

The Effects of Vaping Fluid on Zebrafish Development and Somites

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ABSTRACT:

This experiment was to find out the effects of vaping fluid on zebrafish. It helps scientists infer what would happen to a developing baby if the mother was vaping, since zebrafish share 70% of human's genes. The method used was putting five zebrafish in each well of a twelve well plate. They would be submerged in Instant Ocean in the control wells, vaping fluid with nicotine and flavoring, or vaping fluid just flavoring, but no nicotine. The key results were that zebrafish had curved spines in the wells with vaping fluid and nicotine and the zebrafish embryos turned green in all the wells that had vaping fluid. This led to the vaping fluid without nicotine and flavoring (pre) 0.1 mg/ml data being statistically significant. After collecting this data and seeing the consequences of vaping fluid, the data suggests that women should not vape during pregnancy.

INTRODUCTION:

Danio rerio, the scientific name for zebrafish, are used as model organisms to help scientists figure out information about human health. Zebrafish share 70% of human genetics. The fish also have two eyes, a mouth, brain, spinal cord, intestine, pancreas, liver, bile ducts, kidneys, esophagus, heart, ear, nose, muscle, blood, bone, cartilage, and teeth. Scientists use these fish because if they have anything wrong with them during these experiments scientists can infer humans would have the same reaction. In this experiment, the zebrafish were used to see the effects of a baby if a pregnant mother vaped. Scientists also use zebrafish because they are cheaper and don't take up so much room (Burke, 2016).

Over the past few years, vaping and Juuling have been very popular among teenagers. Multiple people don't know the consequences of vaping and Juuling. E-cigarettes have heavy metals in them, such as nickel, lead, chromium, tin, and aluminum, and chemicals like formaldehyde, which is known to cause cancer. Multiple things in their bodies are put in danger. The brain is still developing until the age of twenty-five, which means vaping can cause damage to the developing brain. Also, respiratory systems can be affected in a negative way. In fact, one Juul pod contains as much nicotine as a whole pack of cigarettes. Nicotine causes a surge to go to the brain that releases dopamine. Dopamine is known as a reward chemical. That means that it feels good or encourages to vape more (Surgeon General, 2019).

In this experiment, zebrafish were used to find out the effects of vaping fluid with and without nicotine and flavoring on the somites of the fish. The independent variable was the vaping fluid. The dependent variables were the mortality rate of the fish and the hatch rate. The control was Instant Ocean. The expectation for this experiment was that the vaping fluid would affect the growth of the somites in the fish.

MATERIALS AND METHODS:

The experiment required certain items to conduct the experiment. The embryos came from UW-Milwaukee's Science Education Partnership. The materials used for safety were safety glasses and rubber gloves. For the fish to stay alive in a safe place, they required a 12-well plate, the incubator, the instant ocean water, a beaker for the clean embryos and liquid disposal, a beaker to dispose of the dead embryos. For disposing of the dead embryos the material that was used was a pipette. Pipettes were also used for transferring eggs to the observation container and manipulating them in the container. To conduct the experiment with vaping fluids, a bottle of chemical solutions were needed. In order to look at the zebrafish, a compound microscope was used. A dry erase marker was used to label the 12-well plate. The depression slide with coverslip was used for viewing the fish up close. During the first day of our experiment, all of the zebrafish were submerged in Instant Ocean. After the second day, the embryos were submerged in the vaping fluid (pre) 0.1 mg/ml with or without nicotine and flavoring. The embryos started to hatch and the eggs were cleaned out with the pipettes and the ones that died were also disposed in the waste beaker. This was repeated for the rest of the experiment. After seeing the mortality and hatched rate, it was recorded down in a data table to get statistics.

RESULTS:



