

The Effects of Vaping Fluid with Nicotine and Flavoring on Zebrafish Embryos

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Abstract:

This experiment studied the effects that vape fluid with nicotine and flavoring has on growing zebrafish embryos. It was thought that exposure to these harmful chemicals at a crucial developmental stage would have serious, permanent damage to the body. In this zebrafish toxicity experiment, the ability to test how different amounts of this vape fluid affects each embryo and hatched zebrafish in different ways was presented. It was hypothesized that if vape fluid with nicotine and flavoring (pre-vape) is put into the environment of zebrafish embryos, the embryos will undergo growth and development issues over time, including somite deformities, lack of pigmentation, and the inability to move. The zebrafish embryos were separated into groups of five embryos. Each group was put in their own well in a twelve-well plate. The different solutions containing vape fluid with nicotine were then injected into each well. The results of this experiment showed that the original hypothesis was not fully supported by the data collected because the vape fluid did not leave a statistically significant mark on the survival rate of zebrafish embryos. These results, when looked into for a longer period of time, may become more significant.

Introduction:

Danio rerio, also known as the zebrafish, is a type of tropical freshwater fish that is genetically related to humans in a wide variety of ways (U.S. Department of Health and Human Services, 2017). Seventy percent of the genes that are found in humans are also found in the zebrafish (Manner, 2018). Zebrafish can be observed and studied to learn many things about the human body. Studying zebrafish is a more practical method than studying other lab animals when it comes to learning about the way humans function and react to different stimuli. This is because zebrafish have many organs in common with humans. Zebrafish are easy and inexpensive organisms to maintain. In a way, they sustain themselves. Female zebrafish produce around 200 eggs each week.

Nicotine ($C_{10}H_{14}N_2$) is a harmful chemical that is found in many addictive substances (Editors of Encyclopedia Britannica, 2019). Some of these products include cigarettes and e-cigarettes. The nicotine in these dangerous products can lead to addiction by affecting the reward system that is found in the human brain. Once someone exposes themselves to nicotine, their brain starts to become reliant on it. On top of the harmful nicotine, the flavoring that is found in many of these products can react negatively when vaporized and produce chemicals that have been proven to be damaging to the body (CDC, 2019). Popcorn lung can be a result of the vape flavoring

and chemicals being inhaled. Becoming reliant on nicotine can cause numerous health issues. Multiple types of cancers can be caused by nicotine addiction.

The negative effects on an unborn baby that come from a woman smoking e-cigarettes during her pregnancy are unknown due to the lack of research. However, embryos exposed to nicotine may experience heart damage, incomplete brain growth and development, as well as, an array of deformities with the baby.

This experiment studied the effects that vape fluid with nicotine and flavoring has on growing zebrafish embryos. It was hypothesized that exposure to these harmful chemicals at a crucial developmental stage can have serious, permanent damage to the body. In this zebrafish toxicity experiment, the ability to test how different amounts of this vape fluid affects each embryo and hatched zebrafish in different ways was presented. A control group was used for comparison to the experimental groups. The independent variable used in this experiment was the different levels of nicotine in each solution. The dependent variable was the effect that nicotine and flavoring have on the survival of zebrafish embryos. The hypothesis was that when exposed to dangerous vaping chemicals, the embryo will have a hard time growing and developing. It is hypothesized that the mortality rate of the zebrafish embryos will increase. It is also hypothesized that brain development will be stunted, along with the overall growth of the zebrafish.

Materials and Methods:

For this experiment, many safety precautions were taken. Goggles and gloves were worn every single day in the lab.

Before the experiment started, the zebrafish embryos were separated into 12-well plates. Initial observations about the fresh embryos were recorded. The well plates were labeled with dry-erase markers. The zebrafish embryos came from the University of Milwaukee's Science Education Partnership Award Program which is sponsored by the National Institutes of Health. In order to keep the zebrafish in ideal temperatures, the well-plates were kept in an incubator when they were not being observed. The incubator was kept at 28.5 degrees Celsius.

