

# TITLE: How Flavored Electronic Cigarette Juice Impacts Embryonic Stages of a Zebrafish

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**ABSTRACT:** *The purpose of this experiment was to show the effects of vanilla flavored e-cigarette juice had on zebrafish. This is important because generations of young people are now exposed to e-cigarette vaping chemicals daily. The procedure involved a well plate filled with different concentrations of chemicals. Observations were made every day, changes of the solution were made, and the number of hatched and living embryos was counted. In conclusion, the experiment results showed that any amount of vanilla oil affected the development of the zebrafish embryos. This experiment used different amounts of oil, in separated well plate spheres, with a control group without vanilla oil. All three groups had a low hatch rate within the first 24 hours. The control group was the only amount of fry to survive. Embryos, whether they are human or zebrafish, may face a terrible outcome when exposed to vanilla flavored e-cigarette juice.*

**INTRODUCTION:** Zebrafish are freshwater creatures that are very useful in experiments because they're extremely similar to humans. Zebrafish share 70% of human genes in their DNA. Zebrafish are very cheap and they also breed quickly: a female zebrafish can breed rapidly, producing over 200 embryos in a day.

E-cigarettes are a device that ejects vaporized nicotine that gives an effect that is 'supposed' to replace cigarettes and be less harmful to humans. The nicotine gets heated up which makes the user inhale the nicotine because it's now a vapor. The nicotine that's present is from the tobacco family, but it's not a part of the same plant that makes cigarettes. In the United States, about 10.8 million adults use e-cigarettes. E-cigarettes can cause type 2 diabetes, increased blood pressure, and increased heart rate (Ross, 2016). Previous studies show that vaping everyday can double your risk of a heart attack. Other studies show that it increases your cardiovascular risk more than cigarettes (Glantz, 2018).

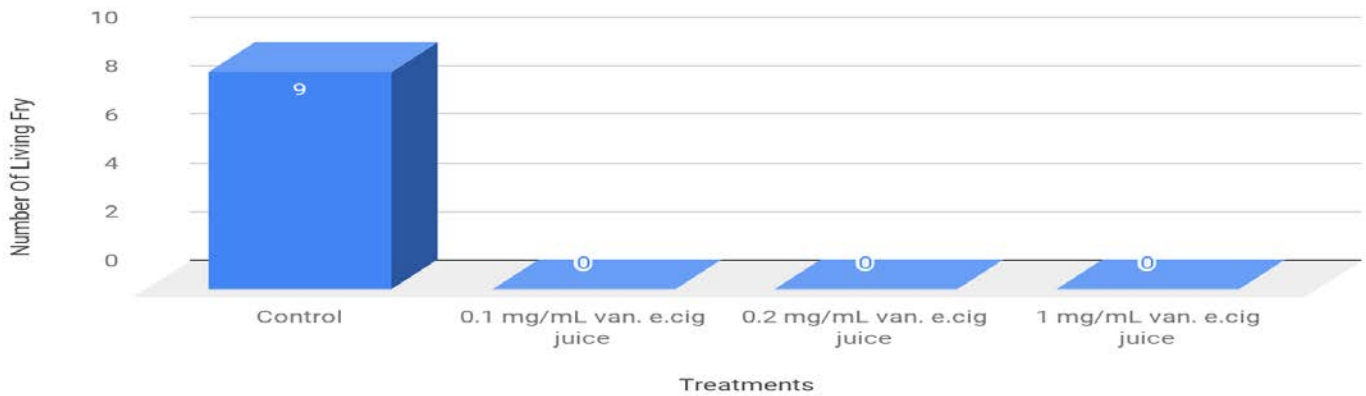
The prediction is that the zebrafish will sustain disorders that will not allow them to live up to their full potential. Based upon recent studies showing the harmful toxins within the chemicals can cause side effects (Ross, 2016). The independent variable is the chemical of vanilla flavoring in e-cigarettes. The dependent variables are the mortality rate and the hatch rate. In this experiment, the data that is being looked for is the significance of just the flavoring of vanilla from e-cigarettes to zebrafish, and not any additional nicotine. If the chemicals in e-cigarettes in the vanilla flavor are placed into the environment that *Danio rerio* live in, then the zebrafish will not undergo full development and full ability. This experiment pertains to environmental health.

