

# The Impact of Nicotine on Zebrafish Embryos

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## Abstract

The purpose of study the effects of nicotine on zebrafish embryos is to their information on the developmental effects the substance has. This information can be used to predict how nicotine will then affect human embryo development. Also the purpose is to figure out which concentration of nicotine poses the most risk in embryo development. Utero exposure to tobacco —nicotine specifically —increases the risk of a child being born prematurely. Also conditions associated with an increased risk of respiratory distress syndrome, cardiovascular defects, cleft lip and palate as well as cognitive and locomotor impairments. Exposure is also associated with childhood leukemia, lymphoma, and brain tumors. (O'Brien, Jennifer. "Tobacco Smoke Affects")

## Introduction

Nicotine, a parasympathomimetic stimulant found in all tobacco products is highly addictive. According to Dr Ananya Mandal, MD, nicotine is commonly used as a relaxant and stimulant. It only takes as little as seven seconds after entering the bloodstream to reach the brain. This can cause problems with cholinergic receptors and disrupt normal brain function until the habit becomes addictive. Having nicotine in the body system can also ease the feelings of pain and anxiety by releasing neurotransmitters into the bloodstream. Regular use of nicotine may result in weight loss as nicotine raises blood glucose levels which decrease appetite in the body. (Mandal, MD Dr Ananya. "Nicotine Effects.")

Danio rerio, commonly referred to as the zebrafish, is a freshwater fish found in slow streams and rice paddies, as well as the Ganges River in East India. Zebrafish eat smaller living organisms and are eaten by larger fish, small amphibians, mammals, and birds. Zebrafish are a common and effective organism to use for research and experimentation because like humans, zebrafish are vertebrates; meaning they have a spinal cord. This matters because the more they are physically like humans, the higher probability they share biological traits and reactions with humans. Also zebrafish embryos develop quickly and are transparent so it's possible to watch the development of the embryo. ("Zebrafish FAQs - University of Oregon.")

The question was, *what impact does nicotine have on the development of zebrafish embryos?* This question was asked because since zebrafish embryos share similar characteristics to those of a human, and that they develop rapidly they were the most appropriate candidate to test. It was hypothesized that the embryos exposed to the highest concentrations of nicotine will develop the most severe developmental defects, health defects such as disfigured spine, and die more rapidly.

## Materials and Methods

- One bottle of 0.05 mg/mL nicotine solution
- One bottle of 0.1 mg/mL nicotine solution
- One bottle of 0.2 mg/mL nicotine solution
- One beaker for dead embryos and liquid disposal
- One sharpie
- One bottle of embryo media solution
- One large bore pipette
- One minimum bore pipette
- One 1.5 mm pipette for transferring and manipulating embryos
- One transfer pipette
- One multi-well plate
- One 28.5°C incubator
- One depression slide
- One stereoscope
- One compound microscope

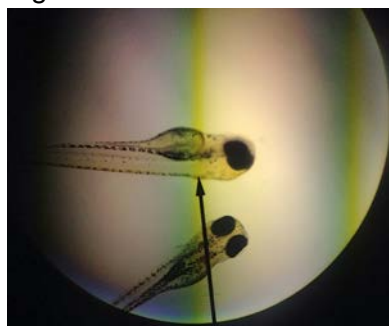
At the beginning of the experiment, each group was given the materials for the lab. On each multi-well plate, the first four wells were labeled A1, A2, A3, and A4. After that one ml of embryo media solution was added to each of the four wells. Once all four wells had embryo media in them, ten zebrafish embryos were added to each well. After all the embryos were transferred from the petri dish to the wells, one ml of 0.05 mg/mL concentration nicotine solution was added to well A2, one ml of 0.0 mg/mL concentration nicotine solution to well A3, and one ml of 0.2 mg/mL concentration nicotine solution to well A4 and were observed. 24 hours later, the embryos were removed from the incubator. Wells were cleaned of dead embryos and solutions were replaced. Additionally, detailed observations on all four wells using both the stereoscope, and the compound microscope were taken. This process was repeated for five days. On a given day one, two, three, or even four of the wells would be chosen and examined under the compound microscope. Once the experiment was over, the hatched embryos were placed into a proper waste container and disposed of properly. Throughout the duration of the experiment, observations were recorded between approximately 12:00-12:30 pm in order to keep results as accurate as possible. The outcomes of the experiment were measured through the observations compared to well A1, which served as the control. Data was recorded which can be seen in tables one and two.

