The Negative Effects of Nicotine on Mortality Rate Of Zebrafish Embryos

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Abstract

Studies show nicotine causes serious illnesses and unhealthy effects on people's heart, lungs, and body. On top of the proven negative effects of nicotine on humans, zebrafish have a similar genetic structure to humans. The effects of nicotine on zebrafish embryos are important because it shows how nicotine affects fetuses and developing embryos. The purpose of the experiment was to expose zebrafish embryos to nicotine and correlate the results to human embryonic development. Increasing amounts of nicotine were added to four wells containing 10 zebrafish embryos each. The zebrafish embryos were monitored for 72 hours. When measuring and controlling the amount of nicotine given to the zebrafish embryos the mortality rate and the effects of nicotine has on zebrafish embryos was clear. The experiment showed that the zebrafish that are in the control solution remain living, whereas, the zebrafish exposed to nicotine have a higher mortality rate. Therefore, the assumption can be made that human fetuses also slowly become closer to death when exposed to nicotine. The way the nicotine kills the zebrafish throughout time shows how the nicotine is going to affect humans.

Introduction

Nicotine is a very addictive and strong substance that affects our bodies in various ways, not in positive ways but in negative ways. Nicotine is even killing people and is known to affect the development of fetuses in the womb. According to Conger (2019), "In addition to raising the risk of birth defects, maternal smoking is closely associated with adverse neurobehavioural, cardiovascular, respiratory, endocrine, and metabolic outcomes in their children that can persist into adulthood." Secondly, nicotine is even killing the children in the womb. According to Ellis et al. (2014), "The smoking of tobacco continues to be the leading cause of premature death worldwide and is linked to the development of several serious illnesses…" Lastly, nicotine harms not only humans but also other mammals and animals. According to Felman (2018), "When humans, mammals, and most other types of animals are exposed to nicotine, it increases their heart rate, heart muscle oxygen consumption rate, heart stroke volume,

and your lungs. These are known as pharmacologic effects.” What will happen if zebrafish embryos are exposed to nicotine?The way the nicotine kills the zebrafish throughout time shows how the nicotine is going to affect humans. In this experiment, the mortality rate of zebrafish was measured to show that nicotine can, in fact, kill embryos. If zebrafish embryos are exposed to nicotine then they will end up dying or having birth defects because according to studies, human fetuses that have been exposed to nicotine have died and been born with birth defects.

Materials and methods

The materials given were: tape, sharpie, multi-well plates, datasheet, pencil, 40 embryos, 28.5°C incubator, depression slide with cover slip, gloves, dissecting and compound microscope, 1 mL of instant ocean/embryo media solution, large-bore transfer pipettes, minimum bore, 1.5 mm for transferring eggs to observation container and manipulating them in the container, transfer pipette, iPad, 4 100mL beakers, and dropper tool, 0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL of nicotine. On the first day, a spawning tank was set up, and feeding brine shrimp in preparation for spawning was completed. On the second day rinsed embryos were given from the teacher. Then, the wells were labeled with names, class hour and the concentrations of nicotine. 10 zebrafish embryos were placed in each of the four wells. Next, the excess fluid from the embryo transfer was removed with a pipette. After that, gloves were put on to prevent nicotine exposure to skin because nicotine is a dangerous chemical to make skin contact with. Following that, 1 mL of the assigned concentration of nicotine was placed in the corresponding wells using a large-bore pipette. The first well was filled with 1 mL of instant ocean/Embryo Media solution (0.0 mg/mL) using disposable pipettes. The remaining wells were filled with the proper amount of nicotine solution 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL) using a large-bore pipette. Next, the embryos were placed in an incubator at 28.5 degrees Celsius overnight. On the third day, the plate with the embryos was taken out of the incubator and The embryos were observed under a dissecting microscope and the observations made of the hatched, dead, and alive were recorded. The dead embryos were removed from the plate using a disposable pipette. In the beginning, all the embryos were moving. When there wasn’t a moving embryo or wasn’t clear if it was moving, the embryo was recorded as dead. When an embryo hatched, it was recorded on the datasheet as hatched. When embryos hatched and were moving or had a heartbeat, they were observed to be alive and recorded as alive on the data sheet. Each time an embryo or fish died, a fine bore was used to remove the dead embryos or fish. Each day, the nicotine was switched out for fresh nicotine by removing the nicotine use on the previous day and replacing it fresh nicotine using a large-bore pipette. Removing the nicotine solution was made easier by tipping the plate

