

# The Effect of Ibuprofen (Advil) on the Survival Rate of Zebrafish

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**Abstract:** These trials were conducted to test the hatching and growth rates of zebrafish embryos. This was mastered by, exposing fertilized zebrafish embryos to varied amounts of Ibuprofen. The first well was set up to expose the embryos to one dosage of ibuprofen for the time length of the experiment. The second well was set up to expose the zebrafish embryos to, two times the dosage and the third was to expose to three times the amount of Ibuprofen. As the experiment was taking place and the embryos grew and developed in the toxicant that was chosen, the number of living was written down and same with the dead. Pictures were taken of any deformities that the group could see and were put in the data section. The total count of hatched and unhatched was taken around 144 hours after fertilization and as a group we came to the conclusion that Ibuprofen does not an effect on the hatching rate of zebrafish embryos. During our observations, we realized that there were some anatomical differences in the group that were exposed to the toxicant that was three times amount the prescribed dosage is. There were some embryos with an enlarged liver and heart. This proves that if taken over the gestation period, there could be some abnormalities with the individual being born.

## **Introduction:**

Ibuprofen is a nonsteroidal, anti-inflammatory that many people take for numerous reasons such as, headaches, dental pain, menstrual cramps, muscle aches, and arthritis. But, has it ever been asked what it does to an embryo when it is developing during a pregnancy? If this medication is taken to many times during the duration of the day, it can cause some farther pain like, abdominal pain, belching, cloudy urine, bloating, heartburn, and so many other things. All these side effects that are listed, are on a person that is already developed and not developing, so what would the side effects be when a person is developing? When examining animals that have taken Ibuprofen during their pregnancy, it was shown that there is a risk of miscarriage, cardiac malformation and gastroschisis on the developing species. There are also some effects on the administer of Ibuprofen and that would consist of premature closure of the fetal ductus arteriosus, oligohydramnios, and there is also even a slight possibility of fetal renal impairment. (Micromedex, Multrum, Kluwer, 2017). During a Canadian study, it was shown that a women that takes Ibuprofen during pregnancy is 2.4 times more likely to have a miscarriage than a women that does not take any Ibuprofen during their pregnancy. (Ekern, 2016). Another study that was taken place, showed that there was lower levels of amniotic fluids, higher blood pressure, some birth defects, and there might even be a slightly higher risk for the mother to be at risk of a miscarriage. (Yarn,2016).

Zebrafish are the most suitable for this type of scientific research since, they mature at a very rapid speed and they develop outside of the mother. These animals are also very useful to study pathological behaviors that will be shown. (Kalueff, Etal 2013). Zebrafish embryos were also chosen because, their embryos are clear, making it easy to watch the development of the fish and they lay hundreds of eggs at a time. With that being said, this experiment is not killing off the population of zebrafish. Lastly, they are easy to work with and they are “cheap”.(YourGenome, 2017). The human body is a lot like a zebrafish in some ways. 75 percent of all human disease are also found in this certain type of fish. This makes research easier done with mimicking the diversity of cancer diseases, muscle, lung, ,and heart diseases,

and many more diseases.(Sarasamma, Etal 2017). Since we can see what street drugs do a human embryo developing, what does prescribed drugs such as, Ibuprofen do to a developing zebrafish? Lastly, it is strongly hypothesized that Ibuprofen will have a negative effect on the survival rate of zebrafish.

### **Materials And Methods:**

- 1 Sharpie
- Roll of Masking Tape
- Ibuprofen (1x dosage bottle, 2x dosage bottle, 3x dosage bottle)
- 50 zebrafish
- 4 mL pipettes
- (one for 1x dosage, one for 2x dosage, one for 3x dosage, and one for dead fish and bad ibuprofen)
- 1 bottle of Methylene Blue
- 1 Waste Beaker
- 1 3x4 Well Plate
- 1 28.5°C Incubator
- 1 Compound Microscope
- Safety glasses may be worn when dealing with Ibuprofen, when desired

