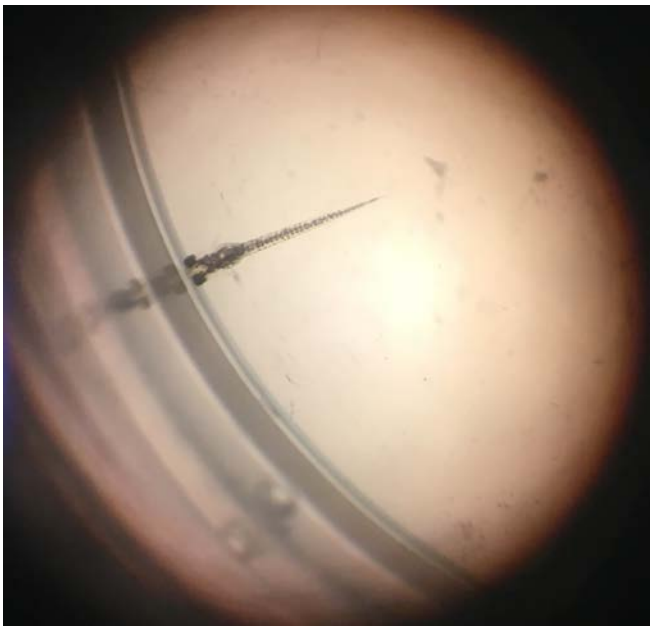

Figure 5: Last photo taken of this experiment, this is one of the two surviving control fish on day 5, note how this fish is developed enough that it is upright.

Discussion

In the end, acetone is toxic to zebrafish and effects their development and hatching. When this experiment began, a question was asked; Will acetone affect the hatching and development of a zebrafish embryo? This question was asked in order to help clarify the safety of using acetone while pregnant. Seeing as acetone is a toxic substance and it is toxic in large concentrations to adult humans, but hardly any research has been published for the public on how acetone would affect an unborn child. Zebrafish are very similar to humans in many different ways, such as the way their bodies function and their DNA (see introduction for more detail).

The results came as a shock, originally it was expected that the majority of fish in the control group and the lower concentration of acetone would survive. Yet, 95% of all 40 fish were deceased. After completing the Chi Square analysis, some pieces began to fit together. The null hypothesis was accepted, meaning that an unknown variable affected the results in some way. For example, the growth of the two hatched control fish was just above the normal growth rate of zebrafish. The hatching period usually takes between 48 and 72 hours (Hill, 2018, Zebrafish Stages), it isn't that much of a difference. But, this may connect back to the Chi Square analysis results, the the unknown variable that caused a larger amount of zebrafish to die in this experiment. Even so, the assumption cannot be made as to why that happened because of the Chi Square analysis results. In the end, the information gathered can still be taken and used in order to make better assumptions and hypothesize with more information in the future. Even though some variable affected the results, the results still showed that the control fish withstood the variable better than the fish in the acetone dilutions. This could lead to the belief that acetone does have some sort of affect on the life expectancy of zebrafish embryos.

Clearly, there were flaws in this experiment. This experiment was affected by something outside of control, but that doesn't mean that cannot be prevented in the future. More care should be taken of the embryos, ensuring that they get new solution and that all broken or dead embryos are removed every day of the experiment. Not only could this be avoided, but many more steps could be taken in order to prove more significant results. For example, using a larger sample size or conducting multiple experiments would improve not only the amount of information gathered from this experiment, but would also improve the results in general. Not only this, but there were most definitely some errors made whilst running the experiment. The embryos were not watched and cared for every day, there was a day when the experimenter was sick. This may have affected the results.

Even so, the trends in the data can be evaluated and the hypothesis can be accepted or rejected. The data had a very clear trend on day 2, with almost all 40 embryos dying. All embryos that we any concentration of acetone died, and 80% of the control fish died as well. Throughout the rest of the experiment the numbers stayed steady, with any control fish that were alive staying alive. From this data, the hypothesis can be accepted for the most part. It was hypothesized that the higher concentrations of acetone would cause a higher mortality rate than the control or the lower concentration of acetone. This did happen in the experiment, but the lowest concentration of acetone also killed the embryos. Even so, due to the Chi Square analysis results, these conclusions cannot be considered significant.

This experiment lead to many further research questions. Considering that it wasn't statistically significant, the experiment could have modifications made in order to answer the same question. But, it could also lead to new research questions. For example, it could lead to researchers asking the question: does acetone affect other types of aquatic wildlife? This would

be straying from the original purpose of the experiment, but it could help to raise awareness of waste and how we are treating the environment around us.

When it comes down to it, the assumption can be made that acetone would negatively affect the development and growth of zebrafish embryos. The zebrafish embryos that were not in an acetone concentration clearly withstood the unknown variable better than the embryos that were in any concentration of acetone. Therefore, we can make the assumption that, if there had been no unknown variable, the embryos in acetone would have had a higher mortality rate or a high rate of birth defects than those in the control solution. Zebrafish have very similar bodily functions and DNA to humans, therefore a zebrafish embryo can be compared to an human fetus. So, when looking at the effects of acetone on zebrafish embryos, it can be assumed that acetone would have similar effects on a human fetus.

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