

**TITLE:**

The Effects of Nicotine-Free Vape Fluid with Flavoring on the Mortality and Hatch Rate of Zebrafish Embryos

**NAMES OF RESEARCHERS:**

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**ABSTRACT:**

*This experiment was conducted to test the effect of nicotine-free vape fluid with dragon fruit flavoring on the mortality and hatch rates of zebrafish embryos. Its goal was to give more insight into how vaping fluid without a nicotine component affects human embryos in the womb. The experiment also questioned if nicotine is the sole reason that embryos, both human and animal, become malformed or die when exposed to vape fluid. In each well of a 12-well plate, five zebrafish embryos lived in varying concentrations of nicotine-free vaping fluid. The results concluded that the toxicant did not have a significant effect the mortality and hatch rates. However, the results did show that the vaping fluid did negatively affect the development of zebrafish embryos and lead to an increase in malformations. Experiments like this can give better insights into how toxicants like vape fluid can affect a developing human embryo inside of pregnant women.*

**INTRODUCTION:**

In this experiment, the effects of flavored vaping fluid without nicotine content were tested on zebrafish embryos. Zebrafish (*Danio rerio*) are tropical fresh-water fish that are often found in the rivers and ponds of India. They are also widely available in pet stores today. Zebrafish are often used in human research due to a number of factors. For instance, they are inexpensive compared to other animal subjects such as mice. They are also used due to their large quantity, in sync development, transparency of the body, swift development, easily identifiable organs, and their development outside of the mother. Zebrafish embryo structures are also similar to human embryos while in the early stages. Humans share 70% of their protein-coding genes with zebrafish, and 84% of human diseases have a zebrafish counterpart (Burke, 2016).

Vaping is the process of inhaling a superheated aerosol mixture into the lungs. The concoction uses extremely small particles that can embed themselves deeper into tissue than the average cigarette. Many of these vapes contain over 200 different chemicals, many of which are theorized to be harmful and carcinogenic. Most e-cigarettes are battery operated and contain a highly addictive substance called nicotine which is also found in cigarettes. The propellant that creates the vapor is also potentially harmful along with the hundreds of other additives in the vaping fluid (Richter, 2018). Other research has found that vaping during pregnancy may also be dangerous for the developing embryo. Nicotine can permanently damage a developing brain and other organs (Hays, 2019).

The vaping fluid that will be utilized in this experiment is one without any nicotine content. The fluid is also post-vape. Therefore, the focus will mainly be geared towards the effects that the chemicals within non-nicotine e-cigarettes (independent variable) have on the mortality and hatch rates (dependent variables) of the embryos. It is hypothesized that the embryos exposed to the toxicant will have a higher mortality rate and lower hatch rate. The yolk will be supplying the embryo with problematic material that will decrease the overall health of zebrafish embryos. The effects of the chemicals on the zebrafish embryos relates to human development. For instance, this experiment can give better insight on how vaping during pregnancy can impact the health of the human embryo.

## **MATERIALS AND METHODS:**

The materials used in this experiment includes one 12-well plate, a dissecting/stereo microscope, a compound microscope, goggles and gloves for each student, multiple disposable pipettes (small (1 mL) and large (3 mL) bores), a depression slide with coverslip, a dry-erase marker, and beakers for waste disposal and clean solutions. Also used will be 5mL samples of embryo media (Instant Ocean), and 5mL samples of a low, medium, and high concentration of flavored vape fluid without nicotine, post-vape. The embryos will also be kept in an incubator kept at 28.5 degrees Celsius. Gloves and goggles are also to be worn during the entire time working with the embryos.

On day 0, the embryos were delivered from UW-Milwaukee Science Education Partnership Award Program (sponsored by the National Institutes of Health), extracted from Instant Ocean, and then placed into the 12-well plate with fresh embryo media (Instant Ocean). A total of five embryos lived in each well. The first column of the well-plate (A1, B1, and C1) was the control group that would only be living in only the Instant Ocean. The second column (A2, B2, and C2) contained the low (0.01mg/mL) concentration of vape fluid. The third column (A3, B3, and C3) contained the medium (0.05mg/mL) concentration of vape fluid. And the fourth column (A4, B4, and C4) contained the high (0.1mg/mL) concentration of the post-vape fluid. All concentrations were in proportion to 1 mg/mL of Instant Ocean). At any time, 5 mg/mL of each solution was in each well.