### **Procedure:**

#### **Day 1**

- A. Obtain around 45 embryos from instructor
- B. Label acquired well plate with name, hour, and toxicant on the masking tape provided. Make sure the label is on top of the lid to the well plate. Then label the dosage or time on top of the well also with the sharpie that is provided.
- C. Divide the zebrafish into groups of exactly 5 in each well. Make sure to absorb the extra water that is placed into the well. **NOTE:** Tip the plate so the embryos go to the bottom and it will become easier to get the excess water out.
- D. Place embryos under microscope and observe. Be sure to write down the number of living hatched and the number of living unhatched.
- E. Add about 200mg of Ibuprofen into all the wells.
- F. After the toxicant is placed into all of the wells, place about 3-4 drops of Methylene Blue in each well. Use a new pipette for this solution, so that all of the other pipettes are not contaminated. This solution will ensure that very minimal to absolutely no bacteria is growing in the wells with the zebrafish.
- G. Lastly, cover the well plate with the lid that has the name, hour, toxicant, and dosage that is being used and put it in the incubator, where the instructor demonstrates, overnight.
- H. Make sure to record the exact number of hatched and unhatched alive embryos.

**Day 2**

- A. Remove the well plate from the incubator and remove the cover.
- B. Obtain a microscope and examine the embryos under the microscope that is provided and remove any dead embryos into the waste beaker.
- C. Count the remaining hatched embryos and unhatched embryos and make sure to record.
- D. Remove the all the waste toxicant and add new into all the wells. **NOTE:** Make sure to not suck up any living embryos or zebrafish that are exposed out of their embryos.
- E. Add about 3 - 4 drops of Methylene Blue to all the wells again and make final observations for the day.
- F. Write down any abnormalities or developmental markers and make sure to take pictures.
- G. Lastly, cover the well plate with the lid that has the name, hour, toxicant, and dosage that is being used and put it in the incubator, where the instructor demonstrates, overnight.

**Day 3**

- A. Remove well plate from the incubator
- B. Obtain a microscope and examine the embryos under the microscope that is provided and write down the remaining hatched embryos and unhatched embryos.
- C. When finished with observations, the remaining zebrafish can be dumped into the waste beaker, where the instructor demonstrates.
- D. Make sure to rinse any equipment that was used .

**Procedure is from SEPA UW-Milwaukee**

**Data:****Data Table I :**

| P- Values And Significant Unpaired T-Test Comparison |   |  |  |
|--|---|--|--|
| Exposure   | Control vs. 1x Dosage                       | Control vs. 2x Dosage                            | Control vs. 3x Dosage                            |
| One Times Dosage                                     | 0.0015<br>Considered to be Very Significant | X  | X  |
| Two Times Dosage                                     | X   | 0.0001<br>Considered to be extremely significant | X  |
| Three Times Dosage                                   | X   | X  | 0.0001<br>Considered to be extremely significant |

Data table one shows the unpaired T- tests and P- values of 200mg of Ibuprofen compared to Instant Ocean Water, as well as the significance of every value in the table. A T- test was used to compare the results of the toxicant on zebrafish embryos and see if it was significant or due to

random chance. P - Values of .05 or less were thought to be significant, with that being said it is shown that Ibuprofen has a significant effect on the developing embryo.

**Data:**

**Data Table II**

| Number of Living Zebrafish Embryos |                    |                            |                           |                          |
|------------------------------------|--------------------|----------------------------|---------------------------|--------------------------|
| Quantity of Dosage                 | # of Starting Fish | # alive 24 hpf (Wednesday) | # alive 48 hpf (Thursday) | # alive 162 hpf (Monday) |
| One times dosage                   | 15                 | 13                         | 11                        | 5                        |
| Two times dosage                   | 15                 | 5                          | 4                         | 0                        |
| Three times dosage                 | 15                 | 0                          | 0                         | 0                        |

Data table two shows the number of living through the duration of this lab.

