



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

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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 malformities. Experiments like this can give better insights into how toxicants like vape fluid can affect a developing human embryo inside of pregnant women.

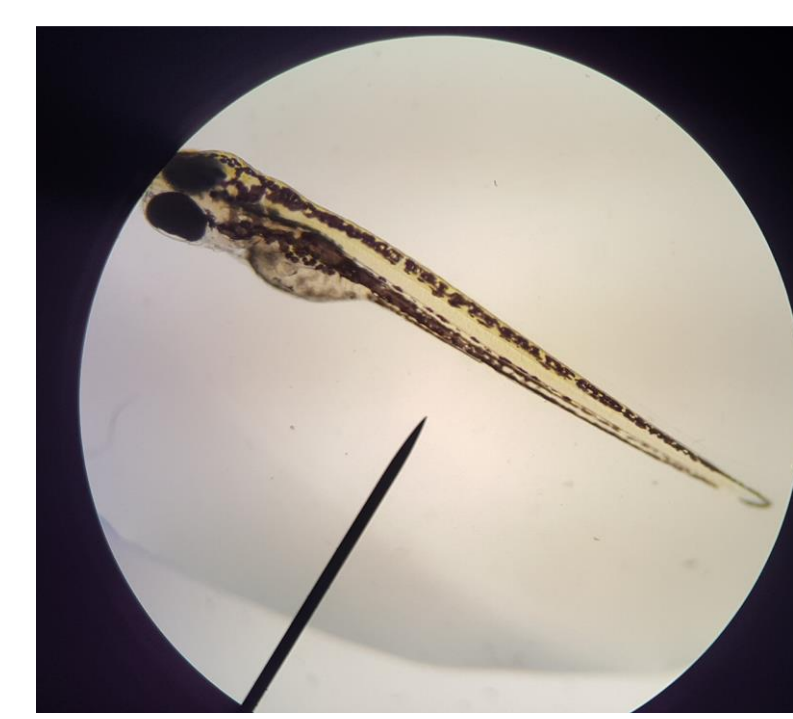
RESULTS: Besides the quantitative data, additional observations were made in this experiment. For instance, embryos in the medium to high concentrations had more significantly enlarged or reduced features and delayed neural responses. By the end of the experiment, the zebrafish from the higher concentrations had less pigment, delayed eye development, malformed/delayed fin development, and slower movement than the control zebrafish (Figure 5-7).

Figure 5:



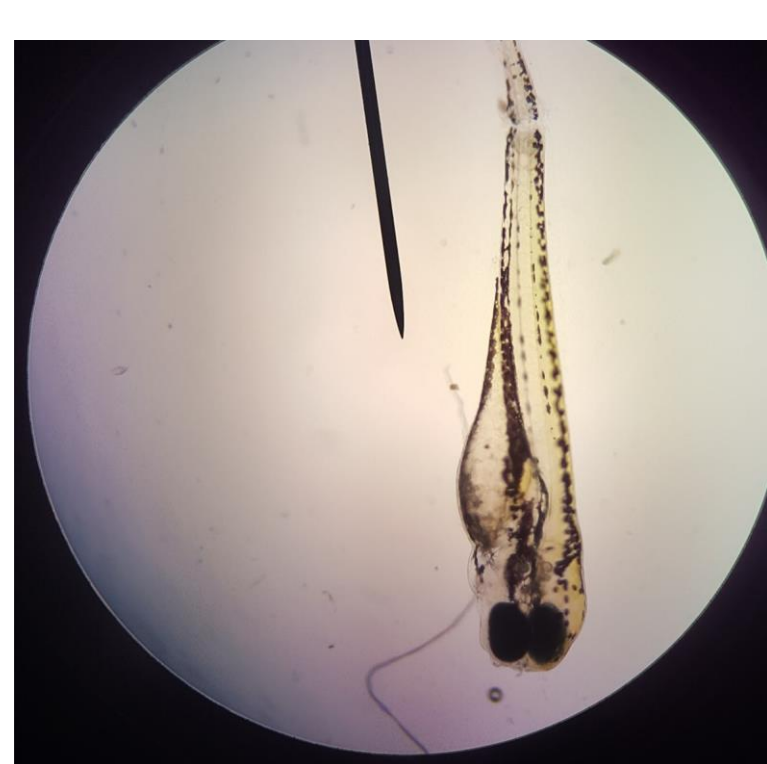
This image above shows a fry from the control group that has developed fins, pigment, and eyes. Pupils are forming in the eyes.

