

## **TITLE: The Effect of Mint Vape Juice with Nicotine on the Mortality and Hatch Rate of Zebrafish Embryos**

**NAMES OF RESEARCHERS: Kendall Elizabeth Laridaen**

**ABSTRACT:** *This experiment was conducted to measure the effects of mint vape juice with nicotine on developing zebrafish embryos. A 12-well plate was used and 10 zebrafish embryos were placed in each well. Three wells were the control, three more rows each contained concentrations of 0.1 mg/mL, 0.2 mg/mL, and 1.0 mg/mL. The data showed very statistically significant evidence that embryo development was affected negatively by the vaping juice. This experiment was important to collect data to show that vaping juice does have negative effect on developing embryos, which may show a related negative impact on human health.*

### **INTRODUCTION:**

**Zebrafish:** Zebrafish (*Danio rerio*) were first used in the 1990/2000 for experimental purposes. They were first used by George Streisinger at The University of Oregon. Fish are more of a useful experimental organism compared to mice because they lay 200 eggs a week and develop outside of the mother's body, unlike mice. Mice have 15 pups a liter every 21 days. A big boost in the use of zebrafish came from the research of mutations to understand the role of genes in normal development. This experiment was led by Christiane Nüsslein-Volhard and Wolfgang Driever in 1995 (Your Genome, 2016).



**Nicotine (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>):** is an addictive substance that can act as a stimulant and sedative to the body. The toxic, colorless or yellowish oily liquid acts as a stimulant in small doses. If nicotine is consumed or inhaled in large doses it can block the actions of the autonomic nerves and skeletal muscle cells. Possible side effects from nicotine are lightheadedness, dizziness, disrupted sleep, heartburn, dry mouth, nausea, increase of blood pressure, problems with brain development, spasms in lungs and joint pain. (Felman, 2018) The chemicals in vape juice include propylene glycol (used to maintain moisture in food) and vegetable glycerin (used as a sweetener) along with nicotine are the main chemical in e-cigarette is vape juice. According to Robert Strongin, that solvent is “like the elephant in the room”. Although the chemicals may seem safe alone, together they are utterly different and are very harmful to the body.

**Hypothesis:** This experiment will be exposing zebrafish embryos to chemicals of mint e-cigarettes. The experiment is an impact on environmental health. The independent variable in this experiment is mint nicotine of three different concentrations such as 0.1 mg/mL, 0.2 mg/mL, and 1.0 mg/mL. The dependent variable that will be measured in this experiment is the hatch rate and the mortality rate of the developing zebrafish embryos. This could cause the developing zebrafish embryos to have a delayed hatch rate or could cause there to be a zero percent mortality rate. If the developing zebrafish embryos are exposed to mint vape juice with nicotine in different concentrations, then it will affect their embryonic development and mortality rate.

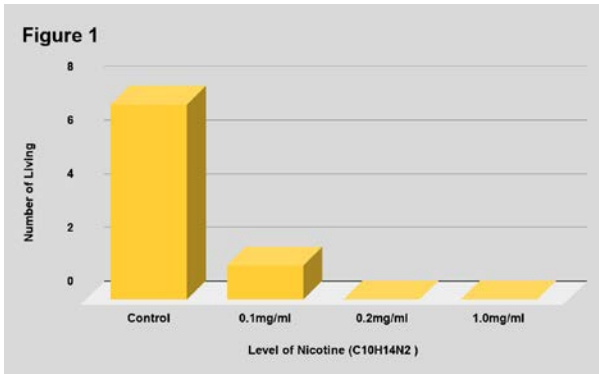
**MATERIALS AND METHODS:** For the experiment on the zebrafish embryos, the following materials were used, chemical solutions, one beaker for dead embryos, one beaker for clean embryos, disposable pipettes, large bore pipettes for transferring eggs to observation container and manipulating them in the container, small bore disposable pipettes for daily solution changes, 12-well plate, 28.5 incubator, depression slides with coverslips, dissecting/stereo microscope, compound microscope, goggles, and gloves. Goggles and gloves were used for safety reasons because chemicals were used in the experiment. The embryos were delivered from UW-Milwaukee Science Education Partnership Award Program which is sponsored by the National Institutes of health. The first step in the experiment was to fill the 12-well plate with several mL of embryo 'Instant Ocean' solution, and then separated the embryos to put 10 in each of the well plates. In this experiment data was recorded on the student's data sheets each day. The embryos were observed under the dissecting microscope each day. After observing the embryos, they were placed in 28.5 C incubator overnight. After the experiment each day, the students cleaned the lab station and make sure everything was ready for the next group of students. Those steps were re-done for each day of this experiment. The teacher then took care of the embryos properly when the experiment was finished and all of the data was recorded.

**RESULTS:** The information was gathered to see how nicotine affects the hatch rate, embryonic development of zebrafish, and mortality rate. The experiment was conducted to see the possible effects on human embryo development. The hypothesis in the zebrafish experiment is that if zebrafish are exposed to mint vape juice with nicotine in different concentrations, then it will affect their embryonic development and morality rate. The independent variable of the experiment was the different amounts of mint vape juice (nicotine). The dependent variable was the number of live fry and hatched embryos at the end of the experiment. The hypothesis in this experiment was correct. All zebrafish in the 0.2mg/mL and 1.0mg/mL mint nicotine vape juice died by the end of the experiment. There was a small amount of zebrafish embryos alive at the end of the experiment in the 0.1mg/mL mint nicotine vape juice and a fair number still alive in the control. The hatch rate and live fry number may have been affected by condensation from the incubator causing nicotine to get into the control.