**Figure I**

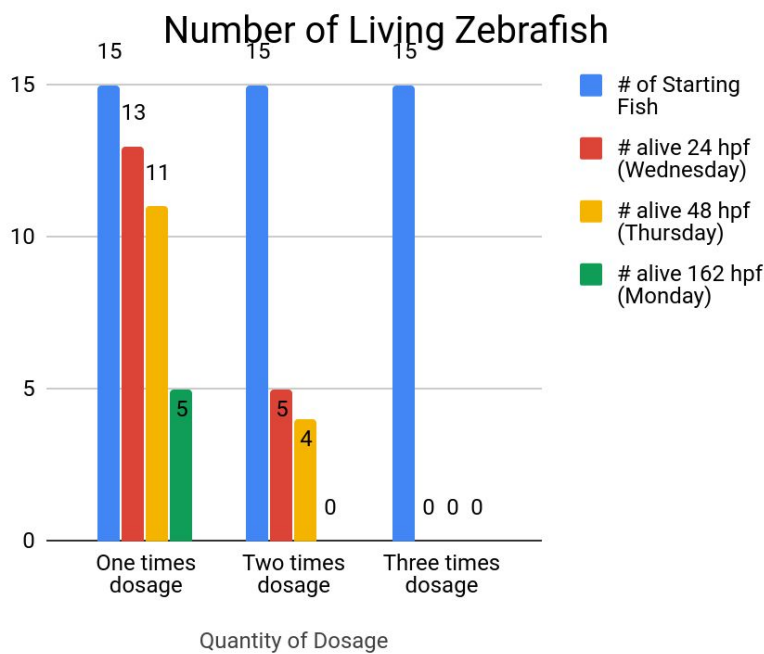


Figure one shows the total number of living embryos during the duration of this lab.

**Data Table III**

| Number of Dead Zebrafish Embryos |                    |                           |                          |                         |
|----------------------------------|--------------------|---------------------------|--------------------------|-------------------------|
| Quantity of Dosage               | # of Starting Fish | # dead 24 hpf (Wednesday) | # dead 48 hpf (Thursday) | # dead 162 hpf (Monday) |
| One times dosage                 | 15                 | 2                         | 4                        | 10                      |
| Two times dosage                 | 15                 | 10                        | 11                       | 15                      |
| Three times dosage               | 15                 | 15                        | 15                       | 15                      |

Data table three shows the number of deceased during the duration of the lab.

**Figure II**

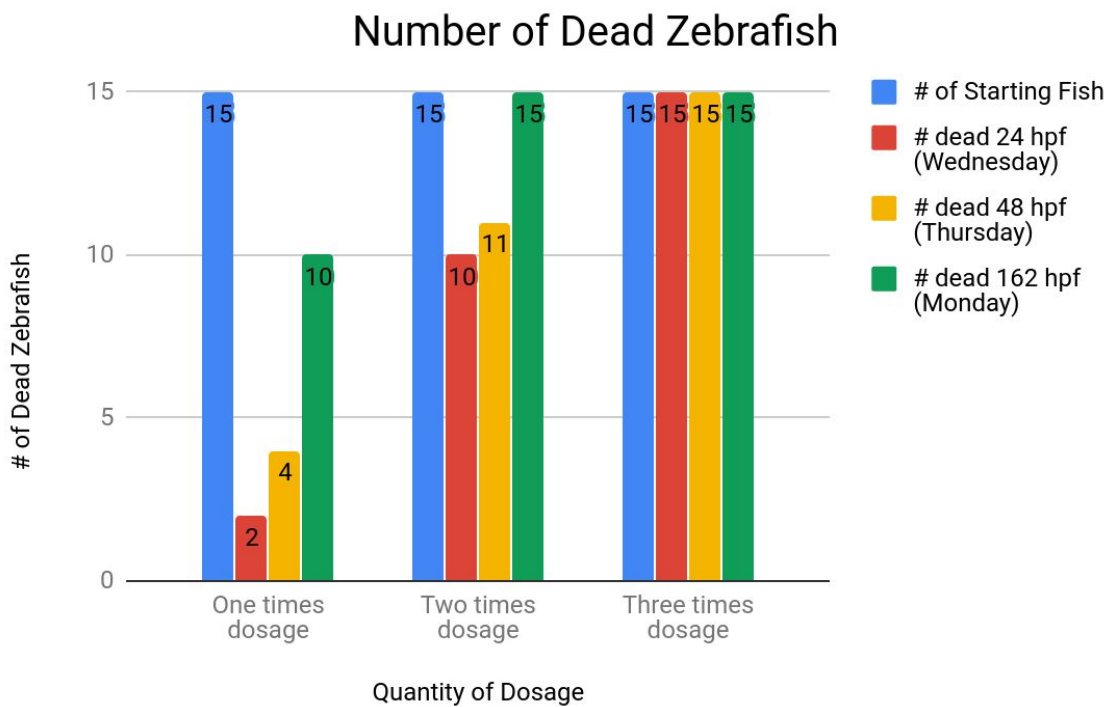
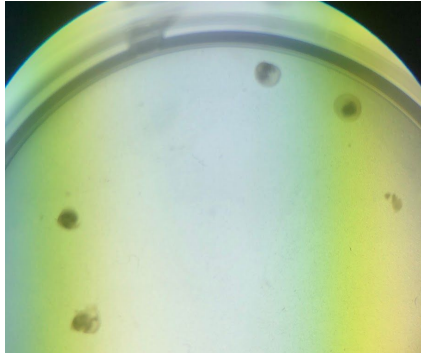


Figure two shows the complete number of deceased during the duration of the lab.

