

The Effect of Vape Juice on Zebrafish Development

Ariana Refinski, Emilie Rochon, Ella Milsted Muskego High School



Abstract

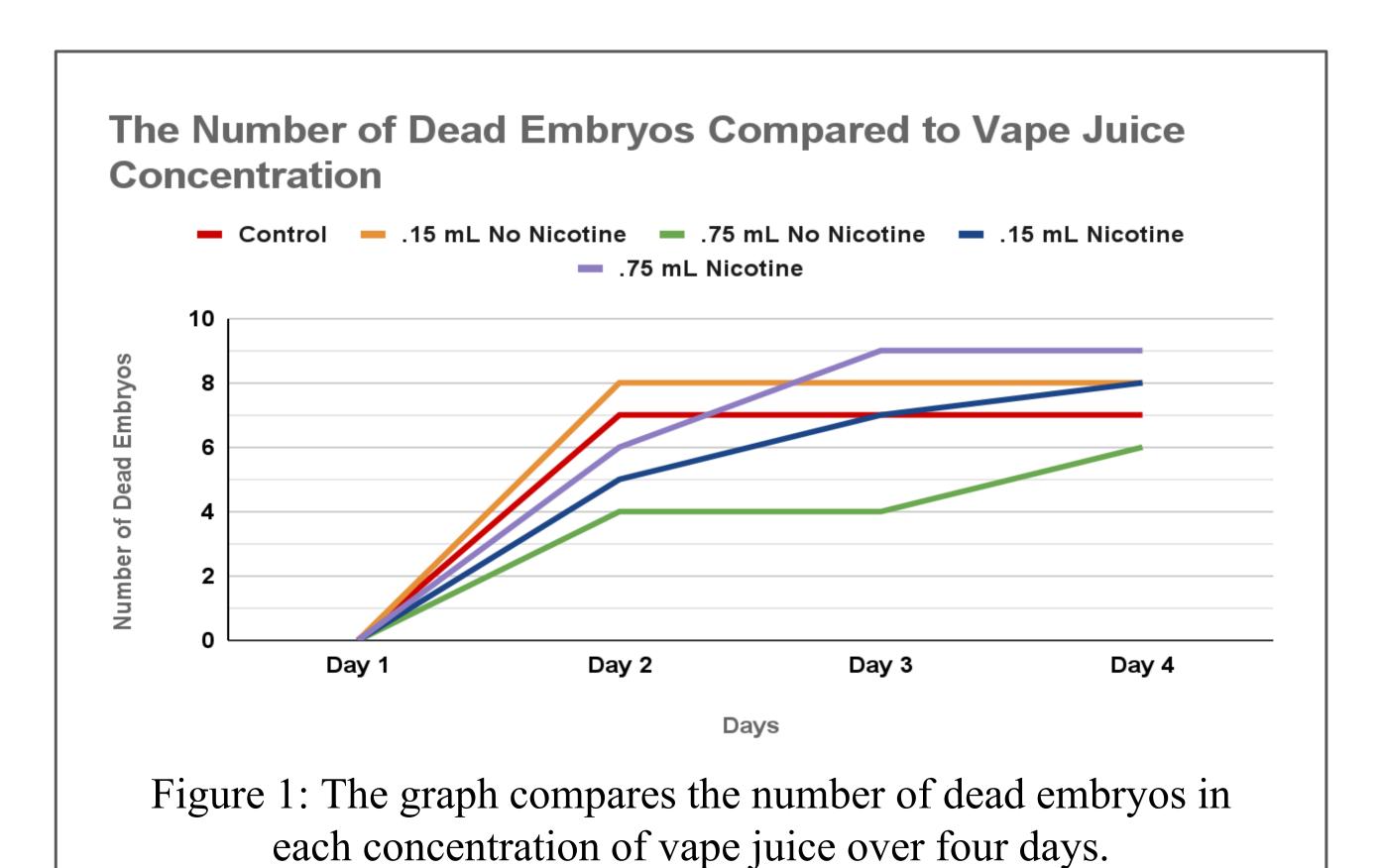
In the past decade, the use of e-cigarettes has become more popular compared to the use of traditional cigarettes because e-cigarettes are viewed as being a "healthier" alternative. However, recent studies show that the chemicals inside of the vape juice used in e-cigarettes are harmful to humans and are projected to cause developmental issues. In this experiment, zebrafish embryos were exposed to *Hell's Vapor* cappuccino vape juice in order to understand how this solution would affect fetal development. This experiment indicated that the presence of vape juice causes developmental abnormalities.

