THE EFFECT OF ETHANOL ON ZEBRAFISH EMBRYOS

The Effect of Ethanol on Embryonic Development in Zebrafish

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Abstract

The focus of this experiment was to investigate the effects of ethanol on developing zebrafish embryos and predict the effects of ethanol on developing human embryos. Ethanol was added in concentrations of 30 mM, 100 mM, and 300 mM to developing zebrafish in a multiwell plate and were observed over 96 hours. Observations showed that ethanol slowed down the hatching rate of zebrafish embryos by approximately 33%, but did not affect the mortality rate. It was also observed that ethanol decreased the heart rate of zebrafish embryos by 12.5%. Some zebrafish suffered from deformities such as lordosis, an enlarged pericardial sac, a decreased heart rate, and abnormally large eyes. However, this experiment's sample size was too small to draw notable conclusions. These results, along with results from future experiments, can be used to learn about the effect of toxicants on embryo development, specifically the side effects of ethanol on human embryos.

The Effect of Ethanol on Embryonic Development in Zebrafish

Ethanol (C_2H_6O) is known to cause harmful complications, deformations, and disorders in both embryos and offspring. This experiment used zebrafish to test the effects of ethanol in a developing embryo. Zebrafish (*Danio rerio)* were chosen for this experiment due to their similarities to humans in neurological matters (University of Sheffield, 2014). Also, exposing human embryos to ethanol for scientific research would be highly unethical. Zebrafish have a life cycle of about three to four months, from embryo to adults with reproductive capabilities (University of Sheffield, 2014). Zebrafish also have a fast spawning rate, transparent embryos, quick development time, hardiness, short life cycle, and externally fertilized eggs (Browder and Iten, 1998). Since zebrafish have a transparent embryonic membrane, it makes it easy to observe the growth progress of the embryos and the internal state. Also, since zebrafish are hardy, they can survive severe environmental changes and are durable. In addition, the embryos hatch 4-5 days after fertilization and only take about 24 hours to take the shape of their final form (University of Sheffield, 2014). Finally, female zebrafish lay their eggs, which occurs often as they lay hundreds of eggs per week, when a male zebrafish is present (University of Sheffield, 2014). The male zebrafish then fertilizes the eggs allowing test zebrafish to be obtained shortly after fertilization.

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Many studies have been conducted that have stated the negative effects of drinking alcohol while pregnant. Alcohol consumption during pregnancy can cause birth defects, poor vision and hearing, premature birth, unhealthy birth weight, physical and mental disorders and speech difficulty (March of Dimes Foundation, 2014). Zebrafish embryos were used to simulate, test, and observe how ethanol affects embryos similar to that of a human. Ethanol mainly affects the neurological sector of the zebrafish (March of Dimes Foundation, 2014). Zebrafish that have been exposed to ethanol have shown decreased motor response when touched compared to the control group of zebrafish (March of Dimes Foundation, 2014), showing that ethanol decreases nerve cell population and function. Muscle fibers inside the zebrafish were also smaller than those of the control group, causing a decrease in motor function (University of Sheffield, 2014). Also, deformities in the spinal cord caused lordosis, or a curving of the spine in the fish, reducing their swimming abilities (University of Sheffield, 2014). Exposing zebrafish embryos to ethanol caused them to suffer from defects in the circulatory system, body structure, and the nervous system. These defects could include learning and memory problems, the death of cells in the nervous system, abnormal tissue formation, and altered reactions to outside stimuli. The abnormalities could be seen at varying levels based on the dosage and concentration of ethanol that the embryos were exposed to (Carvan, et. al, 2004). Recordings of eye development from an electroretinogram (ERG) showed that when exposed to ethanol, the growth in the eyes was severely hindered as compared to a normal zebrafish embryo (Bilotta, et. al, 2002.). The ocular irregularities are not limited to zebrafish as humans exposed to alcohol may suffer from similar problems. In humans, the exposure of fetuses to alcohol could cause deformities classified as fetal alcohol syndrome (FAS). FAS can result in stunted growth after birth, deficiencies in the central nervous system, and malformations in the face. The eyes are the most common indicator of the malformations and are particularly affected by the alcohol. External signs include drooping lower eyelids, increased distance between the eyes, and the inability to focus without squinting. Internal signs include impaired vision and underdevelopment of the optic nerves (Strömland & Pinazo-Durán, 2002). The evidence in previous studies shows that ethanol had adverse effects on zebrafish and humans after they were born.

