

The Effect of Oxybenzone (Benzophenone-3) on Mortality Rate of Zebrafish
(*Danio rerio*) Embryos

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Abstract

Oxybenzone, an estrogenic compound, is commonly used by humans and is not a substance most people avoid, including pregnant women. Oxybenzone is a photo-toxicant; adverse effects are exacerbated in the light (C.A. Downs, 2015) The research performed is used to help determine the potential effects of benzophenone-3 (Oxybenzone) on *Danio rerio* (Zebrafish) by comparing the development/mortality rate of two groups of ten *Danio rerio* embryos exposed to benzophenone-3 to the development/mortality rate of a single group of ten *Danio rerio* embryos. The experiment found that the Zebrafish embryos exposed to Oxybenzone had a higher mortality rate.

Background

If there is a presence of oxybenzone then the mortality rate of zebrafish embryos will increase because estrogenic compounds can lead to death early in life. As stated before oxybenzone is a photo-toxicant as well as an estrogenic compound that is commonly found in sunscreen. It was tested because oxybenzone commonly interacts with aquatic organisms as many people swim while wearing sunscreen.

Zebrafish were used as model organisms because they have a “fully sequenced genome, are able to generate quickly, can produce large numbers of externally fertilized eggs, and they have an optical transparency of embryos and larvae”(Hayden, 2005). A fetus may come into contact with Oxybenzone if the mother ingests the chemical. The variable being tested in this experiment is Oxybenzone because it's commonly found in sunscreen and other hygiene products. 90% of the sunscreen products in Pakistan contain Oxybenzone (Pakistan Observer, 2016). Oxybenzone is often used as active ingredients in sunscreen lotions and personal-care products, such as body fragrances, hair-styling products, shampoos and conditioners, anti-aging creams, lip balms, mascaras, insect repellants, as well as dishwasher soaps, dish soaps, hand soaps, and bath oils/ salts (CIR 2005)

The effect of Oxybenzone on a fetus was tested by comparing the growth and development of a Zebrafish embryo maturing in a well plate coated once with oxybenzone to the growth and development of a Zebrafish embryo maturing in a well plate containing no oxybenzone. The experiment was conducted a day after the egg was fertilized. The experiment ended five days post fertilization. The results of this experiment shows that Oxybenzone increases the mortality rate of Zebrafish embryos by about 20%. This experiment also show the Zebrafish embryos exposed to Oxybenzone developed an abnormally curved spine.

Materials and Methods

For the procedure, first paint a thin layer of sunscreen on bottom of 8 of the 12 wells and record which wells have sunscreen. Second, add 10 zebrafish embryos in each well using a pipette. Third, mix Instant Ocean in wells until well is filled half way. Remove water and remove dead embryos daily. After removing water and dead embryos place clean Instant Ocean into wells. Place wells into an incubator at 28.5 degrees celcius. Record amount of alive and hatched fish.

The materials used in this experiment included randomly selected zebrafish embryos, a well plate containing 12 separate wells to hold the embryos, a water mix Instant Ocean, Sunscreen containing oxybenzone, a Waste Container, and a Pipette.

In the experiment there were some necessary safety precautions to make sure no unnecessary harm was done to any animals or humans. Don't consume any materials because there could be something bad in them. Don't get Sunscreen in your eyes because that will burn your eyes. Lastly don't put alive fish in the waste because then the fish may get infected with fungus. In this experiment to measure the results the amount of zebrafish alive, dead, and hatched were counted twice to ensure accuracy and recorded. To do the statistics the average of sunscreen and no sunscreen wells were calculated each day and graphed to show the correlation of the two variables.

Results

In the experiment it was tested if oxybenzone has an effect on the mortality rate of zebrafish. The independent variable was whether oxybenzone was present or not. The dependent variable was the amount of zebrafish embryos alive and hatched at the end of each day. The controls were the wells with just Instant Ocean and zebrafish embryos, no sunscreen containing oxybenzone. By the end of the five days the average amount of embryos alive and hatched in the oxybenzone wells was 1.4, but in the no oxybenzone wells the average amount alive and hatched was 6.5. It was also found that some of the zebrafish in the wells with oxybenzone had curved spines.

Rows A and B have sunscreen and Instant Ocean. Row C has Instant Ocean.

