The Effect of Vaping Fluid Without Nicotine on the Mortality and Hatching of Zebrafish Embryos

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ABSTRACT: *The purpose of this experiment was to show the effects of vaping fluid without nicotine on developing zebrafish embryos. This experiment is important because it relates closely to how vaping during pregnancy can affect a developing fetus, or, how vaping affects a person's developing lungs, immune system, heart and more. During the experiment, the developing zebrafish embryos were examined underneath a microscope each day. The amount of hatched and living zebrafish were counted, these numbers were averaged out and used to show the overall results of this experiment. A common result from each of the experimental wells include deterioration of the embryos in the higher doses of vaping fluid, as well as the control wells having a consistent but low rate of live embryos that were thriving.*

INTRODUCTION:

Zebrafish are a species of freshwater fish native to South Asia. This type of fish is effective to use in scientific research, experiments and studies given the fact that the adult fish have an average length of 3 centimeters making the adult fish easy to work with and the tiny embryos are ideal to study in small spaces such as well plates. Zebrafish reproduce at a rapid pace, generating about 200 progeny per female (Burgess, n.d.). With this, zebrafish also have a fast paced developmental cycle. They are also cheaper to house than other animals, such as rodents, birds, and rabbits. In comparison, these zebrafish can be tested on the toxicity or adverse effects from drugs in only a five days span (Burk, 2016). In this experiment, the effects of various dosages of vaping fluid without nicotine on the zebrafish embryos were studied. This toxicant is a dangerous substance as it might cause embryos to experience heart damage and incomplete brain growth during the embryo's developmental stages. Many deformities can form once the embryo is hatched or even beforehand (Orzabal, et al. 2019). From previous research and data, it has been concluded that zebrafish embryos are vital and an excellent model to use for learning the basic principles of vertebrate development (Teame, et al. 2019). Throughout the experiment, the questioning factors of the survival probability and the process of development will be studied. Will the vaping fluid toxicant affect the developing embryos before they can hatch? Likewise, will the vaping fluid toxicant affect the zebrafish embryo hatching rate? The hypothesis in this experiment was that if the embryos receive a constant dosage of the vaping fluid daily, they will deteriorate and reduce the production of the embryo cells. The independent variable was vaping fluid without nicotine. The dependent variable was the heart. The control was the clean embryo media solution, and the variables that were held constant were the dosages given, the amount of time outside of the incubator.

MATERIALS AND METHODS:

In this experiment, zebrafish embryos were used. These embryos were obtained from UW-Milwaukee's Science Education Partnership Award Program. This program is sponsored by the National Institutes of Health. In order for this particular research to be conducted, many different materials were used such as a 12 well-plate, disposable large and small pipettes, a microscope, two 250mL beakers, developing zebrafish embryos, an incubator at a constant temperature of 28.5 degrees celsius, as well as the vaping fluid with no nicotine. The 12 well plate was used to house the developing zebrafish embryos. Each was given a different amount of vaping fluid. These dosages consisted of 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL, distributed in each of the wells. This was a significant process in the experiment because it compared and contrasted the effects of the fluid on the embryos, determining the pace and the production while the embryos undergo crucial development. Included in the 12 well plate, 3 columns of wells were given the various dosages, while one column of wells was used as the control, meaning no factors of this well were changed throughout this experiment. Over the course of the 4 inclusive days, the vaping fluid mixture was changed out of each individual well. The embryo media solution was the main substance in the control wells, changed everyday similarly to the experimental wells. Everyday, the well plate of developing embryos was taken out of the incubator for twenty five minutes, and then placed back in. This procedure helped ensure that the zebrafish were able to spend enough time developing in the incubator. To calculate the results of this experiment, a statistical t-test was used. This calculated the probability of the experiment being statistically significant or not.

REFERENCES:

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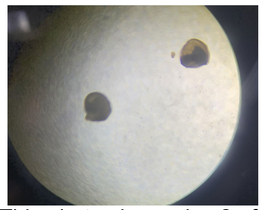
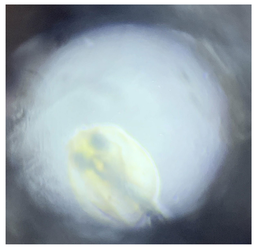
RESULTS:

Photo 1



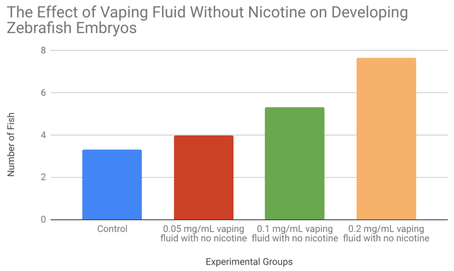
This photo shows day 2 of the experiment in one of the control wells. It depicts two deceased embryos.

Photo 2



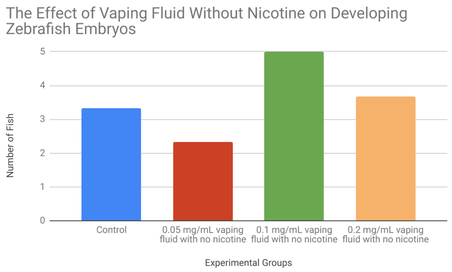
This photo shows an embryo with its chorion still attached in day 4.

Graph 1



This graph shows the average number of live fish on the last day of the experiment.

Graph 2



This graph shows the average number of hatched fish on the last day of the experiment.

Table 1

Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	6	2	2	3	-	-
0.05 mg/mL vaping fluid with no nicotine	5	5	2	4	p = 0.7096	not statistically significant
0.1 mg/mL vaping fluid with no nicotine	3	9	4	5	p = 0.4309	not statistically significant
0.2 mg/mL vaping fluid with no nicotine	2	9	12	8	p = 0.2532	not statistically significant

This table shows the amount of living zebrafish in each well on the last day of the experiment, it also includes the average.

Table 2

Control	6	2	2	3	-	-
0.05 mg/mL vaping fluid with no nicotine	3	3	1	2	p = 0.5391	not statistically significant
0.1 mg/mL vaping fluid with no nicotine	3	9	3	5	p = 0.5262	not statistically significant
0.2 mg/mL vaping fluid with no nicotine	0	6	5	4	p = 0.8911	not statistically significant

This table shows the amount of hatched zebrafish in each well on the last day of our experiment, this also includes the average.

DISCUSSION:

While this experiment took place, there was a significant decrease in living embryos throughout their development process, including the controls. This can be considered the main effect of the various dosages of vaping fluid with no nicotine. In the higher dosages of the vaping fluid, more embryos made it through the entire experiment. However, in the control wells, there was a lower amount of living embryos, but these embryos were thriving. The groups that are being tested in this experiment show various assortments of outcomes of surviving embryos, including the rate of deterioration. However, the outcomes of this experiment did not support the hypothesis that was created at the beginning of the experiment. This statement is reflected in the results shown by the data charts.

The controls were established to be kept outside of the incubator for 25 minutes per day, in order to be kept under observation. There were moments during the experiment in which the control wells were kept outside of the incubator for several minutes longer than need be. This issue may have had an effect on the development of the control embryos, given the fact that their body temperature did not stay consistent. While this error didn't have too much of a significant effect on the experimental groups, there could have been a potential conservation by limiting the time outside of the incubator to prevent the higher risk of death for the embryos.

For a higher probable outcome compared to the outcome of this previous experiment, a consistent amount of time outside of the incubator would have been sufficient. This consistent technique may have prevented more deaths of the embryos. Given 30 or even 40 minutes of time for observation may have been just enough to ensure the production of the embryos in a healthier manner.