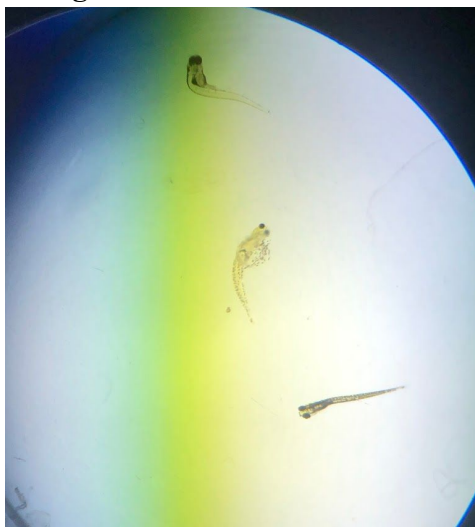
**Figure III**

Figure three shows all the embryos that were exposed to three times the amount of dosage were deceased after 24 hours post fertilization .



**Figure IV**

Figure four shows that the embryos that were exposed to the two times amount dosage had enlarged hearts as well as liver.



## **Results:**

This experiment was conducted to observe the effects of Ibuprofen on the survival rate of zebrafish embryos and see what abnormalities would occur due to the exposure to the chosen toxicant. This was achieved by making sure to observe the number of hatched eggs as well as unhatched living eggs. In addition, pictures were taken of any abnormalities during the development of the embryos and observations were recorded.

The control was tested in Instant Ocean Water and then compared to the toxicant, Ibuprofen and then set in an incubator set at 28.5°C. The groups that were tested against the control were exposed to one times the amount of Ibuprofen that should be taken, two times, and lastly, three times. The survival rate was the dependent variable, which relied on the independent variable. In this experiment, the independent variable did have a significant effect on the dependent variable. Therefore, the amount of Ibuprofen taken during the duration of a developing embryo is a significant factor.

There was an average survival rate of 11% in the toxicant group, while in the control group there was an average survival rate of 78%. There were a couple abnormalities such as, there were quite the amount of enlarged heart and livers, and it looked as if the zebrafish once outside of the embryo became anorexic. Other than that there were not too many things that stuck out to be abnormal.

This experiment was completed to see if there were to be any effects of Ibuprofen on zebrafish while developing. The data collected in this experiment comes to the conclusion that when Ibuprofen is taken in the stages of developing, it may be significant to abnormalities and deaths. The toxicant had an effect on the survival rate, more so on the groups that were exposed to higher quantities, but overall there was a significant effect.

## **Discussion:**

All the data found in this experiment was found to be significant. This would make sense because, the embryos were sitting in not their normal habitat and on the t- tests it shows that this data is extremely significant.

Error and some slight limitations may have had a slight effect on the results from the experiment. While it was possible to see some physical effect of Ibuprofen on the zebrafish embryos, it is impossible to see any neuro, cardio, or even psychological effects on the zebrafish. With the equipment provided from the instructor, it was only possible to see the physical effect. As it is known, there is always going to be error that could have possibly happened. In this case it is suggested to repeat this experiment to confirm.

In this experiment it was found that Ibuprofen was considered to have a significant effect on the embryos. This could be seen as the more you increase the dosage during pregnancy, the more susceptible you are to miscarriage. During this experiment, dosage was the main component instead of time and that may have had a significant impact on how it affected the development of the zebrafish both physically and/or neurology.

This experiment was completed to see the possible effects of Ibuprofen on the embryonic development of zebrafish also, how that could relate to a human developing embryo. In this experiment it is shown that Ibuprofen does have a negative impact on zebrafish embryos. Which if related to the human body, could show that a miscarriage is possible if taken in high dosages. Therefore, ibuprofen will have a negative impact on the development on an embryo.

## Citations

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