Figure 6:



Pictured here is a fry from a medium concentration (0.05 mg/mL) well. It has slightly less pigment and eye development than the high concentration fry.

Figure 7:



This fry comes from a well with a high concentration (0.1 mg/mL) of vape fluid. Its pigment, eyes, and fins are all very underdeveloped.

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

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. Each day of the experiment, which lasted four total days, after observations were taken (under a microscope), 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 were 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.

Figure 1:

Average Quantity of Hatched Zebrafish in Different Concentrations of Vaping Fluid

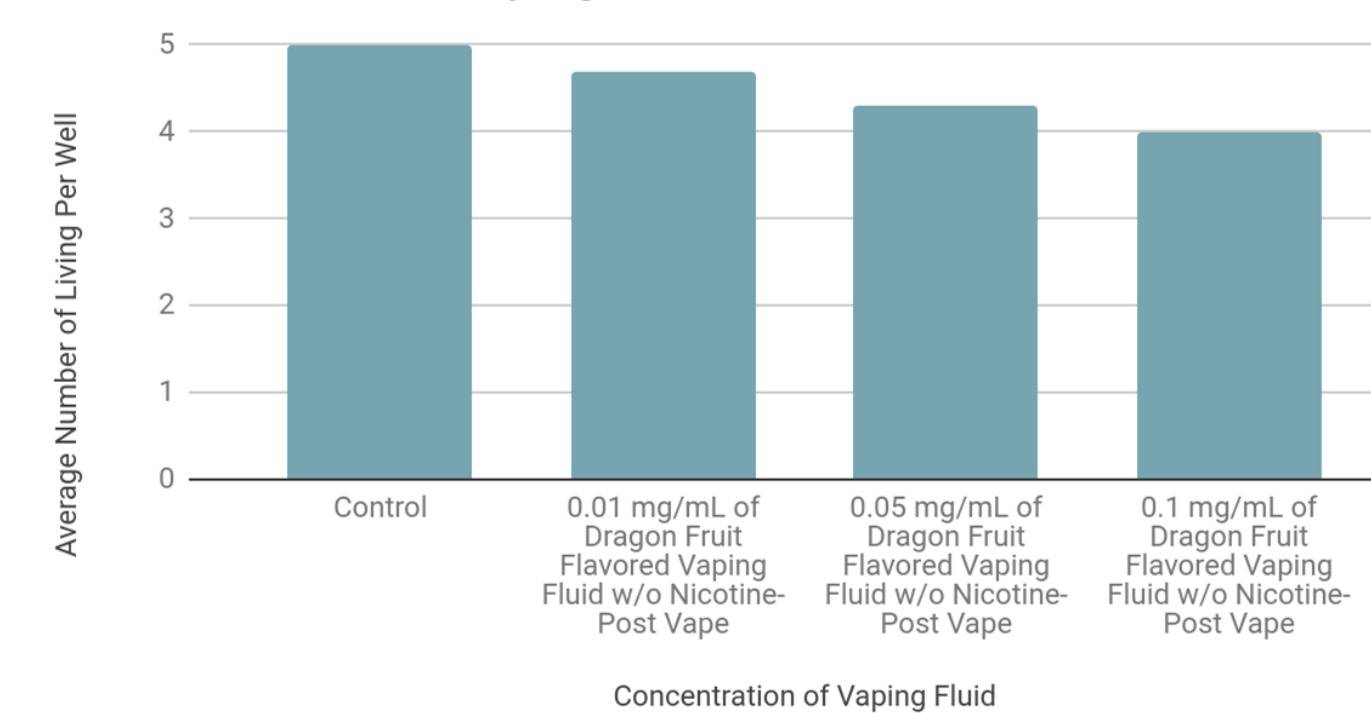


Figure 2:

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 Living Zebrafish in Different Concentrations of Vaping Fluid

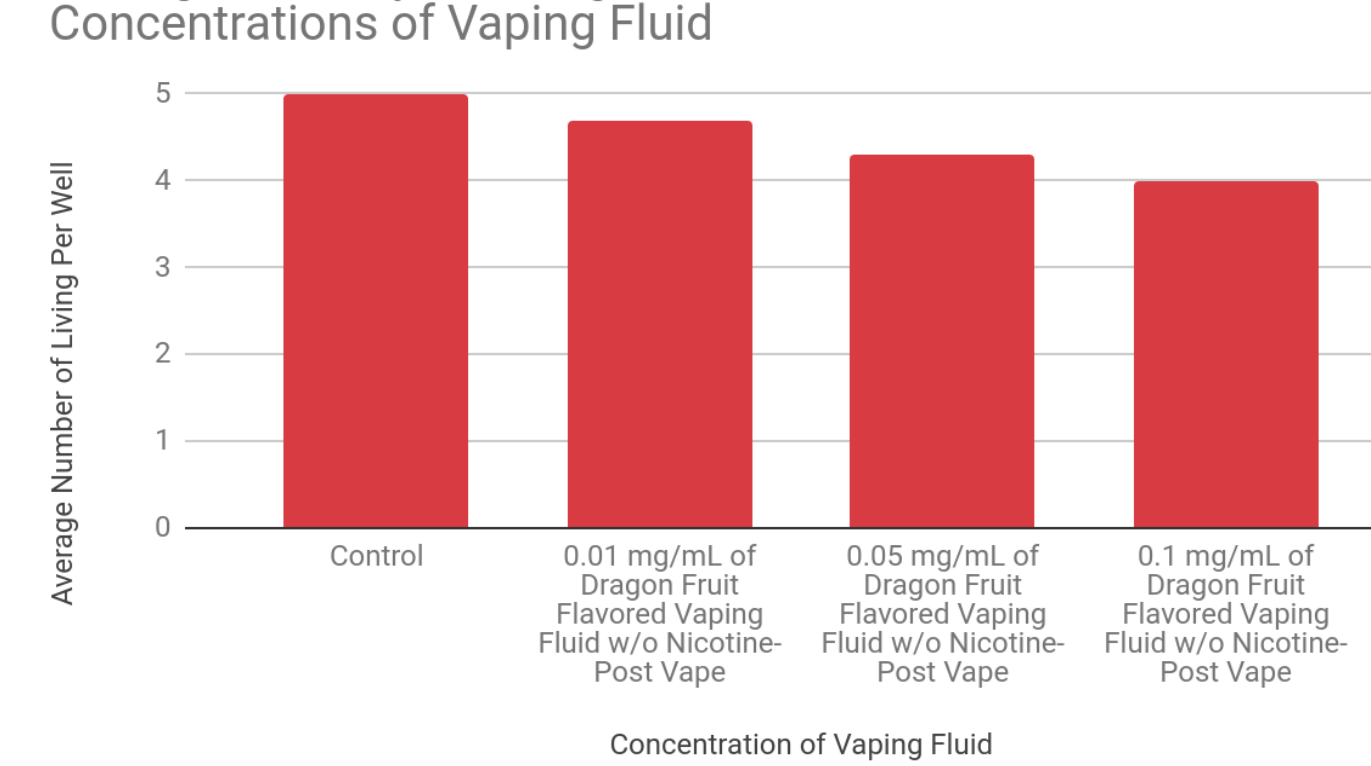


Figure 4:

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

DISCUSSION: 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 (see Figures 1-4).

However, 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, 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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Richter. (2018). "What Is Vaping?" *Center on Addiction - Addiction Science, Prevention & Treatment Research*, 1 Oct. 2018, <https://www.centeronaddiction.org/e-cigarettes/recreational-vaping/what-vaping>.