so the embryos settled so then the liquid could be removed from the top. Fresh nicotine was put back in the wells by measuring 1 mL of the concentration of nicotine needed in each well (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL) using a clean large-bore pipette each time. After this, the plate was put under the dissecting microscope and observations were recorded on the data sheet. Abnormalities and developmental markers were also recorded on the data sheet. Then 1-2 embryos were placed on the depression slide with a cover slip and the embryos were observed with a compound microscope and then recorded down on the data sheet. This process was repeated for all nicotine solutions. After observations were made, photos of the embryos were taken on iPads of their progress each day. After the photos were taken, the embryos were placed back in the well and then placed back in the 28.5°C incubator. This process of recording data, and observing the embryos was repeated for three days (72 hours). On the last day, the amount of dead hatched, and live embryos were recorded. All of the wells were emptied with the embryos but the control group because they were put in with the rest of living fish/embryos. A chi-square analysis was completed on the data to ensure statistical significance.

Results

The research done was to prove that nicotine kills embryos. It was set up with different amounts of nicotine in each well to show that increasing amounts of nicotine can cause different mortality rates. The death of the embryos depended on the effect of nicotine, a higher amount of nicotine causes higher mortality rates. The control group was 0.0 mg/mL. The independent variable was the amount of nicotine and the dependent variable was the mortality rate. Constant variables in the experiment were the size of the well, the incubation temperature, and the amount of fluid in each well. The treatments of nicotine were 0.05 mg/mL, 0.1 mg/mL and 0.2 mg/mL. As the concentration of nicotine increases then the number of zebrafish embryos died. (Shown in figures 1-4 and 6-7) In the 0.0 5mg/mL well an observation of protozoa were shown (Figure 5). The chi-square value was 39.99 and the degree of freedom was 3 and the critical value used was 7.82. This experiment was used to test for the independence of nicotine solution and mortality. The null hypothesis for the experiment was rejected. This means it was true that nicotine was the reason for the death of the embryos.