Introduction

Smoking is a common practice throughout the world. While the popularity of smoking regular cigarettes is in decline, the popularity of e-cigarettes is taking over the world. E-cigarettes have one main difference from regular cigarettes: the presence of "e-juice." E-juice is made up of many known ingredients such as nicotine, vegetable glycerin base, diacetyl, formaldehyde, acrolein, and propylene glycol; however, there are also many unknown ingredients (4). The flavoring additives in e-juice are where possible health problems arise; flavorings, such as cinnamaldehyde, are only meant for oral ingestion and not for inhaling (1). With the increased accessibility to nicotine-containing products, it is essential to be aware of the effects that this substance has on humans, especially those that are still developing. In previous studies, higher concentrations of nicotine found in vape juice causes higher mortality rates in zebrafish embryos (3). Exposure to nicotine also slows motor neuron development as zebrafish embryos grow and develop (2). It is hypothesized that when zebrafish embryos are exposed to various concentrations of e-juice during the early stages of development, those that are subject to higher concentrations will exhibit lethal and sublethal effects such as underdeveloped body sizes, paralysis, and heart problems.

Materials and Methods

Two Falcon dishes were obtained and filled with 3 mL of embryo media and a total of five different concentrations of *Hell's Vapor* cappuccino vape juice (a control of an embryo media solution, .15 mL no nicotine, .75 mL no nicotine, .15 mL nicotine, and .75 mL nicotine). Each solution was placed into 3 wells and 5 zebrafish embryos were added. The embryos were incubated at 28°C for four days. Each day, the Falcon dishes were removed from the incubator and observed underneath a microscope. Each day, quantitative (number of dead embryos) and qualitative (developmental abnormalities) data was collected and recorded, any dead embryos were removed using a pipette, the old media was disposed of using a pipette, and 3 mL of new media was placed in each well. At the end of the experiment, a Fisher test was done to compare the amount of dead and alive embryos.



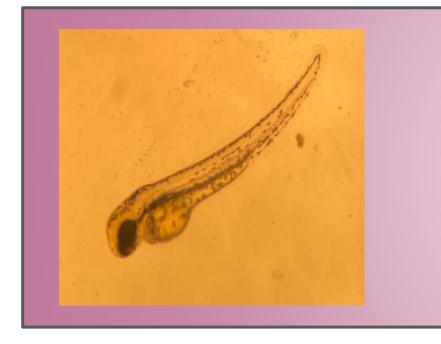


Figure 2: A zebrafish on day three of development in the control solution. No developmental abnormalities are present and normal development is occurring.

Figure 3: A zebrafish embryo on day four of development in the .15 mL, no nicotine vape juice solution. This zebrafish lacks coloration on its body.





Figure 4: A zebrafish embryo on day four of development in the .75 mL, no nicotine vape juice solution. This zebrafish has developed a large cardiac edema.

Results

The independent variable for this experiment was the concentration of vape juice (0 mL, .15 mL, .75 mL). The dependent variable was the developmental characteristics of the zebrafish. The control was the solution of solely embryo media. A Fisher test was done in order to compare the number of dead and alive embryos in the control solution and four different e-juice concentrations. The two-tailed P values were 0.7152 for the 0.75 mL nicotine concentration and 1.0000 for the other three concentrations. The Fisher test proved that there is no significant difference between the mortality rate of embryos in the five solutions because the P-values were greater than .05; therefore, we fail to reject the null hypothesis, which states that different concentrations of e-juice will have no effect on the embryonic development of zebrafish.

Discussion

The purpose of the Fisher test is to compare the proportions of dead and alive embryos in the control solution and the four e-juice solutions. The results of the Fisher test stated that there is no significant difference between the mortality rate in the five solutions. Although developmental issues, such as cardiac edemas and irregular shaped yolks, were commonly seen in the zebrafish exposed to .75 mL of vape juice, there is no statistically significant difference between the developmental health of embryos in the control solution versus the vape juice solutions. Some limitations would be the use of only one brand of vape juice and only having three concentrations of vape juice. Nevertheless, the presence of these large cardiac edemas is alarming. Cardiac edemas were commonly seen in the .75 mL, no nicotine vape solution, which indicates that nicotine isn't the only cause of health issues in e-cigarettes. It can be concluded from these observations that the presence of toxic chemicals in vape juice can affect the development of zebrafish, and likewise humans.

References

- 1. Jaspers, Ilona. "E-Cigarettes and Teen Health." *National Institute of Environmental Health Sciences*, U.S. Department of Health and Human Services, 14 Sept. 2018.
- 2. Subbarao, Tara. *Effects of Nicotine on Embryological Neural Development*. June 2006.
- Thayer, Anthony. Cherry Electronic Cigarette Flavoring With Nicotine and Its Effects on Zebrafish Embryo Development. SEPA Online Journal, 2019.
- 4. "What's in an E-Cigarette?" *American Lung Association*.