

# The Effect of Vanilla-Flavored E-Cigarette Juice on Zebrafish Embryos

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**ABSTRACT:** E-cigarettes are commonly used by people who are trying to quit smoking. Many people, along with pregnant women, may think that e-cigarettes are the healthier choice because they do not contain nicotine. However, an experiment that was done on zebrafish tell a different story. In the experiment, there were 109 zebrafish that were separated into twelve different well plates, which were separated into four groups of three. The first grouping was the Control Group, which had embryo media solution in it, and the proceeding groups had e-cigarette juice within the water, rising quantitatively in solution from left to right. When the experiment commenced, only 40 zebrafish remained. That means only about 38% of the total zebrafish lived. Therefore, it can be deduced that e-cigarette chemicals, even without nicotine, can impair development and even kill developing human embryos.

**INTRODUCTION:** Zebrafish, also known by their Latin name *Danio rerio*, have been able to show significant insight into the early development of human beings. Zebrafish are freshwater organisms, commonly found in slow streams in the Ganges River in East India and Burma (Dougan, 2015). Most emphatic is the fact that zebrafish share nearly 70% of the genes found in humans.

The toxicant that was used in this experiment was e-cigarette chemicals without nicotine. Although vaping is typically marketed toward smokers who are looking to quit, a recent surge in popularity caused these harmful e-cigarettes to fall into the hands of teenagers. Even though they don't contain nicotine, they are still harmful.

This experiment tested the effects of chemicals in e-cigarettes without nicotine on zebrafish embryos. The independent variable in this experiment was the chemicals in e-cigarettes, such as propylene glycol (95% of the e-cigarette juices) and vegetable glycerine. Scientists still do not know how dangerous these chemicals are when vaporized and inhaled into the lungs. The dependent variable was the hatch rate and the mortality rate of the organisms, and the control variable was the untreated embryos in the embryo media solution. The parts and anatomy of the zebrafish that this experiment tested were the brain and the yolk. Therefore, it can be assumed that damage will be done to the fore, middle, and hindbrain, which will usher in the inability to function quickly and effectively. In addition, it is hypothesized that the yolk will become defective and will cause the zebrafish to lose protein.



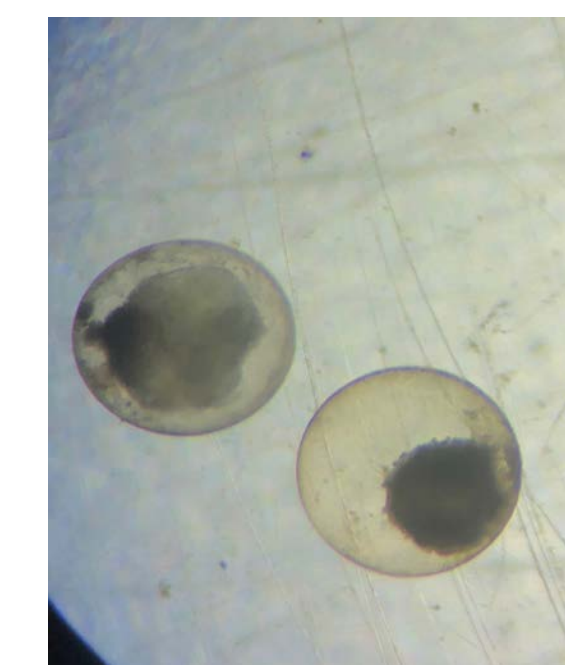
**MATERIALS AND METHODS:** The materials we used for this experiment include a 12 well plates, 28.5 degree incubator, one compound microscope, disposable pipettes, disposable gloves, one beaker for dead embryos and liquid disposal, another beaker for clean embryo media solution, one bottle of chemical solution, depression slides, Dissecting/stereo microscopes, and dry-erase markers for labeling. These materials were presented to the Mauston High School by UW-Milwaukee's Science Education Partnership award (SEPA).

The well plate, mentioned before, consisted of three control wells with embryo media solution in Column 1 and vanilla-flavored e-cigarette juice (nicotine free) in Column 2, 3 and 4. The concentration placed in Column 2 was 0.1 mL/mg of vanilla-flavored e-cigarette juice; the concentration in Column 3 was 0.2mL/mg, and the concentration placed in Column 4 was 1 mL/mg. Each day, one zebrafish from the control Column would be sucked out using a disposable pipet and observed under a compound microscope. After a picture was taken of the zebrafish that was not treated with any chemicals, another zebrafish would be sucked out, using a different pipet, from one of the columns that had e-cigarette chemicals in it. Afterwards, it would be observed under the microscope and be photographed. Next, it would be compared to the zebrafish that was in the embryo media solution. In order to describe if the results of the experiment were statistically significant, an online calculator, GraphPad QuickCalcs was used.

## RESULTS:



This is a zebrafish embryo from the *Control Column* on the second day of the experiment. As can be seen, there is no pigment, therefore it is healthy.



