

# The Effect of Ethanol on Zebrafish Embryos

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## Abstract:

The purpose of this experiment is to demonstrate the harsh effects of ethanol exposure on zebrafish. Then, also understand the effects of the exposure of alcohol on humans. After submerging the zebrafish embryos in different ethanol concentrations, we found that the ones with a higher concentration resulted in a higher number of deformities, discoloration and a faster development. Therefore, we have a greater understanding of the effects of ethanol on humans and their development along with Fetal Alcohol Syndrome.

## Introduction:

Exposure to ethanol during development leads to birth defects, learning and memory deficits, visual problems, skeletal deformities, and cell death in the CNS. The US National Library of Medicine conducted a study on the effects of ethanol on photoreceptors and visual functioning for zebrafish. They found that, "Embryos treated with 1.75% and 2% ethanol had numerous morphological problems, including a dorsally curved body, swollen heart with blood sometimes pooling in the chambers, rounded forebrain, irregular jaw, and smaller eyes". In humans, we can see some of the same effects as the zebrafish. Drinking alcohol while pregnant results in children being born with physical, behavioral and learning problems. This condition is called fetal alcohol syndrome (FAS). According to the American Academy of Pediatrics, "FAS is arguably the most common known nongenetic cause of mental retardation in the United States". The zebrafish show FAS-like effects and have become a great model for testing certain developmental toxicants, such as ethanol. We are investigating the effects that ethanol has on the development of zebrafish. **We hypothesize that, if we expose the zebrafish to different volumes of ethanol, then the one with the highest volume of ethanol will show the most deformities in their embryonic development.**

Solutions:	Control 100% embryo media	Treatment #1 30% ethanol	Treatment #2 15% ethanol	Treatment #3 5% ethanol
Day 1:	-yolk is yellow	-yolk is less yellow	-yolk is less yellow	-yolk is less yellow
Day 2:	-1 dead -yellow	-1 dead -big/dark head	-1 dead -dark head	-1 dead -less yellow
Day 3:	-alot more yellow -tail is way longer	-bigger head -tail is deformed -yolks are odd shaped	-tail is bunched up and deformed -very dark with big eyes -yolk shape is weird	-looks normal -similar to control -yolk shape is deformed
Day 4:	-1 hatched -looks healthy	-6 hatched -very clear -bumps are very big compared to control -body is smaller than control	-1 dead -tail is curved	-look most normal -yolk is less yellow -deformed yolk

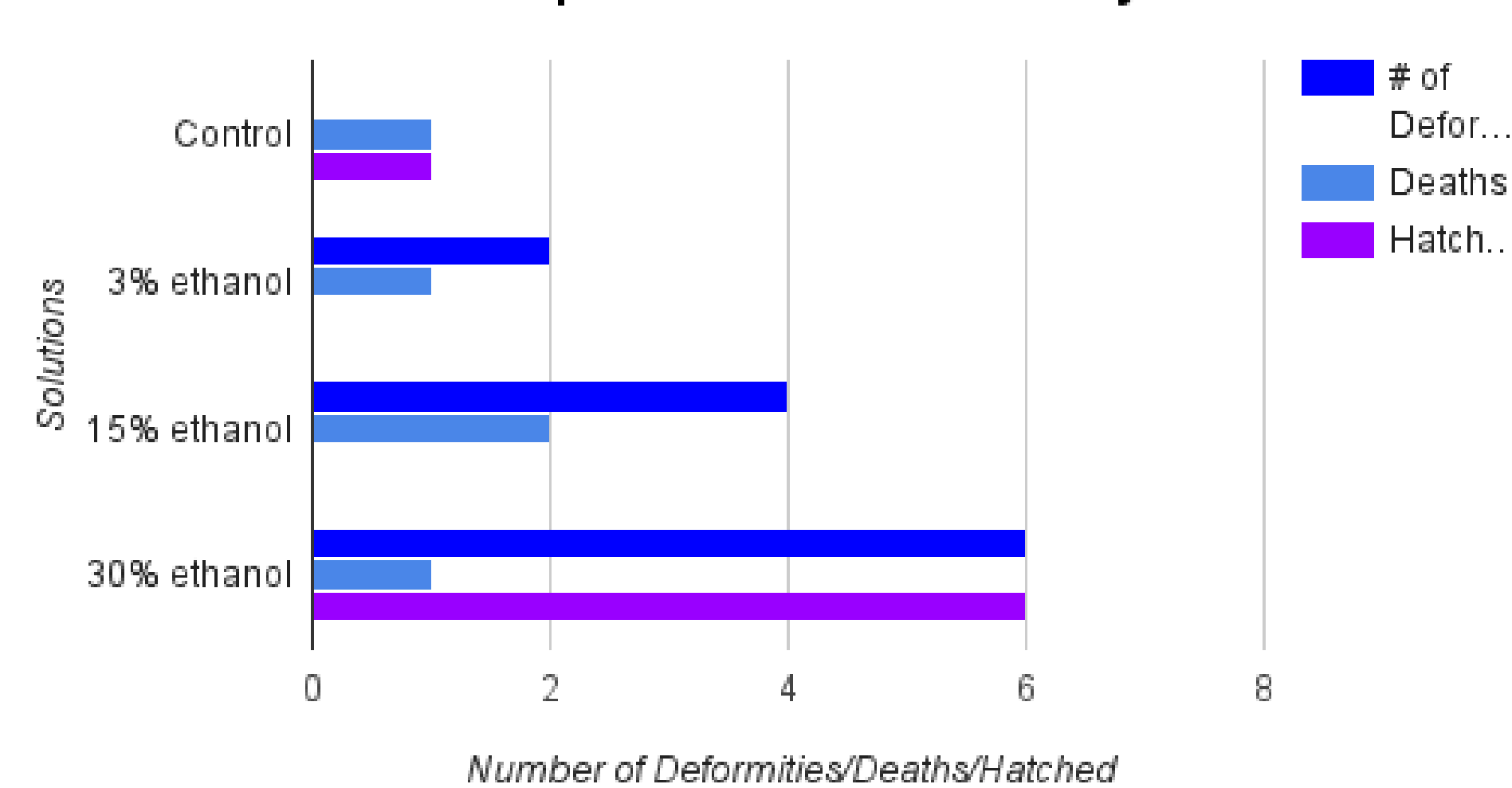
## Materials:

- Embryo medium
- Ethanol
  - Treatment #1: used 3 mL of a 15 mL Ethanol with 35 mL Embryo Media solution
  - Treatment #2: used 3 mL of a 5 mL Ethanol with 45 mL Embryo Media solution
  - Treatment #3: used 3 mL of a 1.5 mL Ethanol with 48.5 mL Embryo Media solution
- 4 Falcon dishes
- 1 Microscope
- Pipettes
- 28 Zebrafish embryos
- Incubator (28.5 °C)

## Methods:

- We placed seven zebrafish embryos in 4 separate falcon dishes. The control falcon dish consisted of 50 mL of the embryo media, the second falcon dish consisted of 15 mL of ethanol and 35 mL of media (30%), the third dish contained 5 mL of ethanol and 45 mL of media (15%), and the last dish contained 1.5 mL of ethanol and 48.5 mL of media (5%).
- In each falcon dish we placed 3 mL of the solution into the dish.
- We observed the zebrafish embryos Monday through Thursday and recorded any observations (deformities, deaths, and hatchings) in a data table. We took pictures of the embryos to keep track of their progress.
- At the end of everyday, we used a pipette to remove the old solution and replace it with 3 mL of fresh solution.
- After recording, we placed the dishes in an incubator set at 28.5°C and let them sit overnight.
- We repeated this process for four days.

Ethanol Impact on Zebra Fish Embryos



Day 2



Day 3



Day 4



## Results:

Independent variable: Ethanol concentration  
Dependent variable: Differences observable characteristics in embryo development

Control: used 3 mL Embryo Media

1.5 mL Ethanol with 48.5 mL Embryo Media solution

We found that although treatment #1 had the most deformities (large head, misshaped tails, large bumps, smaller body) it also had the highest number of zebrafish hatched (6) by day 4.

Treatment #2 also had many deformities such as clear embryos, deformed/bunched up tails, dark coloring, larger eyes, and odd shaped yolk. One embryo died, and zero hatched. Treatment #3 looked most similar to the control group and zero hatched.

## Discussion:

From our results, it is clear that due to the exposure of ethanol the development of zebrafish was altered. With this we can conclude that our results supports our hypothesis and shows the possible risks of ethanol exposure to human embryos. Looking at our data it is clear that the 30% ethanol solution showed the most deformities. Most of the embryos in that solution hatched, but were much smaller, had bigger heads and eyes. These deformities were not as common in the lower level solutions and not apparent in the control. This supports our hypothesis, in which we believed that the fish in the higher ethanol solution would show the most deformities. In our prior research, we had found that birth defects were common in children whose mothers consumed high amounts of alcohol during pregnancy. The children showed physical deformities as well which led us to believe that the zebrafish would show the same. There might be some limitation or error to our experiment however. There is a chance that we did not get the prior solution completely out before adding the fresh solution. This may have caused the solution to have more or less ethanol than what we had planned. Also, sample size could have been a limitation since we only focused on a limited number of embryos. We only observed the embryos for four days, which could have also been a limitation.

## Resources:

"Ethanol Effects on the Developing Zebrafish: Neurobehavior and Skeletal Morphogenesis." *Ethanol Effects on the Developing Zebrafish: Neurobehavior and Skeletal Morphogenesis*. N.p., n.d. Web. 15 Jan. 2017.  
"Effect of Ethanol Exposure on Zebrafish Embryos." N.p., n.d. Web. 2 Feb. 2017.  
Matsui, Jonathan I., Ana L. Egana, Todd R. Sponholtz, Alan R. Adolph, and John E. Dowling. "Effects of Ethanol on Photoreceptors and Visual Function in Developing Zebrafish." *Investigative Ophthalmology & Visual Science*. U.S. National Library of Medicine, Oct. 2006. Web. 24 Jan. 2017.

Deformities Over Time

