

**TITLE:** The Effects of Nicotine and Vanilla Flavoring Used in Vaping Liquid on Zebrafish Development and Hearts

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**ABSTRACT:**

*Vaping has begun to affect the world by coming out with different flavors. Vanilla, one of the more powerful flavorings, had been used in this experiment involving zebrafish embryos. Another chemical was nicotine, which is very common in vape liquid and cigarettes. With these two being mixed together in vape liquids, it is difficult to determine which one is more harmful. The zebrafish were observed over three days in either embryo media solution, nicotine-vanilla mixture, vanilla, or nicotine. The dependent variables in the experiment were to see if any of the chemicals had an effect on the size of the heart, heart rate, embryonic development, also the hatch and mortality rate of these zebrafish. All controls had survived and hatched, but both vanilla alone and the mixture had all deceased embryos. Nicotine had a few survive and hatch, with one of them having a significantly slower heart rate than one in the control group. The size of the heart from the nicotine zebrafish seemed to be smaller also. These results show that the vanilla flavoring may potentially be more harmful than nicotine alone. It has a great effect on embryos and the development over time. Along with unknown chemicals being created with the mixture, nicotine causes heart defects during embryonic development. This experiment demonstrates the toxicity of flavorings used in vape juice compared to nicotine alone.*

**INTRODUCTION:**

*Danio rerio*, also known as zebrafish, are very popular in scientific experiments because they are similar to humans. For example, humans and zebrafish both have a heart, liver, pancreas, gallbladder and many other organs. They are commonly used at UW-Milwaukee in research experiments. This experiment will examine the effects of vaping chemicals on zebrafish. The zebrafish is a special animal to biologists because its embryos are transparent, making them easy to study. Since the 1930s, scientists have been using zebrafish in experiments. Additionally 70% of human genes are found in zebrafish. A model organism is a small group of research organisms that serve as proxies for understanding the biology of humans according to Jane Marion Oppenheimer in the 1930s.

Two chemicals will be tested in this experiment. One is nicotine, an additive made of nitrogen from several plants, mainly tobacco. This chemical is mainly used in cigarettes and is linked to multiple types of cancer (NIDA, 2018). It is almost as difficult to give up as heroin. It affects the rhythm of one's heart rate, and clots blood more often than normal. Over time, as the intake of nicotine stays, tolerance begins to build up. Liquid nicotine is proven to be somewhat safer, but it still contributes to cancer (Felman, 2018). The other chemical is vanilla. It is an addicting flavoring added to vape. It's created with propylene glycol and vegetable glycerin, which then gets mixed with nicotine. As the mixture occurs, 50%-80% of new chemicals are created. When taken in, it triggers irritant receptors in your body (Becker, 2018).

The investigation will examine the effects of the environmental factors of development of zebrafish embryos. The expectations for the project are that the shape of the heart will become deformed over time, along with the heart rate develop to be irregular. One group of zebrafish will be developing in vanilla flavored vape juice, consisting of mainly nicotine and vanilla. Another group will be in nicotine and a different group will be in vanilla, which will help show the effects of the additives to the fish. The control will be embryos growing in water with a little bit of salt, to have the same feel as an ocean. Then monitor the heart rate, shape/size of heart, development and growth, hatch and mortality rate of the embryos.

## **MATERIALS AND METHODS:**

The materials that were used were : zebrafish embryos, bottle of 0.2mg/mL vanilla solution, bottle of 0.2mg/mL nicotine solution, beaker for dead embryos or liquid waste, beaker for embryo media solution, dry erase marker, disposable large bore pipettes , disposable small bore pipettes, 12-well plate, 28.5C incubator, dissecting microscope, goggles and gloves.

On the first day of setting up the experiment, the embryos for the experiment were obtained from the teacher, which are shipped from UW-Milwaukee. Then three rows of wells were filled with several mL of chemicals being tested, nicotine and vanilla flavoring. The next step was to divide the embryos so there were 10 embryos in each well. After discarding any dead ones, the exact number of live ones in each well was recorded on the data sheet. Then the well plate cover was labeled with what chemicals are in each row of three. Finally for day one, embryo media solution was added in the row of three with the control group of zebrafish. After adding the solution, the well plate was returned to the incubator. For the second day, the well plate was taken out of the incubator. With a disposable pipette, dead embryos were removed and put into the waste beaker. Then the remaining living embryos were counted, along with the hatched fish, and recorded observations. The old solutions were then removed from each individual well. After that, the old solutions were replaced with fresh solution using a clean pipette for each different chemical. The well plate finally returned to the incubator and the lab station was cleaned up. Day three and four had the same steps as day two. Once day four arrived, the disposed of the organisms were done properly. The final step was to figure out the results with the t-test. This test compares two groups' means. One variable between the two groups is used to compare them. After the calculations, two fish were chosen to have their heart rates counted and compared.