This is a dead zebrafish embryo that was affected by 1 mL/mg of vanilla-flavored e-cigarette juice. These two embryos are clearly unhealthy as can be seen by their pigmentation.

Vanilla	Well 1	Well 2	Well 3	Average	Probability	Result
Control	3	6	8	5.7	-	-
0.1mg/mL	6	4	5	5.0	p = 0.6918	Not statistically significant
0.2mg/mL	0	0	0	0.0	p = 0.0175	Statistically significant
1 mg/mL	0	0	0	0.0	p = 0.0175	Statistically significant

This table displays the analytics of the experiment, while denoting the statistical significance of how the living zebrafish were affected by the vanilla-flavored e-cigarette juice.

Vanilla	Well 1	Well 2	Well 3	Average	Probability	Result
Control	3	5	2	3.3	-	-
0.1mg/mL	0	0	0	0.0	p = 0.0194	Statistically significant
0.2mg/mL	0	0	0	0.0	p = 0.0194	Statistically significant
1 mg/mL	0	0	0	0.0	p = 0.0194	Statistically significant

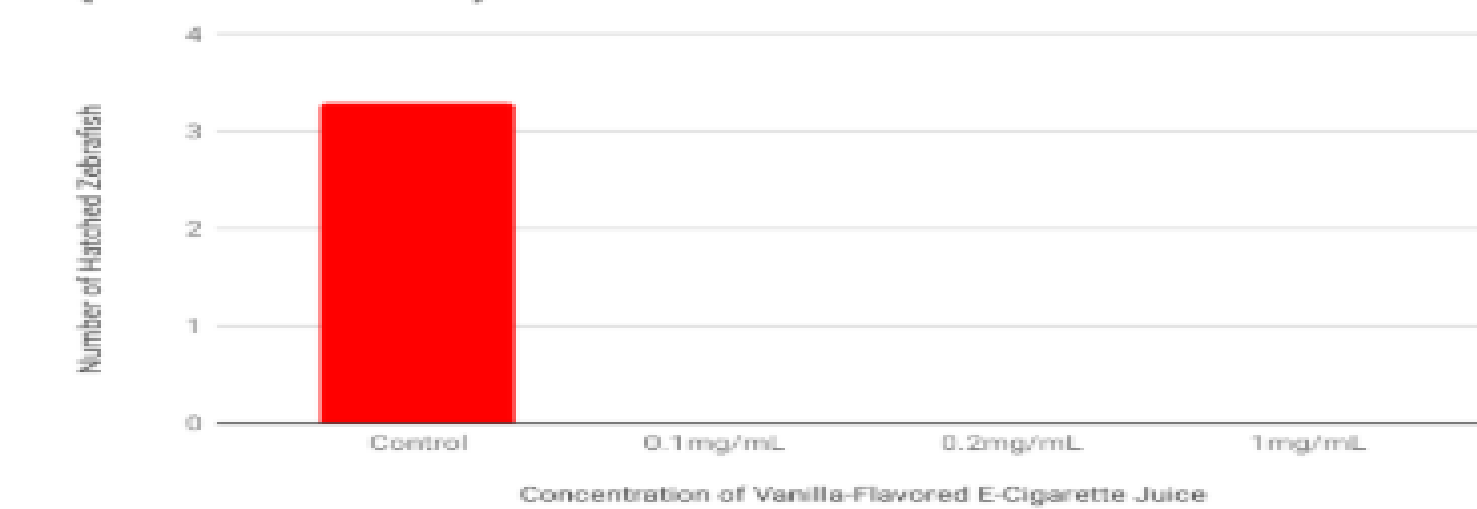
This table displays the analytics of the experiment, while denoting the statistical significance of how the hatched zebrafish were affected by the vanilla-flavored e-cigarette juice.

The Effects of Vanilla-Flavored E-Cigarette Juice (Nicotine-Free) on Living Zebrafish



This bar graph shows the final number of living zebrafish, not in relation to their hatch rate, after the experiment was finished.

Effects of Vanilla-Flavored E-Cigarette Juice (Nicotine Free) on Hatched Zebrafish



This bar graph shows the final number of living zebrafish, in relation to their hatch rate, after the experiment was finished.

**DISCUSSION:** Since the yolk sac of the zebrafish that were influenced by the e-cigarette chemicals could not be seen because of how negative the effects of e-cigarettes are (most of the zebrafish died), the original hypothesis cannot be directly negated nor directly validated. Contrary to popular belief, the chemicals in e-cigarettes are not helpful when one is trying to quit smoking normal cigarettes. Instead, a person struggling with addiction might be ingesting a chemicals such as propylene glycol and vegetable glycerine. The latter is not inherently bad, for it is found in many healthy foods, such as vegetables, but when it is vaporized and ingested into the lungs, there is no telling what could happen. Based on this experiment, it can be assumed that these chemicals, even though they are not inherently harmful, can become very harmful when it is ingested.