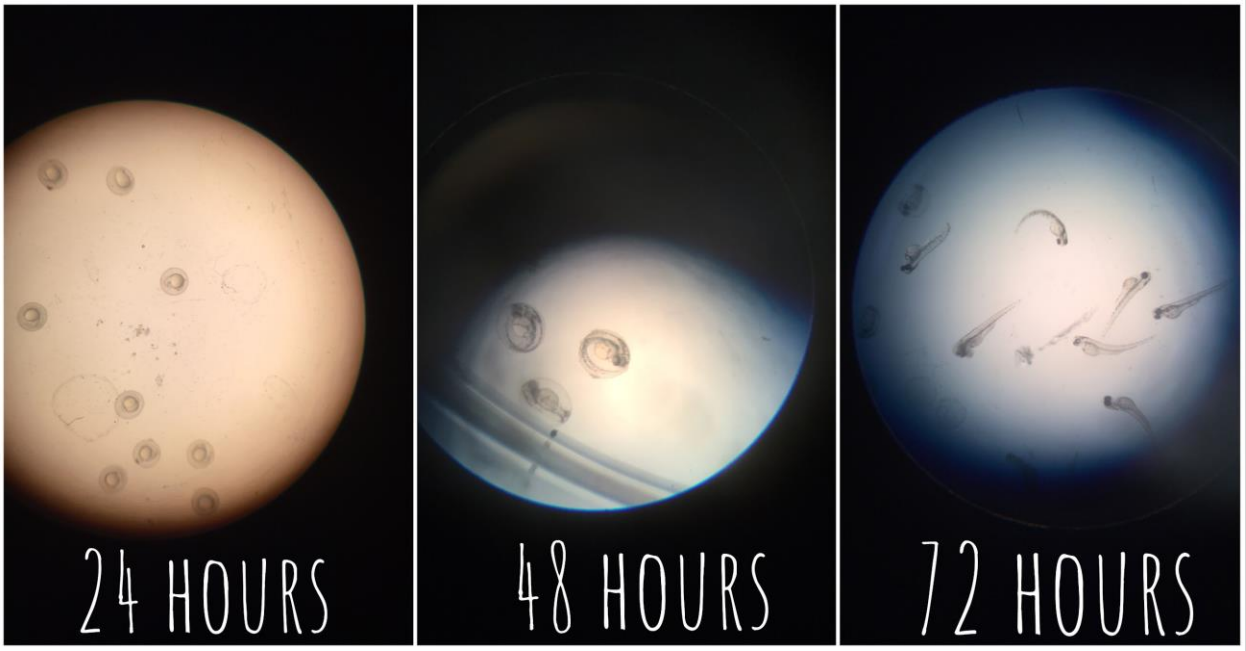

Figure 1: 0.0 mg/mL of Nicotine

Figure 1 shows the development of embryos over 72 hours in the well with 0.0 mg/mL of nicotine

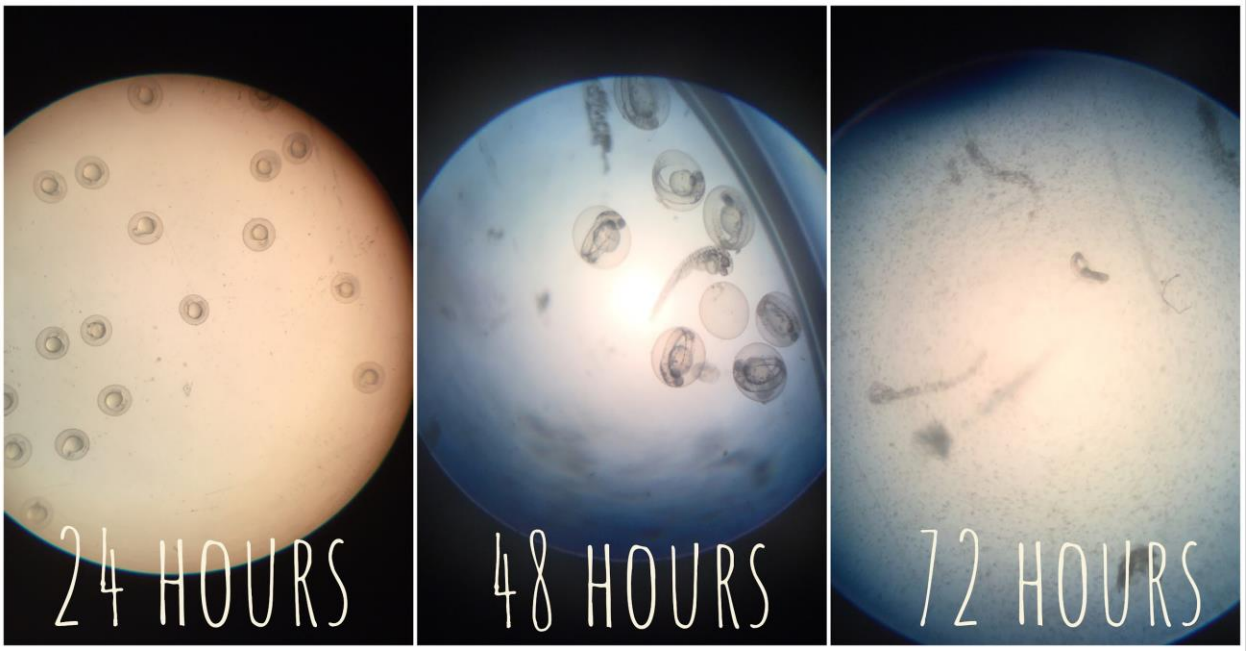

Figure 2: 0.05 mg/mL of Nicotine

Figure 2 shows the development of embryos over 72 hours in the well with 0.05 mg/mL of nicotine