The purpose of this experiment is to explore the effects of ethanol on zebrafish embryos, allowing us to see how these chemicals affect a developing human embryo. This experiment was conducted to find out if ethanol will affect a developing embryo and if so how the embryo is

affected. The null hypothesis states that if zebrafish embryos are exposed to different concentrations of ethanol solutions, then the zebrafish will develop normally. My alternate hypothesis stated if zebrafish embryos are exposed to different concentrations of ethanol solutions, then most of the embryos will suffer from cardiovascular complications because ethanol in developing embryos causes them to develop irreversible circulatory complications. The final purpose of this experiment is to understand the connections between humans and their offspring and the consequences of mothers drinking alcohol while pregnant.

Methodology

Materials

- 96 live zebrafish embryos
- 30 disposable wide-bore pipettes
- 30 disposable fine-tip pipettes
- 1 dissecting microscope
- 2 100 mL beakers
- 82°F incubator
- 3x4 well plate
- Instant ocean embryo media solution
- Ethanol solutions at concentrations of 30 mM, 100 mM, and 300 mM

Procedure

After our zebrafish were fertilized, we transferred 1 mL of each solution into the corresponding well using a large-bore pipette as follows:

300 mM Ethanol	100 mM Ethanol	30 mM Ethanol	Control (Water)
300 mM Ethanol	100 mM Ethanol	30 mM Ethanol	Control (Water)
300 mM Ethanol	100 mM Ethanol	30 mM Ethanol	Control (Water)

After we transferred the solutions, we then used a new large-bore pipette to put 8 clear and round embryos (living) into each well. Next, we placed the cover over our well plate and put it in the incubator that was being maintained at 82°F.

After 24 hours, we removed our well plate from the incubator and observed the number of living, dead, and hatched embryos using a dissecting microscope. Next, we made qualitative

observations and took photographs. We then used fine-bore pipettes to remove deceased embryos and placed them into the 100 mL beaker using a new pipette for every solution. After that, we drained each well and replaced it with 1 mL of fresh solution using a fine-bore pipette, once again using a new pipette for every solution. Then, we recorded the heart rate of the zebrafish by counting the heartbeats in 10 seconds and multiplying it by 6. Finally, we took more qualitative data and photographs/videos.

After 48 hours, we repeated the same procedure done at 24 hours and recorded more quantitative and qualitative data.

After 72 hours, we repeated the same procedure done at 24 hours and recorded more quantitative and qualitative data.

After 96 hours, final observations and conclusions were made.

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Table 1

The number of embryos that survived and hatched in each solution over time.

Well and Treatment	24 hours post fertilization		48 hours post fertilization		72 hours post fertilization		96 hours post fertilization	
Key	# fish alive	# hatched	# fish alive	# hatched	# fish alive	# hatched	# fish alive	# hatched
A1 - 300 mM Ethanol	6	0	6	0	5	5	3	3
A2 - 100 mM Ethanol	7	0	6	4	5	4	5	5
A3 - 30 mM Ethanol	7	0	7	2	7	6	7	7
A4 - Control	7	0	7	4	7	6	6	6
B1 - 300 mM Ethanol	7	0	7	5	3	3	3	3
B2 - 100 mM Ethanol	7	0	5	2	5	5	5	5
B3 - 30 mM Ethanol	8	0	8	0	7	7	1	1
B4 - Control	7	0	7	2	5	5	3	3
C1 - 300 mM Ethanol	6	1	6	3	4	4	4	4
C2 - 100 mM Ethanol	8	0	8	1	4	4	4	4
C3 - 30 mM Ethanol	8	0	8	2	5	5	2	2
C4 - Control	8	0	8	2	7	7	7	7