Day 1:

Amount of embryos alive in each well

	1	2	3	4
A	10	10	10	10
B	10	10	10	10
C	10	10	10	10

Day 2:

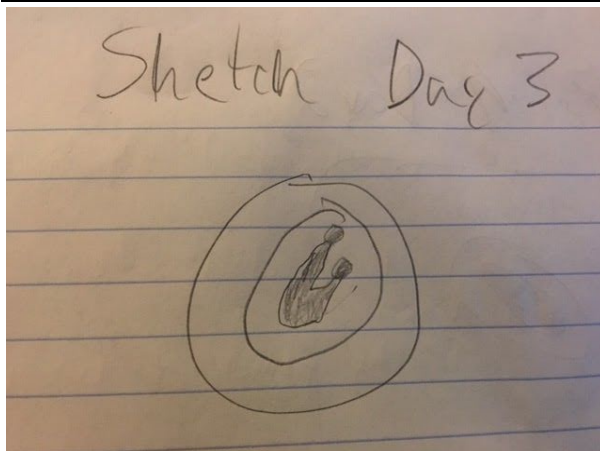
Amount of fish alive in each well

	1	2	3	4
A	9	10	10	8
B	7	9	8	10
C	8	9	8	7

Day 3:

Amount of fish hatched or alive and unhatched in each well (Hatched/Alive and Unhatched)

	1	2	3	4
A	0/9	0/9	0/9	1/6
B	1/5	0/9	0/8	1/9
C	0/8	0/9	0/8	0/5



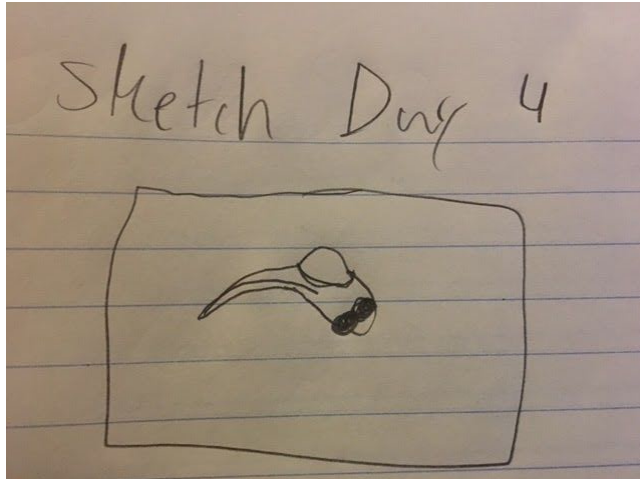
In this picture a zebrafish is starting to form in the egg.

Day 4:

Amount of fish hatched or alive and unhatched in each well (Hatched/Alive and Unhatched)

	1	2	3	4
A	4/2	4/5	4/1	1/6
B	2/0	1/1	1/1	8/1

C	4/4	6/2	6/2	3/2
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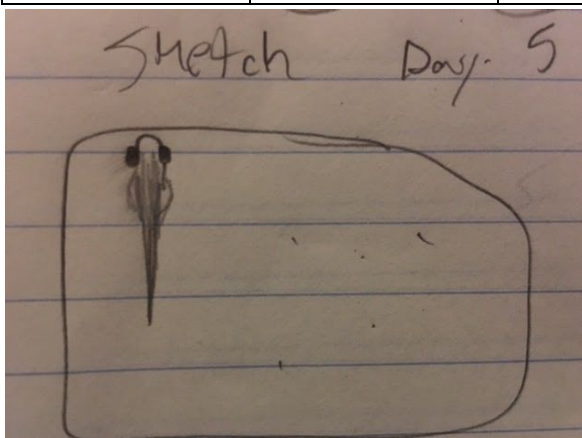


In this picture is a hatched zebrafish, it's spine is curved.