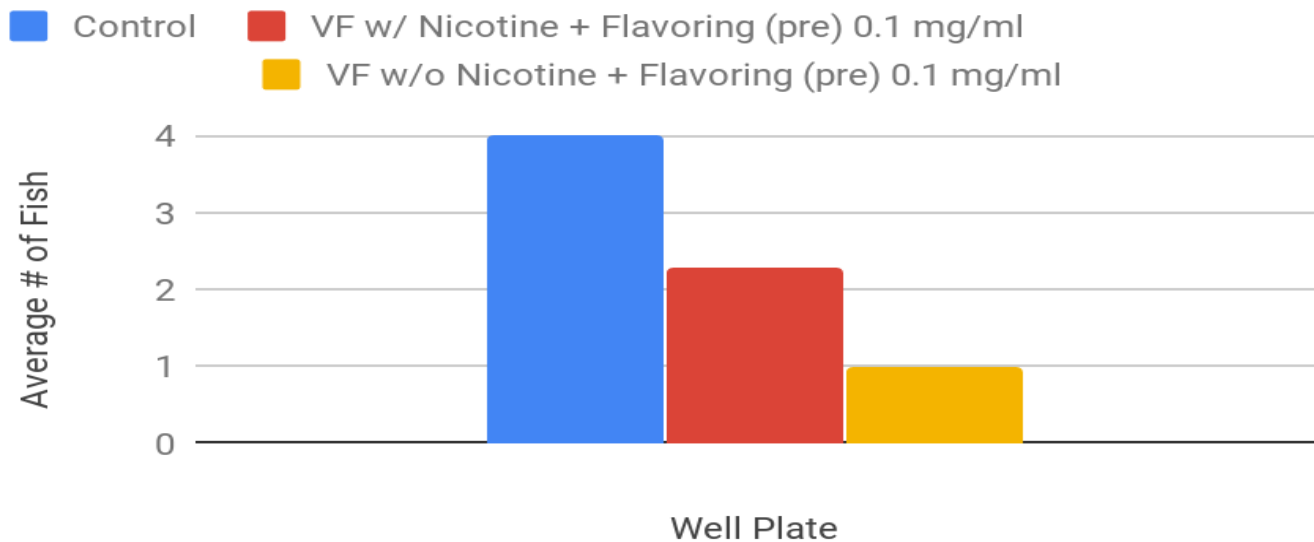
Figure 1: A green zebrafish in its egg casing

Researchers in the past have found out that zebrafish share 70% of human genetics. This helps scientists test chemicals and environments to figure out if it will have any bad side effects on humans. Zebrafish also grow up faster than humans can, so scientists can get results quicker and safer than testing on humans. The hypothesis of this experiment was would vaping fluid with and without nicotine plus flavoring have an effect on the growth of the zebrafish somites. During the experiment, the zebrafish who were submerged in the nicotine and flavoring developed curved spines. Also, in the experiment the B row zebrafish developed a parasitic environment. At the end of the experiment, the fish exposed to vaping fluid row had high mortality rates, curved spines, and parasites, while the C row had none.

Treatment	Well 1	Well 2	Well 3	Well 4	Average	Probability	Result
Control	3	5	5	3.0	4.0	-	-
VF w/ Nicotine + Flavoring (pre) 0.1 mg/ml	5	0	2	2.0	2.3	p = 0.1891	not statistically significant.
VF w/o Nicotine + Flavoring (pre) 0.1 mg/ml	2	2	0	1.0	1.3	p = 0.0105	statistically significant.

During the experiment, the data table showing the zebrafish survival count shows that the Vaping Fluid with and without nicotine overall had a lower survival rate. During the second day of the experiment, the zebrafish in the vaping fluid solutions had at least one dead in each well. The dead zebrafish, were being eaten away by parasites. The living fish were also being eaten away by the parasites, which may have affected the results.