**MATERIALS AND METHODS:** The materials needed for this experiment are: zebrafish embryos, embryos media solution, a microscope (both compound and stereo/dissecting), multiple pipettes, one bottle of 0.1 mg/mL, 0.2 mg/mL, and 1 mg/mL of vanilla flavoring without nicotine, goggles, gloves, a well plate, two beakers, depression slide with coverslip, a dry-erase marker, large bore pipettes, small bore pipettes, and a 28.5°C incubator. On December 10th, 2018 Mauston High School received *Danio rerio* from UW-Milwaukee's Science Education Partnership Award Program whom are sponsored by National Institutes of Health. Immediately, 10-15 embryos were placed into a 4 x 3 well plate. In A1, B1, and C1 embryo media solution filled those well plates. In A2, B2, and C2 0.1 mg/mL of vanilla was placed. In A3, B3, and C3 0.2 mg/mL of vanilla was filled. In well plates A4, B4, and C4 1 mg/mL of vanilla was placed. Before starting the experiment, gloves and goggles were worn through the whole process regarding the safety concerns. The safety concerns were that the vanilla substance would chemically burn human eyes and skin. On the first day observations were made and at the end of the day, the well plate was placed into an incubator overnight of 28.5°C. The next day observations were made under a dissecting/stereo microscope, a change of embryo media solution, and a change of all three of the solutions of vanilla happened with a pipette. The well plates were filled halfway with

their new filling. Embryos that were dead were placed into an extra beaker. After the dead embryos were removed, counting took place each day to determine the amount of how many zebrafish embryos were still living to compare the results. This procedure was repeated for the next three days. To compare results, the statistical t-test could have been used to compare the results of the hatch and mortality rate.

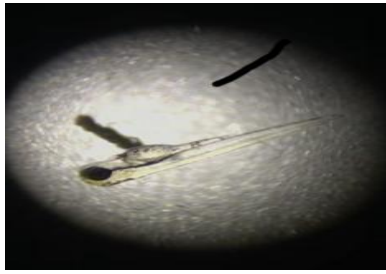
**RESULTS:** The results of this experiment showed that the hypothesis stated earlier was supported by the data collected. If the chemicals in e-cigarettes in the vanilla flavor are placed into the environment that *Danio rerio* live in, then the zebrafish will undergo full development and full ability. The reasoning for this experiment was to research the effects on zebrafish embryos by presenting them with vanilla chemicals. The independent variable is the vanilla chemical in e-cigarettes. The dependent variables are the morality and hatch rate. The embryos of the zebrafish that were living in the environment of vanilla flavoring of e-cigarettes all died. In all three divisions of the vanilla, 0.1 mg/mL, 0.2 mg/mL, and 1 mg/mL, no embryos survived to make it to the hatching stage.

**How Vanilla Flavored Electronic Cigarette Impacts the Lives of Zebrafish Embryos.**



Treatment -	Well 1 -	Well 2 -	Well 3 -	Average -	Probability -	Result -
Control	7	12	0	9	-	-
0.1 mg/ml van. e-juice.	0	0	0	0	p = 0.0030	Very Statistically Significant
0.2 mg/ml van. e-juice.	0	0	0	0	p = 0.0030	Very Statistically Significant
1 mg/ml van. e-juice.	0	0	0	0	p = 0.0030	Very Statistically Significant

**Table 1:** This data, and graph table, shows the final number of living fry in the end of the experiment. Comparing the controlled wells to the contaminated wells, it seems that large amounts of Vanilla Electronic Cigarette Juice had a fatal impact on the *Danio Rerio* living probability.



**Figure 1:** This photo displays the structure and anatomy of a zebrafish living in the control group.

**DISCUSSION:** The patterns that were shown in this experiment were that no zebrafish embryo could sustain life within the environment of vanilla chemicals. Thus, there is no data on hatch rate of zebrafish in the vanilla solution because no embryos proceed into the hatching stage. Embryos in the Embryo Media Solution remained alive and continued to grow. The results that were obtained supported the hypothesis and in this way it makes them statistically significant to the experiment. The significance of this experiment revealed the truth about harmful toxins produced in e-cigarettes. The results formed in this experiment gave the conclusion that toxins that are consumed by someone who vapes while pregnant will potentially harm the growth of an embryo. To gather further results on this trial, what can be done is to research the exact cause of death of the embryos within the vanilla solution. Possible errors conducted in this experiment could've been not using the exact amount of vanilla in each well plate which may have interfered with the results.

#### **REFERENCES:**

“First Evidence of Long-Term Health Damage from Ecigs: Smoking E-Cigarettes Daily Doubles Risk of Heart Attacks.” *Center for Tobacco Control Research and Education*, 24 Feb. 2018, [tobacco.ucsf.edu/first-evidence-long-term-health-damage-ecigs-smoking-e-cigarettes-daily-doubles-risk-heart-attacks](http://tobacco.ucsf.edu/first-evidence-long-term-health-damage-ecigs-smoking-e-cigarettes-daily-doubles-risk-heart-attacks).

Ross, John. “E-Cigarettes: Good News, Bad News.” *Harvard Health Blog*, Harvard Health Publishing, 8 July 2016, [www.health.harvard.edu/blog/electronic-cigarettes-good-news-bad-news-2016072510010](http://www.health.harvard.edu/blog/electronic-cigarettes-good-news-bad-news-2016072510010).

“Where Does Vaping Nicotine Come From?” *South Beach Smoke Electronic Cigarettes*, 26 Mar. 2017, [www.southbeachsmoke.com/blog/where-does-vaping-nicotine-come-from.html](http://www.southbeachsmoke.com/blog/where-does-vaping-nicotine-come-from.html).