A compound microscope was used each day to provide a magnified view of the embryos. The dissecting microscope gave an even more close up view of each individual embryo after they were placed on depression slides. A 3mL pipette was used to transfer the embryos onto the depression slides. A bottle of embryo media solution was used for the environment of the zebrafish embryos in the control group. Two bottles of vape fluid with nicotine and flavoring pre-vape, one with a 0.01 mg/mL concentration and one with a 0.05 mg/mL concentration, were used in the environment of the zebrafish embryos in the experimental groups. When the solutions were changed each day, 1mL pipettes were used to remove the old solutions from the well-plates. The old solutions and dead embryos were then put in a beaker that was set aside for waste. The

zebrafish embryos had a total of 144 hours of exposure to these chemicals. The mortality and hatch rates were recorded each day to provide insight on how fatal these chemicals truly are to developing embryos. To determine if our results were statistically significant, they were put into a website called GraphPad.

Results:

This experiment was conducted to help support the theory that nicotine has harmful and damaging effects on developing zebrafish embryos. These zebrafish embryos also provide insight on what may happen to a growing human embryo, when the mother smokes cigarettes or e-cigarettes. This is because zebrafish have seventy percent of the same genes as humans do. It was hypothesized that exposure to these harmful chemicals at a crucial developmental stage can have serious, permanent damage to the body. Therefore, in order to test this hypothesis, different concentration levels of the vape fluid with nicotine and flavoring, pre-vape, were tested as the independent variable. The dependent variable, the mortality and growth rate of zebrafish embryos was then recorded.

The results of this experiment do not show that the hypothesis was supported by the data collected. If low concentrations of vape fluid with nicotine and flavoring, pre-vape, are put into the environment of zebrafish embryos, the embryos will not undergo major growth and development issues. Such issues may include somite deformities, lack of pigmentation, and the inability to move (Figure 1 and Figure 2). These results show that the nicotine and the chemicals in the pre-vape fluid has many negative effects on developing embryos, but in order to recognize significant effects, the experiment must be further studied.

Table 1

Treatment	Well 1	Well 2	Well 3	Well 4	Average	Probability	Result
Control	5	5	5	5	5	-N/A	-N/A
.01 mg/mL Vaping fluid with nicotine and flavoring (pre-vaporization)	5	5	4	5	4.75	0.3559	not statistically significant.
.05 mg/mL Vaping fluid with nicotine and flavoring (pre-vaporization)	3	4	5	5	4.25	0.1682	not statistically significant.

Table 1 shows the final number of living zebrafish after the experiment was conducted.

Figure 1

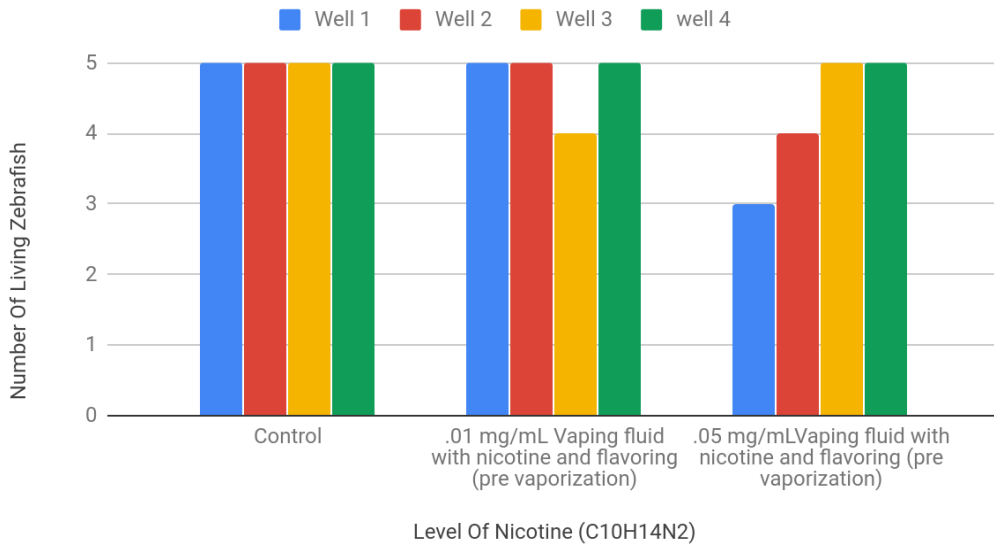


Figure 1 supports Table 1, showing the number of living zebrafish after the experiment was conducted.