Average Survival Rate for Zebrafish



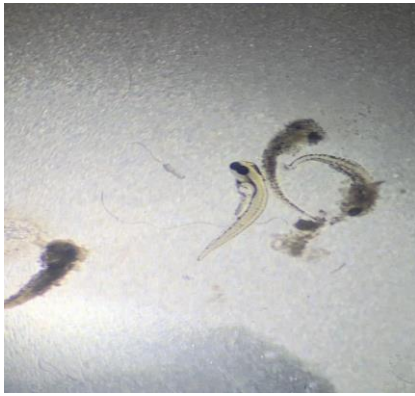


Figure 2: "Parasite infection in well row B

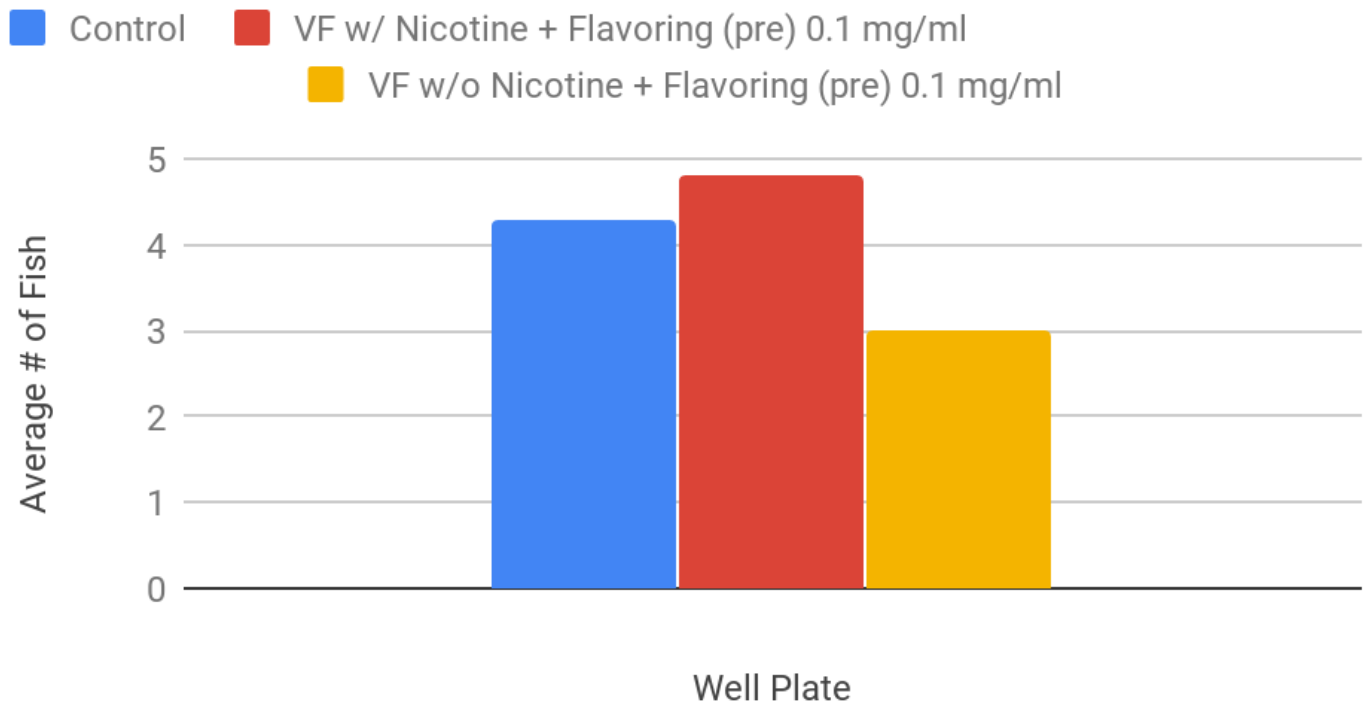


Figure 3: Zebrafish curved spines in row A

While doing the experiment the hatch rate of the zebrafish was pretty even throughout, except the vaping fluid without nicotine. The vaping fluid without nicotine had a lower hatch rate than the other two environments. Using the Graph Pad t-test. This experiment shows that vaping fluid without nicotine and flavoring is statistically significant towards the data or the living zebrafish in the end. The Graph pad t test also showed that the data of vaping fluid affecting the hatch rate of zebrafish is not statistically significant.

Table 2							
Treatment	Well 1	Well 2	Well 3	Well 4	Average	Probability	Result
Control	3	5	5	4.0	4.3	-	-
VF w/ Nicotine + Flavoring (pre) 0.1 mg/ml	5	5	5	4.0	4.8	p = 0.3903	not statistically significant.
VF w/o Nicotine + Flavoring (pre) 0.1 mg/ml	2	4	2	3.0	2.8	p = 0.0686	not statistically significant.

Average Hatch Rate In ZebraFish



DISCUSSION:

At the beginning of the experiment, the zebrafish were all submerged in different fluids. The A row was vaping fluid with nicotine and flavoring, the B row was without nicotine with flavoring, and the C row was the instant ocean solution. During the experiment the zebrafish in the A and B rows developed curved spines. Also, the B row fish turned green. The hypothesis of this experiment was to see if vaping fluid with and without nicotine plus flavoring would have an effect on the growth of the zebrafish somites. The data supported the hypothesis because it was the effect of the vaping fluid on the zebrafish that caused the deformities.

Something that could have gone wrong during the experiment was not cleaning the well plate well enough causing the parasites to spread. This may have affected these results because the parasites could have consumed the living zebrafish causing the mortality rate to go up. Despite the errors caused in the experiment, the results could infer that vaping fluid with nicotine and flavoring do the most damage on the growth of the somites. The results are relevant because this could also happen to a baby if the mother vaped. Scientists can do further experiments by making it longer or by taking more precautions. An example could be switching the zebrafish to a different well-plate after every day.

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