On day 1, checks were made under the microscope to see if any zebrafish were deceased or hatched. Empty egg sacs from hatched embryos were removed using pipettes as were any deceased zebrafish. Observations were taken under a high-powered microscope. Each day of the experiment, which lasted four total days from when the embryos were delivered, after observations were taken, the fluids were replaced in each well. They were replaced using pipettes that were disposed of after each column to avoid contamination. This was to remove any waste that had generated from zebrafish embryos. Then, the zebrafish embryos were left to develop in their temperature controlled (28.5 degrees C) incubator. On day 2, the same steps were repeated. Then, on the last day of the experiment, a final round of observations was taken before the zebrafish were euthanized. A t-test was conducted using the site QuickCalcs, to calculate the statistical relevance of the zebrafish mortality and hatch rates.

## **RESULTS:**

The goal of this experiment was to test the effect of non-nicotine (flavored) vape fluid on development of zebrafish embryos. This could give further insight into the dangers those pregnant women who vape during pregnancy impose upon their developing fetus. This is important due to many smokers switching over to what they deem as a safer alternative because of the nicotine in cigarettes that is widely known to potentially cause birth defects. It was predicted that zebrafish embryos exposed to the vaping fluid would have a higher mortality rate and a lower hatch rate. In this experiment, the independent variable was the non-nicotine vaping fluid with flavoring (post-vape). The dependent variables were the mortality and hatch rates of the embryos. The control zebrafish were kept in an embryo media solution (Instant Ocean). However, this was proved to be false as the data in this section reveals that the vaping fluid did not have a significant effect on the mortality and hatch rates of the embryos (see Figures 1-4).

Besides the quantitative data, additional observations were made in this experiment. For instance, as the concentration of vaping fluid increased as did the signs of malformation in the embryos. As an example, embryos in the medium to high concentrations had more significantly enlarged or reduced features and delayed neural responses. The control zebrafish would flee from foreign objects placed into the container at a rapid pace while those from higher concentrations had a hard time maintaining balance and showed little to no reaction from the insertion of foreign objects. By the end of the experiment, the zebrafish from the higher concentrations had less pigment, delayed eye development, malformed/delayed fin development, and slower movement than the control zebrafish (Figure 5-7).

Figure 1:

Average Quantity of Living Zebrafish in Different Concentrations of Vaping Fluid

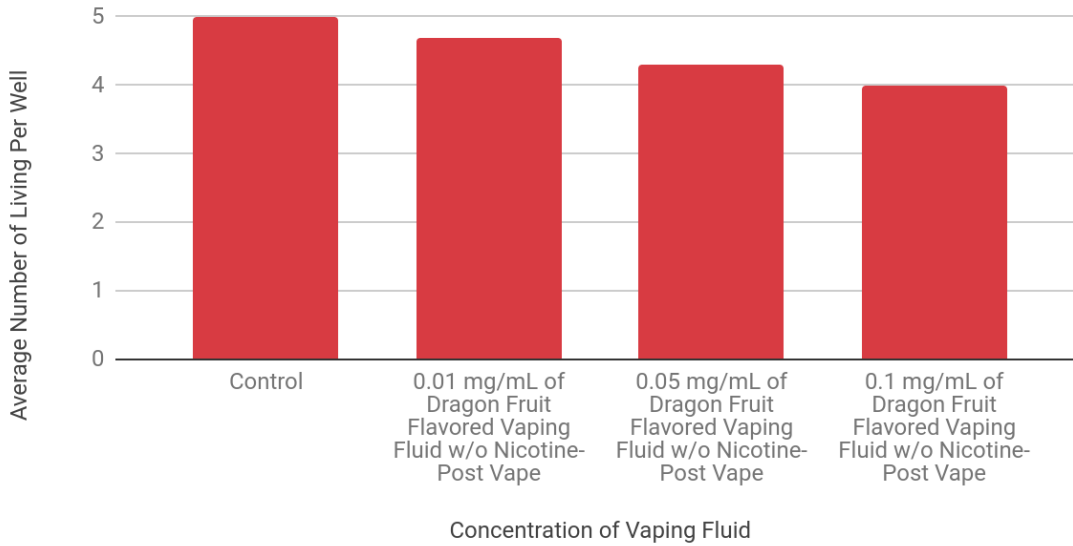


Figure 2:

Treatment	Well 1	Well 2	Well 3	Average of Living	Probability	Result
Control	5	5	5	5	-	
0.01 mg/mL of Dragon Fruit Flavored Vaping Fluid w/o Nicotine-Post Vape	5	4	5	4.7	p= 0.3739	Non-statistically significant
0.05 mg/mL of Dragon Fruit Flavored Vaping Fluid w/o Nicotine-Post Vape	4	5	4	4.3	p= 0.1161	Non-statistically significant
0.1 mg/mL of Dragon Fruit Flavored Vaping Fluid w/o Nicotine-Post Vape	4	4	4	4.0	-	No t-test result available due to perfect data set

Figure 3:

### Average Quantity of Hatched Zebrafish in Different Concentrations of Vaping Fluid

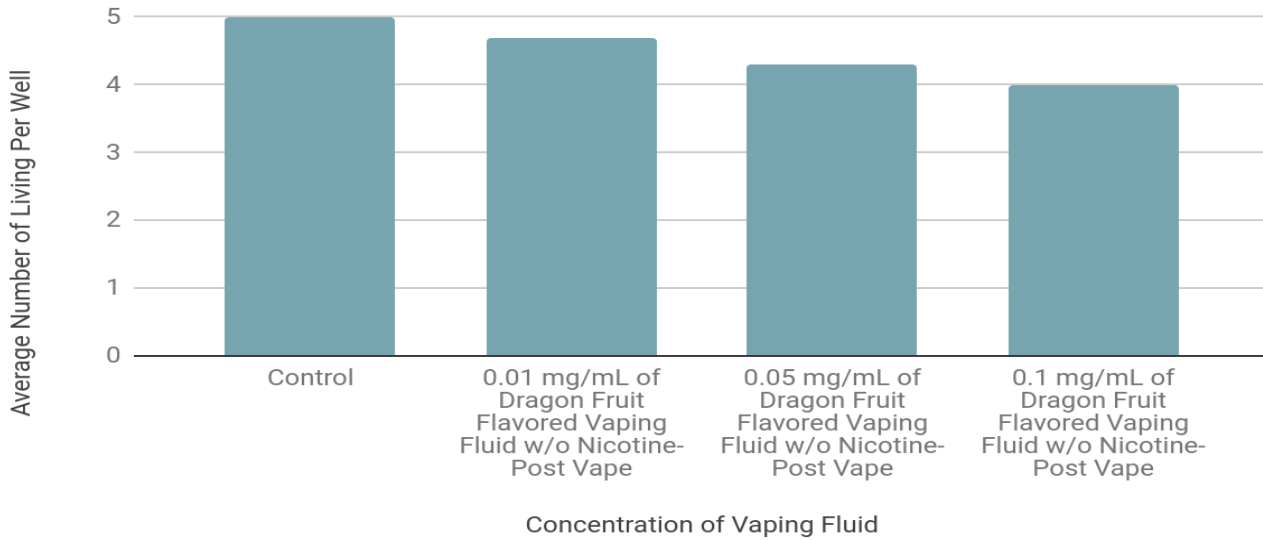
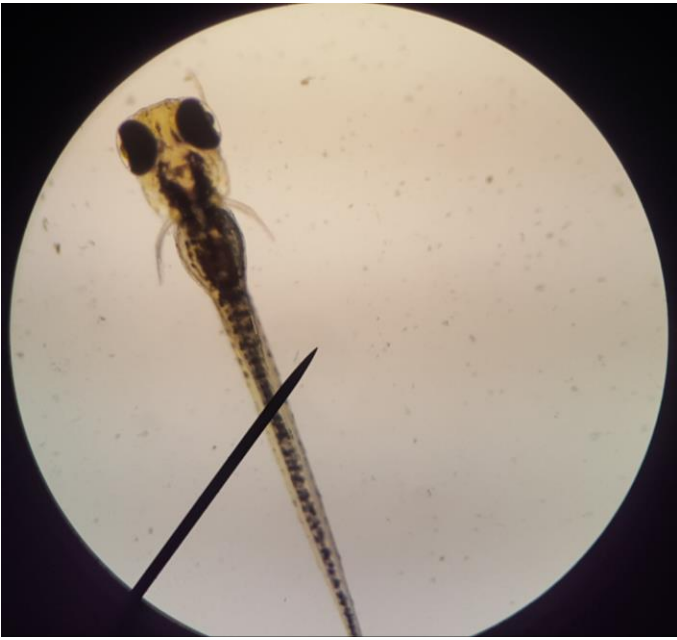