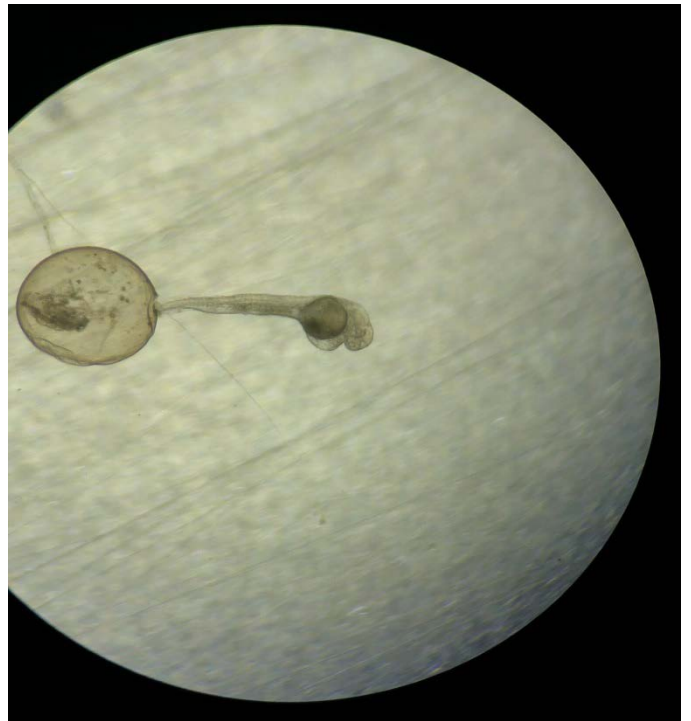
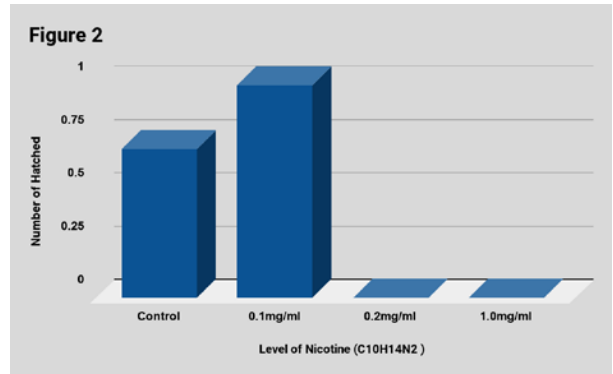
Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	6	9	7	7.3	-	-
0.1mg/ml	2	1	1	1.3	p =0.0031	very statistically significant.
0.2mg/ml	0	0	0	0.0	p =0.0011	very statistically significant.
1.0mg/ml	0	0	0	0.0	p =0.0011	very statistically significant.

Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	0	1	1	0.7	-	-
0.1mg/ml	1	0	2	1.0	p = 0.6433	not statistically significant.
0.2mg/ml	0	0	0	0.0	p = 0.1161	not statistically significant.
1.0mg/ml	0	0	0	0.0	p =0.1161	not statistically significant.

### Final # live fry



### Final # hatched embryos



**DISCUSSION:** This experiment was hypothesized to negatively affect development, mortality rate and hatch rate of zebrafish embryos in three different concentrations of mint vape juice with nicotine. The hypothesis was supported by the data that was collected through the experiment. The highest concentrations had the biggest effect on the embryos. The concentrations of 1.0 mg/mL and 0.2 mg/mL killed all 10 zebrafish embryos in each of the wells. The remaining concentration of 0.1 mg/mL caused deformities in the hatched and unhatched embryos. This proves that mint vape juice with nicotine is potentially harmful to human embryos.

## **REFERENCES:**

Becker, Rachel. "Vape Flavors Can Make Nasty New Chemicals in Your e-Liquid." *The Verge*, The Verge, 18 Oct. 2018, [www.theverge.com/2018/10/18/17996706/vape-juice-flavoring-electronic-cigarettes-chemicals-ingredients-irritating-e-liquid](http://www.theverge.com/2018/10/18/17996706/vape-juice-flavoring-electronic-cigarettes-chemicals-ingredients-irritating-e-liquid).

Felman, Adam. "Nicotine: Facts, Effects, and Addiction." *Medical News Today*, MediLexicon International, 11 Jan. 2018, [www.medicalnewstoday.com/articles/240820.php](http://www.medicalnewstoday.com/articles/240820.php).

Your Genome. "Tiny Fish, Big Splash: the Story of the Zebrafish." *Stories*, The Public Engagement Team at the Wellcome Genome Campus, 13 June 2016, [www.yourgenome.org/stories/tiny-fish-big-splash-the-story-of-the-zebrafish](http://www.yourgenome.org/stories/tiny-fish-big-splash-the-story-of-the-zebrafish).