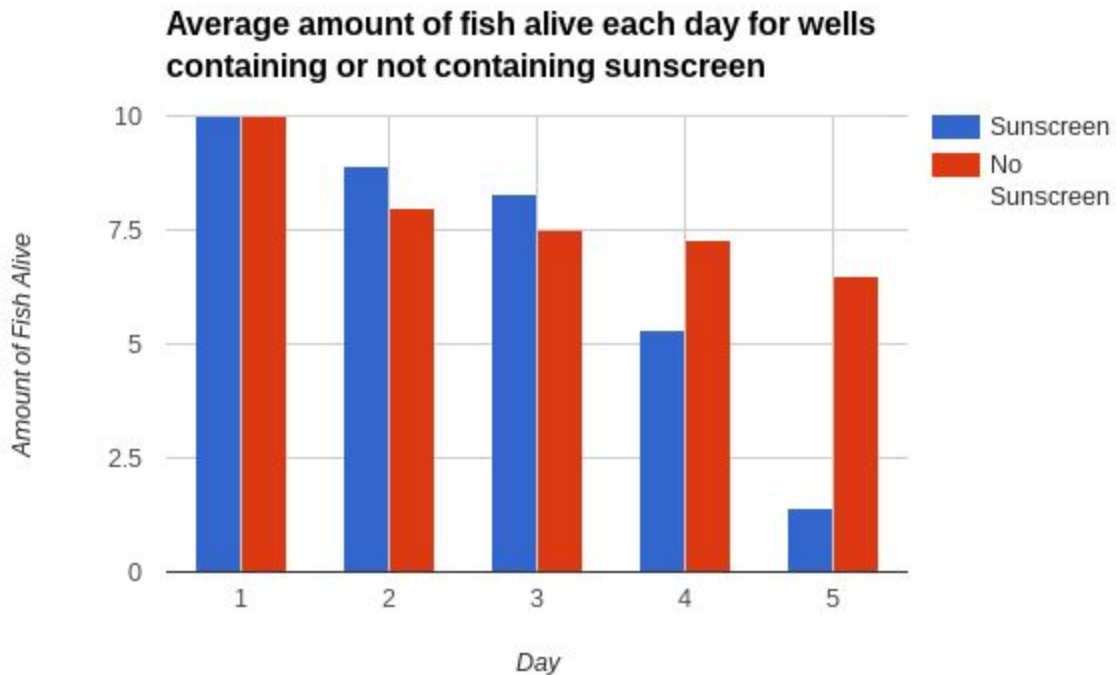
Day 5:

Amount of fish hatched or alive and unhatched in each well (Hatched/Alive and Unhatched)

	1	2	3	4
A	1/0	6/0	0/0	2/0
B	0/0	0/0	1/0	1/0
C	7/0	6/0	8/0	5/0



In this picture is a hatched zebrafish it is sitting still and has a normal spine as it was in a well with no sunscreen.



As the days went on the amount of fish alive in wells with sunscreen decreased drastically, but the amount of fish in wells without sunscreen stayed fairly consistent.

Discussion

From this experiment it was found that oxybenzone did have an impact on the mortality rate of zebrafish embryos. The difference of 6.5 zebrafish on average in each well without sunscreen compared to 1.4 zebrafish on average in each well with sunscreen is a significant difference. The hypothesis stated that if there is a presence of oxybenzone then the mortality rate of zebrafish embryos will increase because estrogenic compounds can lead to death early in life. The experiment showed that Oxybenzone did have an effect on zebrafish embryo mortality rate because a higher percentage of zebrafish embryos that were exposed to sunscreen and oxybenzone died. However in another experiment that tested oxybenzone and zebrafish embryos it was stated, “There was no difference in mortality between control and exposed eleuthero-embryos” (Blüthgen, 2012). One source of error could’ve been failing to conduct the experiment on the day of fertilization might of caused the Oxybenzone to have less of a negative effect on the Zebrafish embryos. As expressed by Blüthgen, “the highest mortality occurs within the first 24 h” (Blüthgen). Another source of error could’ve been that the amount of sunscreen in the well was not measured because it was painted on the bottom, so it could not be measured. This could’ve caused different

results in our experiment because there were different concentrations of oxybenzone in each well causing a higher mortality rate because of more oxybenzone. One more source of error could've been that sunscreen has more than one chemical in it, so the chemicals other than oxybenzone could've had an effect of the zebrafish embryos and caused the mortality rate to increase.

The results of this experiment are important because it shows how the sunscreen that humans use can affect the environment and the other animals in that environment. For example when swimming everyone typically applies sunscreen to make sure they don't get sunburned, but then when swimming in the lake or ocean the sunscreen often will rub off because of the water and will go into the water. This data shows that if the sunscreen or oxybenzone gets to the embryos of fish it could kill many of them and this could cause a decline in the population of fish which are necessary for food or necessary to keep the environment healthy. Another reason the results are important is because they show how oxybenzone could possibly affect humans if it comes into contact with the developing embryo or fetus. Now although this is unlikely it is still possible for it to happen, so pregnant women may need to stay away from sunscreens containing oxybenzone to make sure that nothing happens to the baby.

References

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