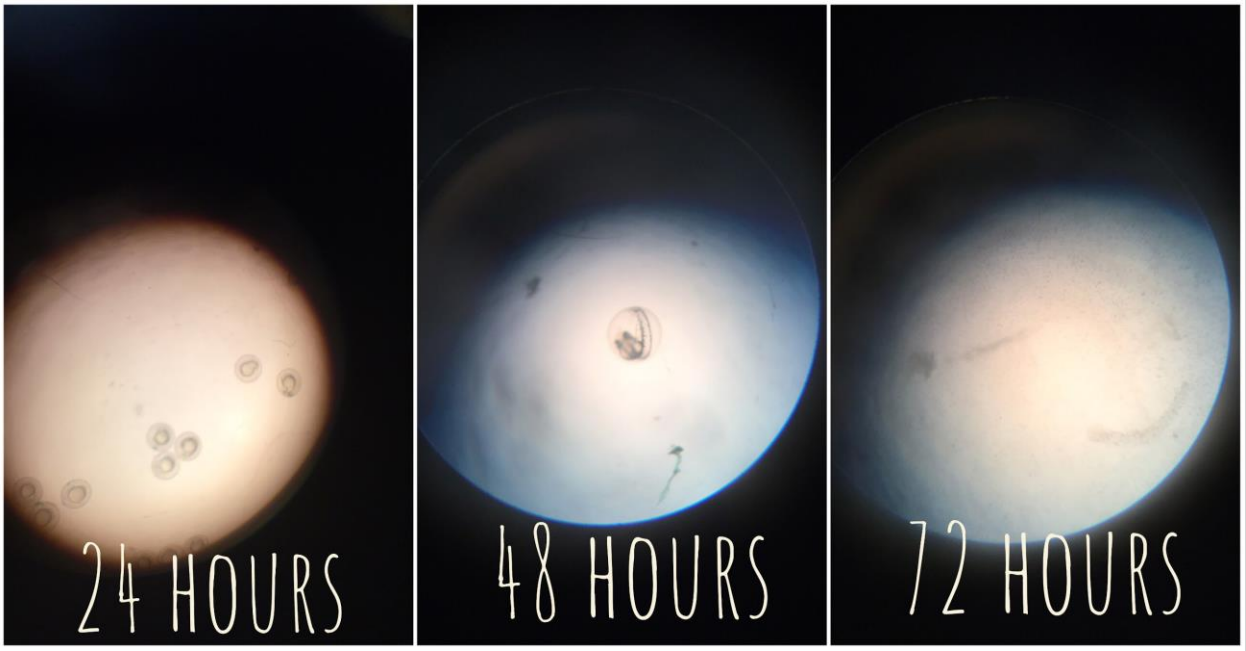

Figure 3: 0.1 mg/mL of Nicotine

Figure 3 shows the development of embryos over 72 hours in the well with 0.1 mg/mL of nicotine. After 72 hours the embryos died