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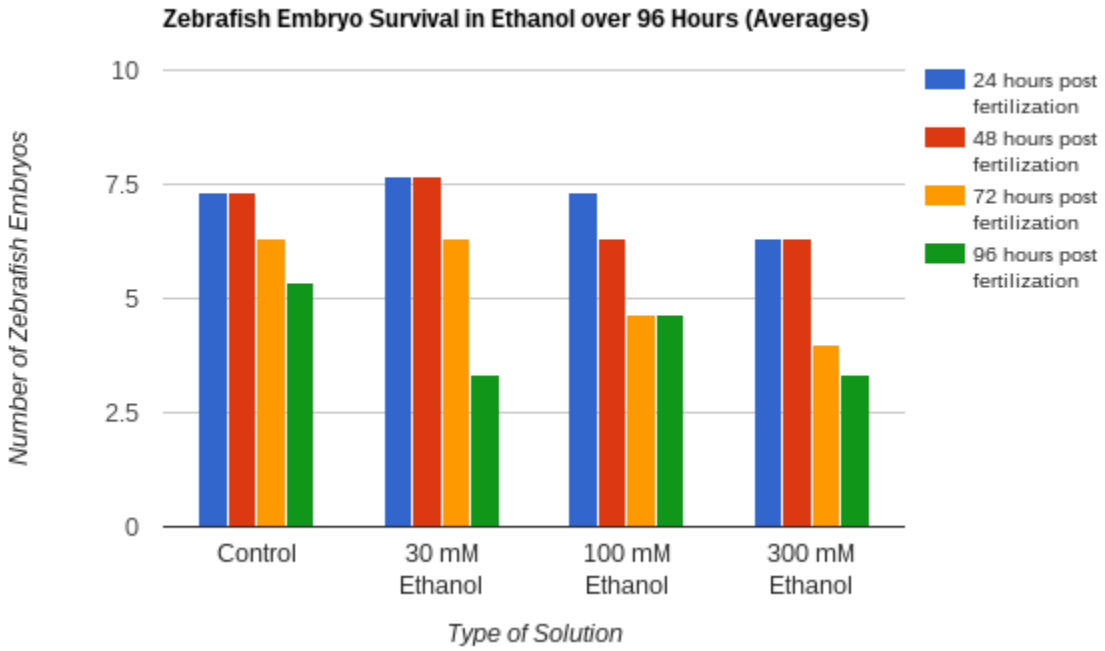
Graph 1

The mean number of surviving zebrafish over time after being exposed to ethanol.



Graph 2

The mean number of hatched embryos that were exposed to ethanol.