## **RESULTS:**

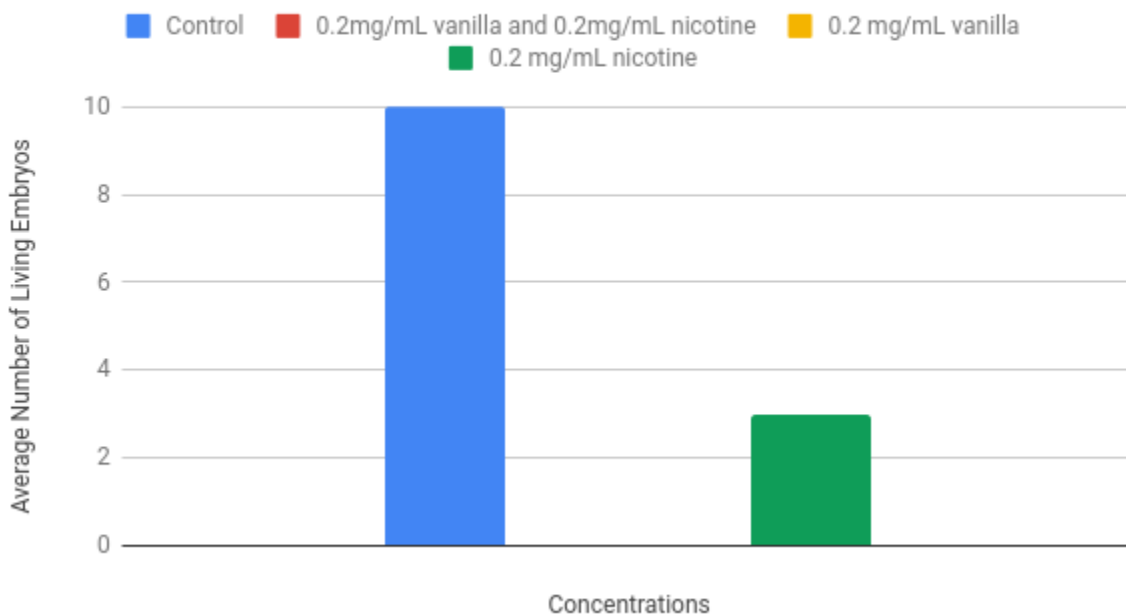
The purpose of the experiment was to see how nicotine and vanilla flavoring, commonly used in vape liquid, affects the human body and embryonic development. Specifically looking at the size of the heart also the heart rate. The predicted outcome was that the size of the heart would decrease and the heart rate would become irregular and slower than one of a healthy zebrafish due to the nicotine, vanilla, or the even mixture of both.

Each well had exactly ten embryos and were counted every day to see the progress of development. The control group had perfect data, with ten alive and hatched by the last day of the experiment. On the other hand, all thirty embryos within the three wells of only the vanilla solution had passed away by the third day. None of the embryos had hatched, but the nicotine solution had an average of three alive by the final day. The average of living fish was extremely significant data compared to the control group. The average of hatched fish was five for nicotine. While the average of living zebrafish had significance, the average of hatched embryos was not quite significant. Finally, for the mixture of vanilla and nicotine wells, it was very similar to the vanilla concentration. There were no living zebrafish, but the average of hatched fish was only one. This data was also extremely significant compared to the control group. After counting the heart rate of a fish selected from the control group, within one minute it was 135 bpm (beats per minute). Then one zebrafish from one well filled with nicotine had a heart rate of 90 bpm. It was much slower and the size of the heart had seemed smaller than the control fish's heart.

**Table 1 : Living Zebrafish**

Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	10	10	10	10	-	-
0.2mg/mL vanilla and 0.2mg/mL nicotine	0	0	0	0	-	-
0.2 mg/mL vanilla	0	0	0	0	-	-
0.2 mg/mL nicotine	2	4	3	3	0.0003	extremely statistically significant

**Averages of Living Zebrafish**



**Table 2 : Hatched Zebrafish**

Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	9	10	8	9	-	-
0.2mg/mL vanilla and 0.2mg/mL nicotine	1	0	2	1	p = 0.0006	extremely statistically significant
0.2 mg/mL vanilla	0	0	0	0	p= 0.0001	extremely statistically significant
0.2 mg/mL nicotine	2	9	4	5	p = 0.0742	not quite statistically significant.