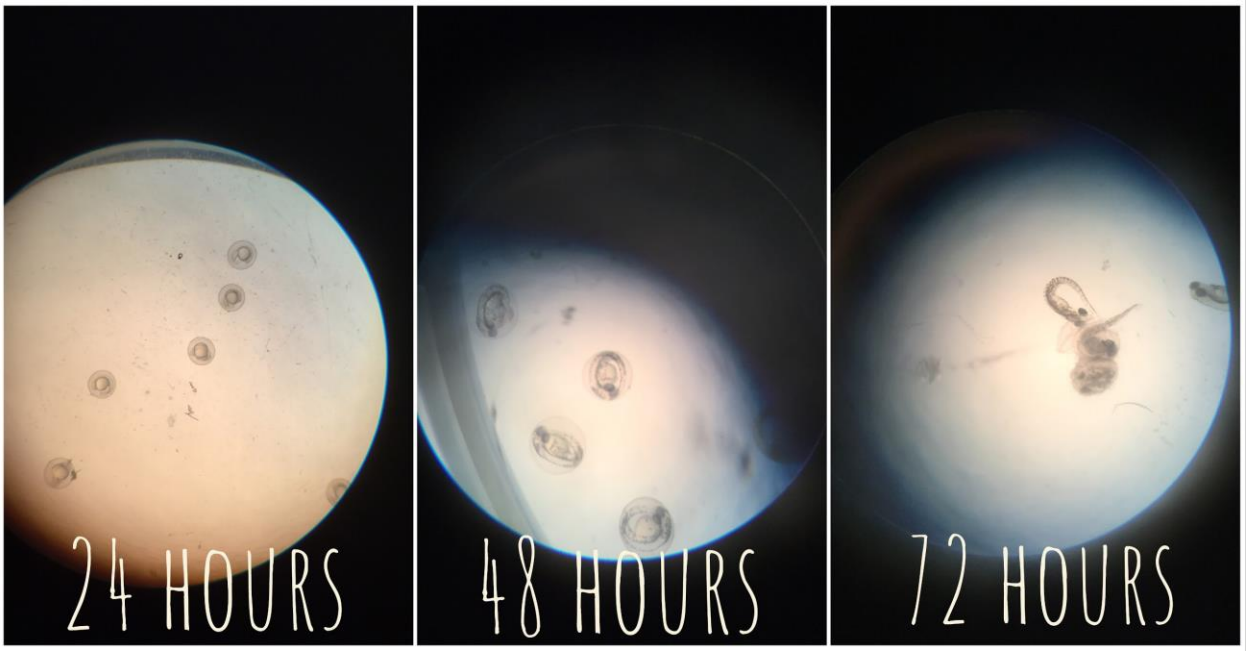

Figure 4: 0.2 mg/mL of Nicotine

Figure 4 shows the development of embryos over 72 hours in the well with 0.2 mg/mL of nicotine. After 72 hours the embryos died