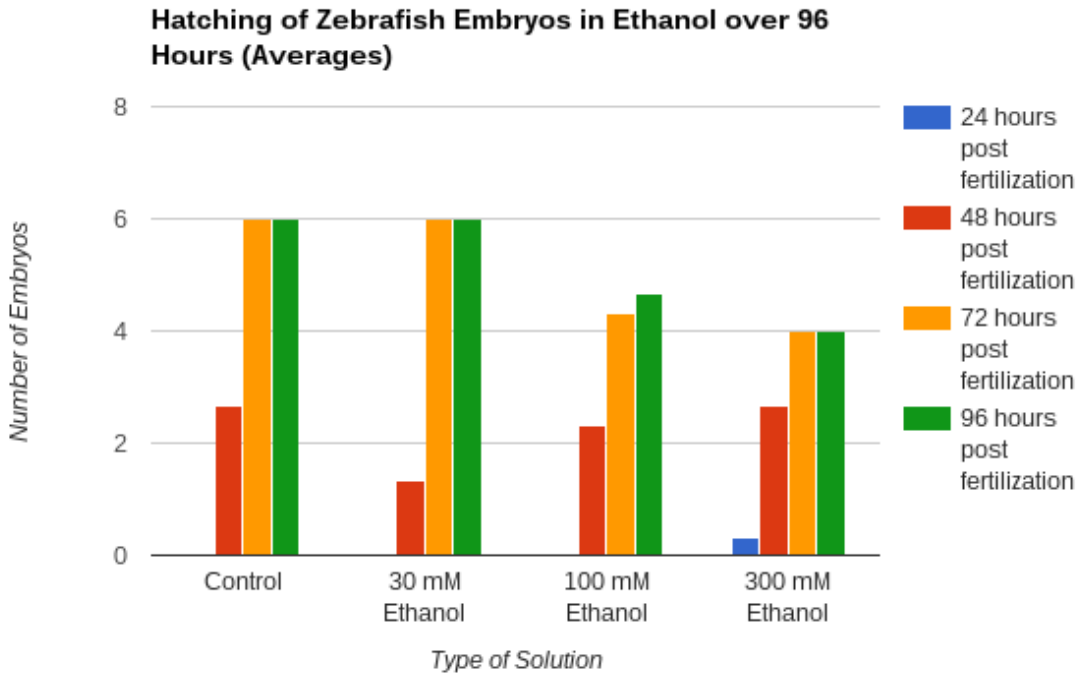


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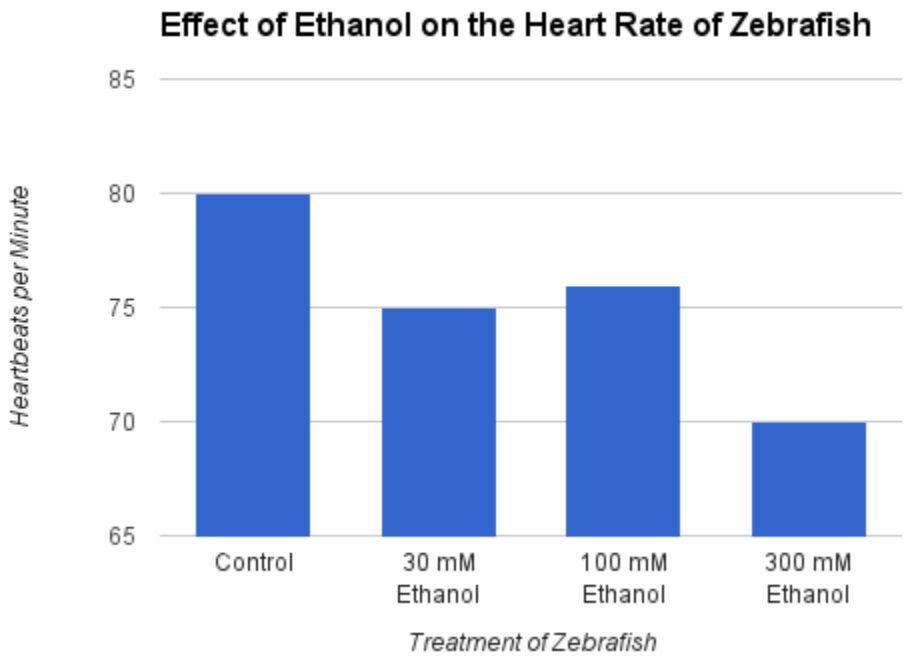
Table 2

Effect of Ethanol on the Heart Rate of Zebrafish

Treatment	Heartbeats per Minute
Control	80
30 mM Ethanol	75
100 mM Ethanol	76
300 mM Ethanol	70

\Graph 3

Effect of Ethanol on the Heart Rate of Zebrafish



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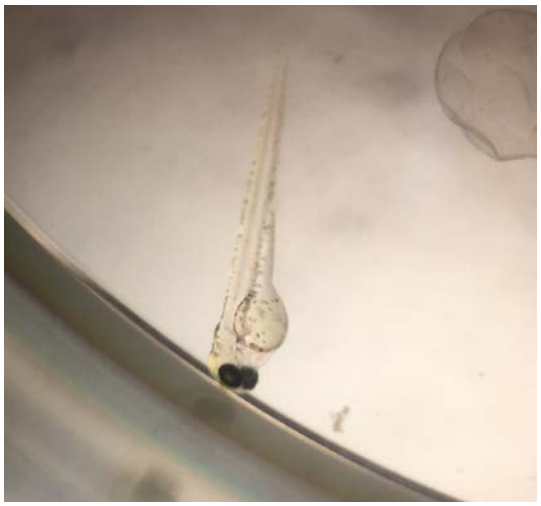

Figure 1. A healthy hatched zebrafish in the control at 48 hours.

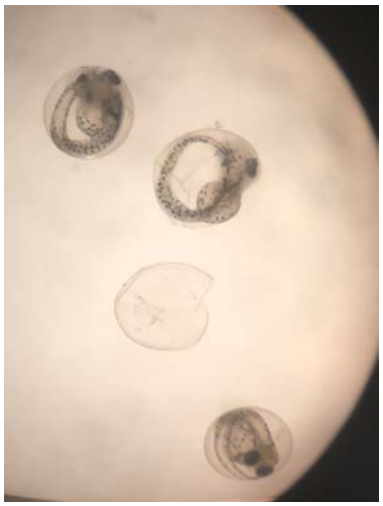

Figure 2. Developing embryos in 300 mM Ethanol at 48 hours. Many of them display curved spines, or lordosis.

Figure 3. Unhatched zebrafish embryos in control at 24 hours. They are round and have clear centers, showing they are in good health.