Figure 4:

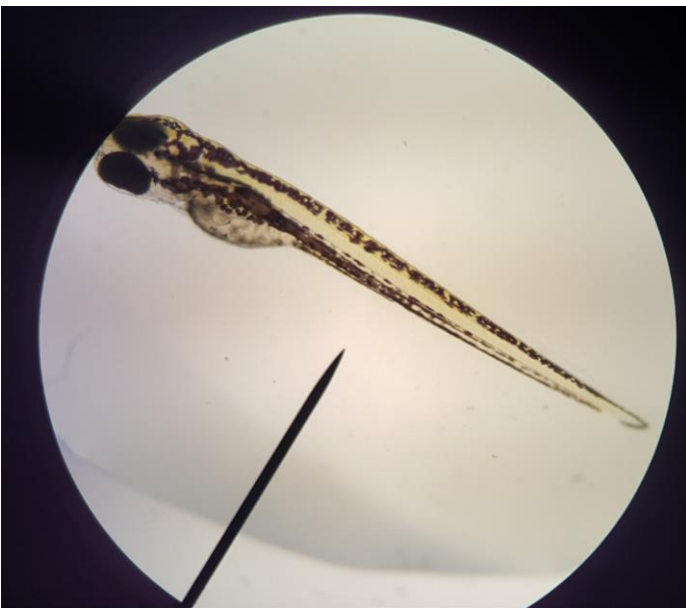
Treatment	Well 1	Well 2	Well 3	Average of Hatched	Probability	Result
Control	5	5	5	5	-	
0.01 mg/mL of Dragon Fruit Flavored Vaping Fluid w/o Nicotine-Post Vape	5	4	5	4.7	p= 0.3739	Non-statistically significant
0.05 mg/mL of Dragon Fruit Flavored Vaping Fluid w/o Nicotine-Post Vape	4	5	4	4.3	p= 0.1161	Non-statistically significant
0.1 mg/mL of Dragon Fruit Flavored Vaping Fluid w/o Nicotine-Post Vape	4	4	4	4.0	-	No t-test result available due to perfect data set

Figure 5:



This image above shows a fry from the control group that lived only in the embryo media (Instant Ocean). It has developed fins, pigment, and eyes. The pupils are forming in the eyes.

Figure 6:



Pictured here is a live fry from a medium concentration (0.05mg/mL) well. It has less pigment and eye development than the control zebrafish, but slightly more than the fry from the high concentration (0.1mg/mL) well.

Figure 7:



Pictured here is a hatched zebrafish from a well with a high concentration (0.1mg/mL) of vape fluid. Its pigment, eyes, and fins are all very underdeveloped. Compared to the control zebrafish (Figure 5), this organism's development is stunted.

**Note:** All three photos were taken on day 3 of the experiment.

### **DISCUSSION:**

In this experiment, zebrafish embryos were placed in several concentrations (0.01mg/mL, 0.05mg/mL, and 0.1mg/mL) of nicotine-free (flavored) vape fluid. This was to test their effect on the mortality and hatch rates of the zebrafish embryos in relation to how human embryos react to vape fluid. After reviewing the results of the experiment, the hypothesized negative effects on the mortality and hatch rates of zebrafish embryos in vape fluid was not supported by the data. The hypothesis was negated by the discovery that there was very little change to the mortality and hatch rates between the control and the different concentrations of vape fluid. The embryos in the wells all hatched at approximately the same time, and they died at approximately the same rate (see Figures 1-4).

While the quantitative data shows the hypothesis to not be supported by data, the qualitative data shows that the vape fluid did have an effect on the development of the zebrafish embryos (see Figures 5-7). For instance, as stated in the results section, there were signs of malformation in certain features of the zebrafish that increases as the concentration of vape fluid increases. If this experiment were to be repeated, it should look more into how nicotine-free flavored vape fluid affects the development of these certain features in zebrafish embryos. These experiments could help further answer some questions about the effect vape fluid has on the development of human embryos during pregnancy.

## REFERENCES:

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