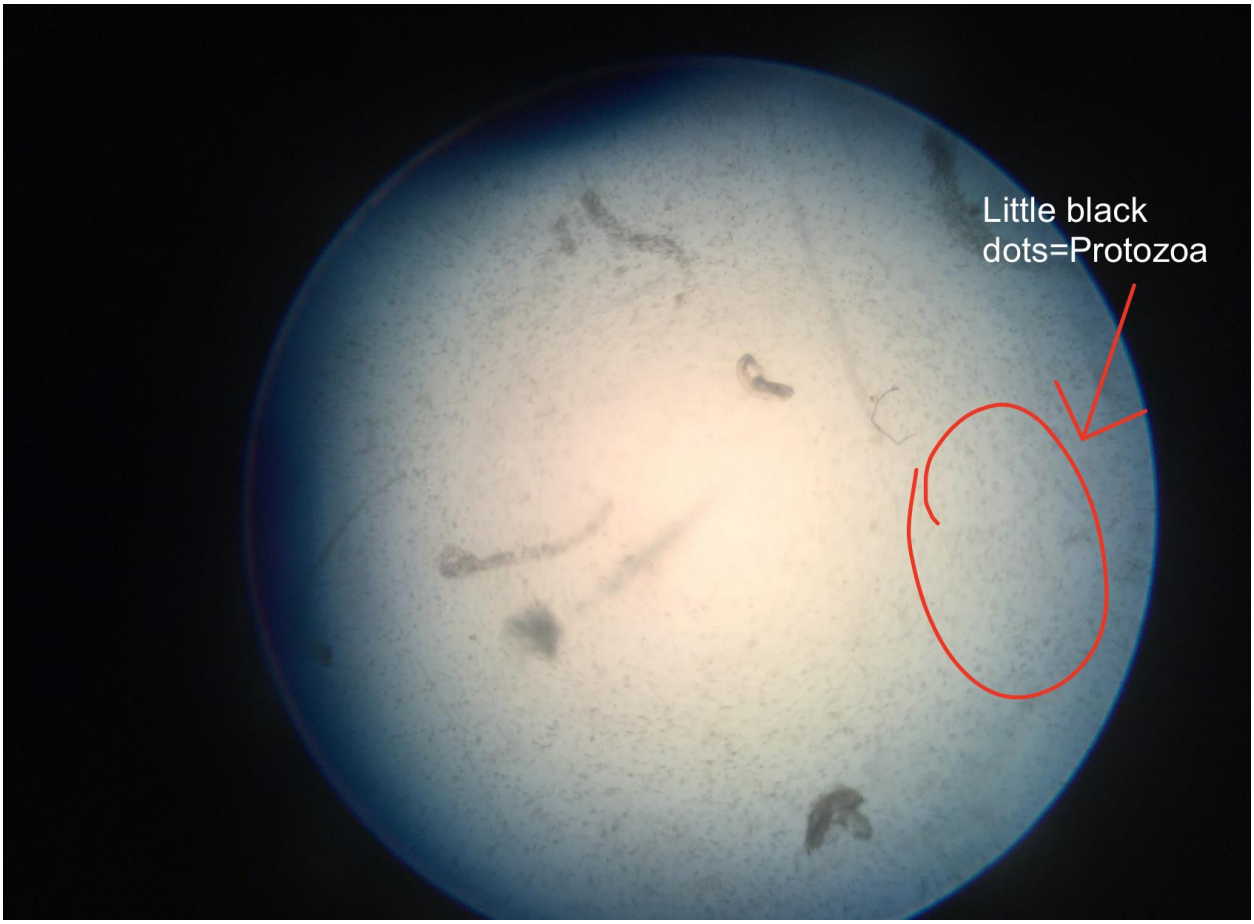

Figure 5: Protozoa in 0.05 mg/mL Nicotine Well

Figure 5 shows the Protozoa feeding off of the zebrafish embryos in the 0.05 mg/mL nicotine well

Nicotine Affecting the Hatching and Mortality Rate of Zebrafish Embryos Over 72 Hours

Treatment	# of starting fish	24 hours post fertilization		48 hours post fertilization		72 hours post fertilization		96 hours post fertilization	
Nicotine		# Hatched	# Live	# Hatched	# Live	# Hatched	# Live	# Hatched	# Live
0.0	10	0	10	2	10	10	10		
0.05	10	2	10	9	10	10	0		
0.1	10	0	10	2	10	3	0		
0.2	10	0	10	0	10	5	0		

Table 1: Nicotine Affecting the Hatching and Mortality Rate of Zebrafish Embryos Over 72 Hours