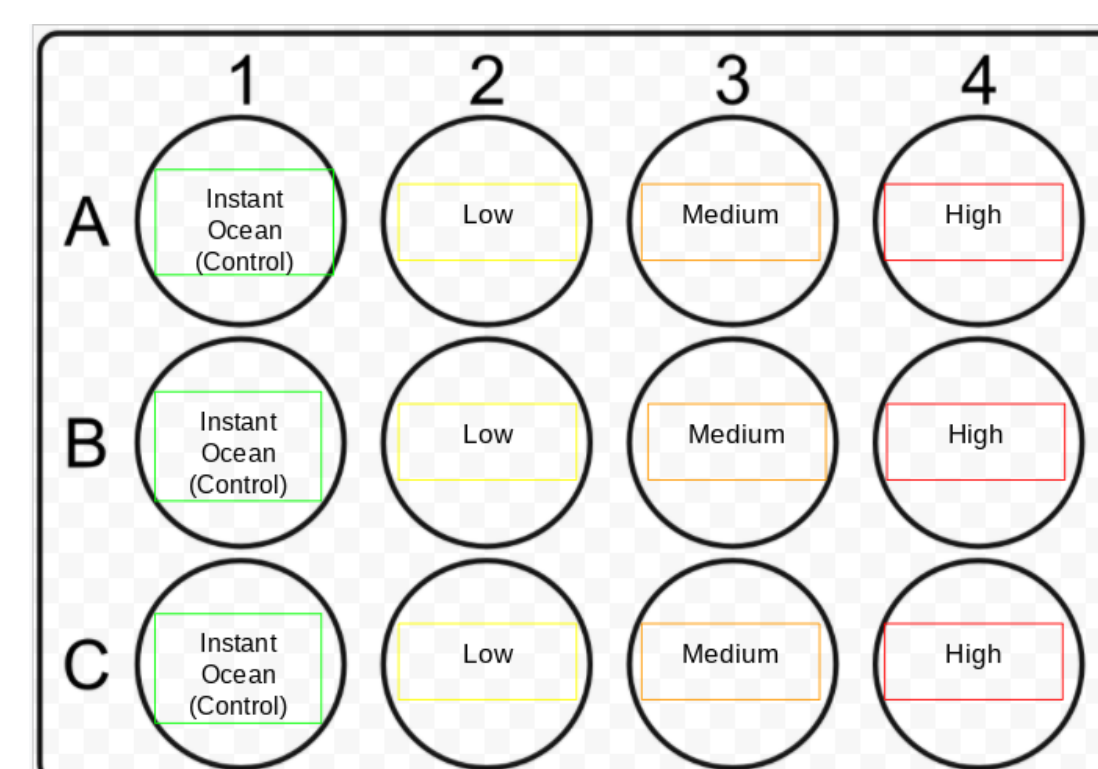
INTRODUCTION: In this experiment, the effects of flavored vaping fluid without nicotine content was tested on zebrafish embryos. Zebrafish (*Danio rerio*) are often used in human research due to a number of factors. For instance, they are used due to their large quantity, inexpensiveness, in sync development, transparency of the body, swift development, easily identifiable organs, and their development outside of the mother. Humans share 70% of their protein-coding 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. Many of these vapes contain over 200 different chemicals including highly addictive nicotine (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 focus of this experiment will be observing the effects of chemicals within non-nicotine e-cigarettes (independent variable) on the mortality and hatch rates (dependent variables) of zebrafish 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 the zebrafish embryos.

MATERIALS AND METHODS:

On day 0, the embryos were delivered from UW-Milwaukee Science Education Partnership Award Program, extracted from Instant Ocean, and then five embryos were placed into each well of a 12-well plate with fresh embryo media (Instant Ocean). The 12-well plate was set up as pictured on the right.



Concentration	Concentration of post-vape vape fluid
● Low	0.01 mg/mL
● Medium	0.05 mg/mL
● High	0.1 mg/mL

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.