## Results

In the experiment conducted, the independent variable was the concentrations of nicotine used (0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL) with the embryos. The Zebrafish embryos were exposed to the concentrations of nicotine resulting in premature hatching, and embryos being underweight. The dependent variables were; number of embryos (ten in each well), number of embryos hatched, and appearance of embryos. Nicotine concentrations impacted the number of embryos. There were a total of 39 embryos on the first day, and by day five a total of 20 embryos had died, only 19 embryos remained alive. None of the remaining 19, were found in well A4, because all embryos had died in that well by day five. Embryos exposed to 0.1 mg/mL

and 0.2 mg/mL began to hatch on the second and third day of the experiment. Most embryos were not hatched by day three and four, while some of the embryos did begin to hatch the. Premature hatches had been found in higher concentrations. The experiment resulted with embryos exposed to nicotine in higher concentrations to have noticeable appearance differences. In well number A4 which held 0.2 mg/mL of nicotine concentration, embryos were noticeably underweight and smaller compared to those in the control group.

Figure 1



Well A1- Day 3

Figure 2



Well A4- Day 3

(table one)

**Number of Embryos Alive in Different Concentrations of Nicotine Over One Week**

		Day 1	Day2	Day 3	Day 4	Day 5
Concentration of Nicotine	Well #	0 hpf	24 hpf	48 hpf	72 hpf	120 hpf
Control	A1	9	9	9	9	9
0.05 mg/mL	A2	10	10	9	9	7
0.1 mg/mL	A3	10	8	7	7	3
0.2 mg/mL	A4	10	9	9	8	0

(table two)

**Number of Embryos Hatched in Different Concentrations of Nicotine Over One Week**

		Day 1	Day2	Day 3	Day 4	Day 5
Concentration of Nicotine	Well #	0 hpf	24 hpf	48 hpf	72 hpf	120 hpf
Control	A1	0	0	5	9	9
0.05 mg/mL	A2	0	1	9	9	7
0.1 mg/mL	A3	0	1	7	7	3
0.2 mg/mL	A4	0	0	1	8	0

## **Discussion**

This experiment was able to conclude that the hypothesis was indeed correct. It was hypothesized that embryos exposed to higher concentrations of nicotine would experience the most death, hatch prematurely, and be underweight. The zebrafish embryos in well A4 with 0.2 mg/mL nicotine solution were the most severely underweight, hatched prematurely, and were some of the first to die in great numbers —see figure two for reference . By the end of the experiment there were no living embryos in well A4.

On the 5th and final day, there was a time gap between observations that was greater than 24 hours, instead it was 48 hours. The experiment ran into a new week and no observations took place over Saturday and Sunday. There was also a miscount in the control, when the experiment was set up there were only nine embryos placed into well A1, instead of the ten embryos placed all other wells. These mishaps in the experiment, especially the day five observations, could have created improper or inaccurate end results. The results from the experiment show a connection to human embryo development. Nicotine exposure to human embryos has commonly caused premature birth, which was shown also in zebrafish as the embryos in wells A2 and A3 had hatched as soon as the second day. Also some of the hatched embryos were underweight and had noticeably smaller tails than those in the control. is also a common phenomenon in human embryo exposure to nicotine.

## **Works Cited**

O'Brien, Jennifer. "Tobacco Smoke Affects Early Human Embryonic Development." UC San Francisco. N.p., 15 Mar. 2012. Web. 27 Feb. 2017.

Mandal, MD Dr Ananya. "Nicotine Effects." News-Medical.net. N.p., 02 Dec. 2013. Web. 15 Feb. 2017.

Zebrafish FAQs - University of Oregon