Table 1 shows that over time the embryos in higher concentration wells died

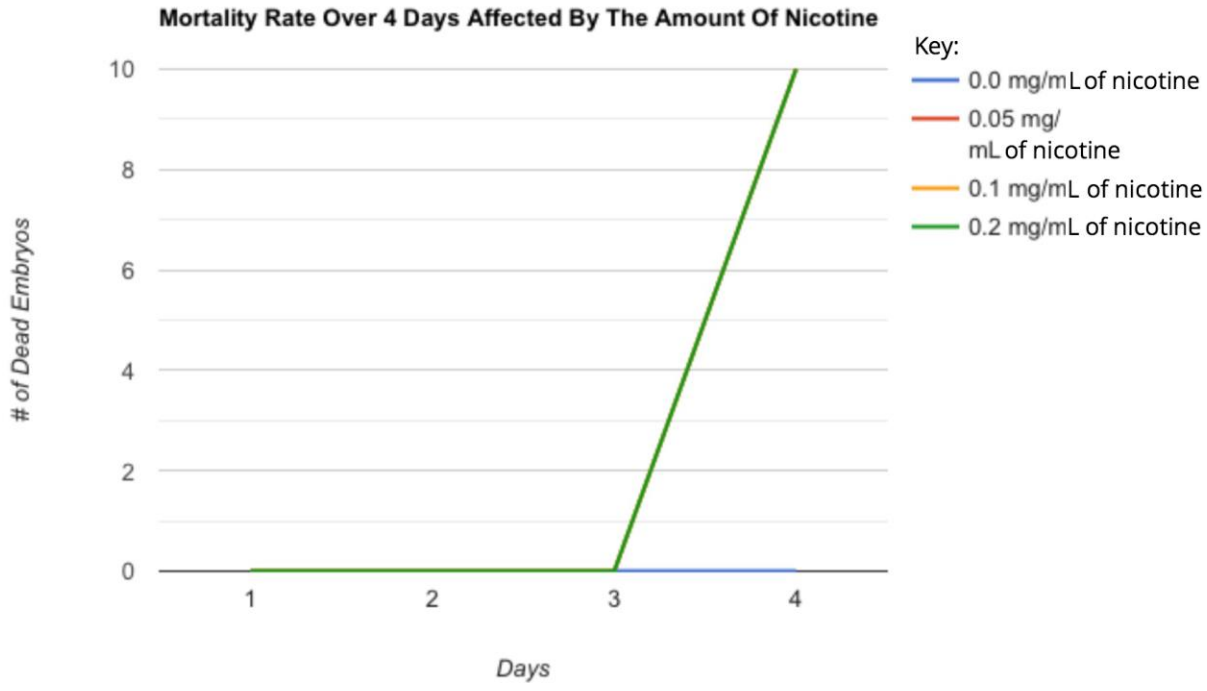

Figure 7: Mortality Rate Over 4 Days Affected by the Amount of Nicotine

Figure 7 shows all of the embryos in the control group (0.0mg/mL) lived on all 4 days and the embryos in the 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL nicotine concentration all died on day 4 and because of that, there are overlapping lines on the graph.

Discussion

Studies have shown that nicotine has killed and even affected fetuses in the womb. In the experiment conducted, results showed that the embryos in concentrations with nicotine all died. The hypothesis was “If zebrafish embryos are exposed to nicotine then they will end up dying or having birth defects because according to studies, human fetuses that have been exposed to nicotine have died and been born with birth defects.” The hypothesis can be confirmed because the embryos in the nicotine solutions had all died on the fourth day. The things that were learned from this experiment is that nicotine can kill embryos. The error in the project was the protozoa. Protozoa are parasites that feed off of dead and living organisms. These parasites are considered to be an error to the experiment because they're not really supposed to be there. They could’ve came in with original embryos or in the water that the embryos came in. They’re also considered to be an error because they started feeding off of the embryos, which alters the final results of the experiment. If the protozoa weren’t there, the chance of zebrafish embryos still being alive was still possible. Next time, another experiment should be performed instead of just one to get further research data to support the hypothesis. If there was another experiment performed would there be a way to get rid of or have no protozoa in any of the wells? Although the chi-square value showed that the nicotine and mortality rate are dependent. It should be noted that in the wells with the concentrations of nicotine there were numerous protozoa which may have impacted our results in those wells.

Resources:

Adam, F. (n.d.). Nicotine: Facts, effects, and addiction. Retrieved November 18, 2019, from Medical News Today website: https://www.medicalnewstoday.com/articles/240820.php

Ellis, L. D., Soo, E. C., Achenbach, J. C., Morash, M. G., & Soanes, K. H. (2014). Use of the zebrafish larvae as a model to study cigarette smoke condensate toxicity. PLOS ONE, 9(12), e115305. https://doi.org/10.1371/journal.pone.0115305

Krista, C. (2019, March 4). Nicotine’s effect on the developing embryo studied in new model. Retrieved November 18, 2019, from Scope website: https://scopeblog.stanford.edu/2019/03/04/nicotines-effect-on-the-developing-embryo-studied-in-new-model/