Figure 2

Nicotine Treatments (C10H14N2)

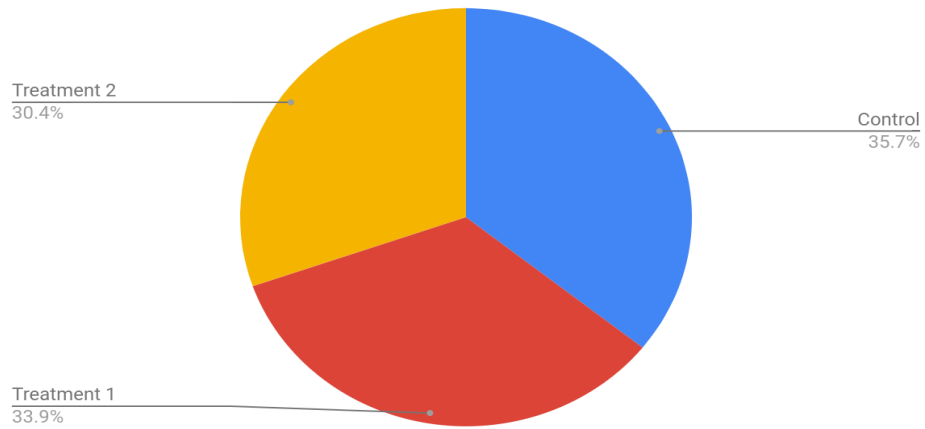


Figure 2 shows how effective each treatment is on harming the zebrafish.



Figure 3: A hatched embryo, from experimental group C, has experienced slight somite development issues from the harmful chemicals in its environment.



Figure 4: A freshly hatched embryo, from control group A, has not undergone any developmental or pigmentation issues.



Figure 5: A hatched embryo, from experimental group B, has experienced a lack of pigmentation from the harmful chemicals in its environment.

Discussion:

The pattern that was recognized throughout this experiment was that the longer that the embryos were left in the nicotine solutions, the more deformities they had to undergo. Another pattern that was recognized was when the zebrafish have a higher amount of the nicotine and flavoring in their environment, their developing bodies have

a hard time growing in the correct way. Some of the developing embryos that were placed in the nicotine solutions were unable to develop straight somites, which are clearly visible body segments that are present in the embryonic stage of vertebrates (Figure 1). Prior to the start of this experiment, it was hypothesized that exposure to these harmful chemicals at a crucial developmental stage can have serious, permanent damage to the body. The results that were discovered do not strongly support this hypothesis.

The results of this toxicology experiment can be linked back to the introduction. However, the results do not significantly support the idea that vape fluid with nicotine and flavoring, pre-vape, can have an array of negative effects on developing embryos. The harmful vape fluid can have damaging effects on brain development. The brain, when exposed to these harmful chemicals, will not be able to fully form. It has been shown that exposure to these harmful chemicals at this crucial stage in the life cycle may have permanently damaging effects for the duration of the rest of the life cycle. While all of these ideas may be correct, this experiment does not fully support them. Further study in this area would need to be conducted, in order to have significant supporting results of the hypothesis.

To further study this area and collect data that strongly supports this hypothesis, the zebrafish that hatched could be studied in more depth and for a longer time period. This could be done to help create an understanding of the long-term results. Reactions to stimuli, continued growth and development, and ability to move are just a few examples of what could be individually studied, after this experiment is concluded.

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