## Averages of Hatched Embryos

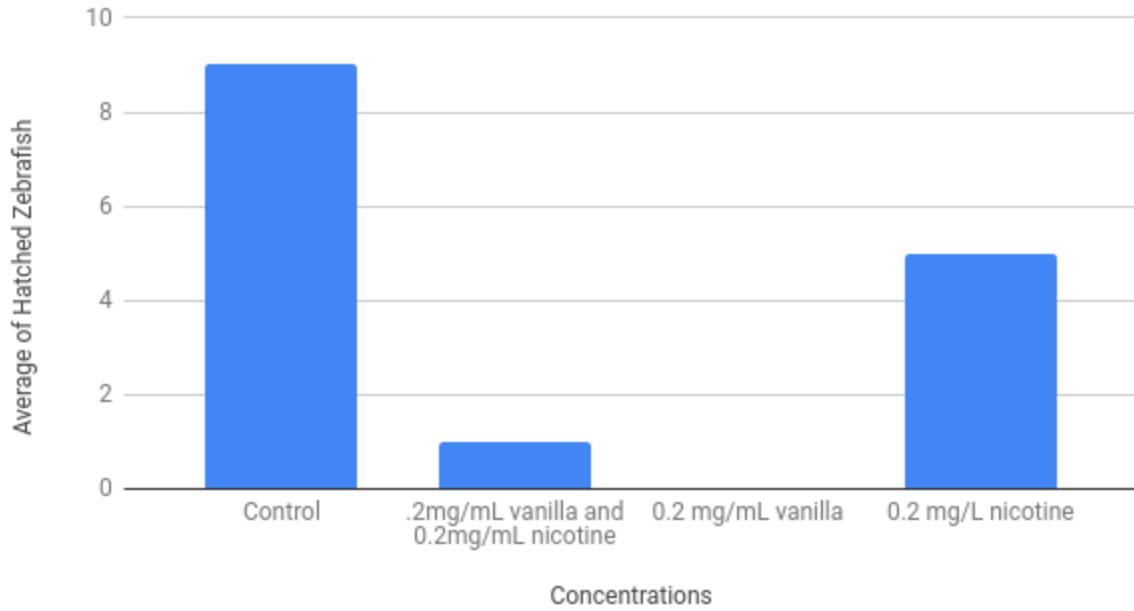


Figure 1 : One healthy zebrafish in the control group.



Figure 2 : A deceased embryo due to the vanilla concentration.



Figure 3 : One living zebrafish surrounded by two deceased zebrafish in the nicotine concentration.



Figure 4 : Two deceased zebrafish in the mixture of nicotine and vanilla concentrations.

## DISCUSSION:

These results had shown the severity of these chemicals on zebrafish embryos and the results can be applied to human embryonic development. The mortality rate was similar between the wells filled with vanilla flavoring, and the nicotine-vanilla mixture wells. None had survived by the last day, but zebrafish in the nicotine wells had some surviving and hatched. Despite some mortality, the living zebrafish were unable to move on their own. Their spines had begun to curve when they hatched. The nicotine seemed to change their growth in a few areas of the body. The heart was smaller in size, and the heart rate was 90 bpm. That's about 45 beats slower than one of a normal zebrafish.

Some errors could've occurred doing this portion of the experiment. With counting the control's heart rate twice, there could be a few beats missing due to how fast its heart was beating. And a lot of recounting had been done when figuring out how many were alive or hatched each day. There could also be a possible mistake with the amount of each chemical put into each well. It could possibly be too many drops of the solution or too little.

With the concluded results, it shows how damaging the vanilla flavoring is to an embryo, along with a mixture of vanilla and nicotine. Both are potentially unhealthy for any pregnant women, but the vanilla is shown to be more harmful than the nicotine alone. Some had survived and hatched in the nicotine wells, with other issues than immobility. Compared to those three wells, vanilla didn't have any survivors and neither did the mixture. These results support the conclusion that the flavorings used in vape liquid are potentially more harmful than nicotine itself. Continuing experiments from here should look at how vaping liquid affects an adult

model organism rather than an embryo. Then it'll show significance to someone other than pregnant women and their offspring.

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