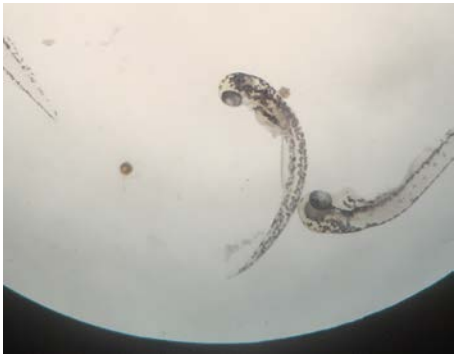

Figure 4. A hatched embryo in 300 mM Ethanol at 72 hours. It displays lordosis.

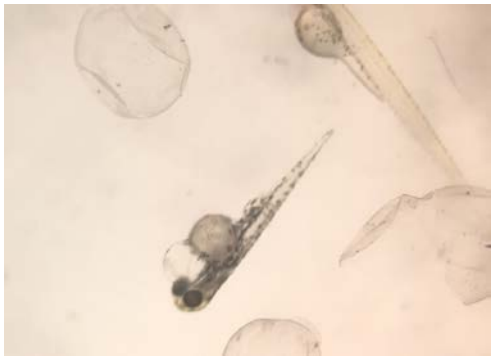

Figure 5. A hatched embryo in 100 mM Ethanol at 72 hours. It has an abnormally large pericardial sac.

Figure 6. A hatched embryo in 30 mM Ethanol at 72 hours. It has developed abnormally large

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Results

After analyzing our data, a few trends were evident. Until 96 hours after fertilization, the zebrafish exposed to ethanol did not show a clear increase in mortality rate compared to the control fish. This is depicted in *Graph 1*. Both 30 mM Ethanol and the Control had similar hatching rates, whereas 100 mM Ethanol and 300 mM Ethanol had decreased hatching rates. This shows that embryos with exposure to ethanol have lower hatching rates than the controls, as seen in *Graph 2.* Ethanol also had an effect on the heart rate of zebrafish embryos. As ethanol concentration went up, the heart rate of the zebrafish decreased. This is seen in *Graph 3*. Some zebrafish also display lordosis, an enlarged pericardial sac, and abnormally large eyes, as seen in *Figures 2, 4, 5, and 6.*

Discussion

After reviewing my results, I conclude that ethanol had a negative effect on the health of the developing zebrafish embryos. My results suggest that zebrafish exposed to the higher concentrations of ethanol had a lower hatching rate and survival rate compared to the control. Also, exposing the embryos to ethanol reduced their heart rate as seen in *Graph 3*. This is consistent with the well-known hazardous effects of ethanol on developing embryos. Though most zebrafish in the ethanol solutions survived and hatched, they developed perilous mutations such as lordosis, or curving of the spine, abnormally large and disproportionate eyes, enlargement of the pericardial sac, and many other unsafe variations, as seen in *Figures 2, 4, 5, and 6.* These are examples of Fetal Alcohol Syndrome (FAS), a condition in which infants are born with physical and mental defects as a result of their mother drinking alcohol while pregnant. In my hypothesis, I stated if zebrafish embryos are exposed to different concentrations of ethanol solutions, then most of the embryos will suffer from cardiovascular complications because ethanol in developing embryos causes them to develop irreversible circulatory complications. I accept my hypothesis because exposing the embryos to ethanol resulted in various cardiovascular complications such as an enlargement in the pericardial sac, a decrease in heart rate, and other issues that may have led to the embryos' death.

The accuracy of our results may have been affected by numerous possible errors occurring during both preparation and experimentation. The presence of a toxic contaminant and other anomalies related to the zebrafish may have caused the effects to be hindered. Also, the ethanol used was a solution and may have inaccurate concentrations of ethanol. More human

errors include miscounting of the zebrafish, extracting living fish deemed to be dead, and damaging embryos while transporting them to their wells using the large-bore pipette. These errors could have caused results of this experiment to be unreproducible and therefore must be repeated for validity of the data. While repeating the experiment, emphasis should be put on being careful with the embryos and keeping all solutions very consistent.

The data gathered in this experiment can lead to various other experiments, such as:

- What other substances cause mutations in embryos? What concentration of these substances cause the most deformities and mutations?
- What other compounds, such as materials used in food packaging, cause mutations in embryos?
- What other species have similar effects due to exposure to ethanol as an embryo?

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