TITLE: How Vaping Fluid with Nicotine Post-Vaporization Affects Developing Zebrafish Embryos

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Abstract: *The purpose of doing this experiment is to test if vaping fluid with nicotine will affect a developing zebrafish embryo. The method used when doing this experiment was having 10 zebrafish in each well of a 12-well plate and having 4 wells for each test group and control. Vaping fluid with nicotine and dragon fruit flavoring post-vaporization at a 0.01 mg/mL concentration was placed in the second row of wells and then vaping fluid with nicotine and dragon fruit flavoring post-vaporization at a 0.1 mg/mL concentration was placed in the third row of wells. The control solution was regular instant ocean water. While doing this experiment, the main focus point was the hatch rate and the number of living zebrafish. The key result was negative impacts on the nervous system and changes in the color of the zebrafish. This experiment showed that vaping while pregnant could potentially have negatively affected the embryos in different ways.*

Introduction: Zebrafish, or as they are scientifically known, *Danio rerio*, are tropical freshwater fish that are part of the minnow family. They are popular aquarium fish and the fish are omnivorous, meaning they eat many different things from sand and mud, insects, plants, algae, and fish scales.

The reason why zebrafish are so popular within medical research is due to several factors, including their short life span and the transparency of their embryos. The reason scientists use zebrafish for experiments is because humans share over 70% of DNA with zebrafish and because of this they have many organs that are similar which have similar purposes in their bodies. These similarities make research testing more accurate because the organs will react in a similar way as human organs. The zebrafish organs react in a similar way as us humans would so when scientists test the effects of a toxic substance like vaping on a zebrafish would be the same as testing it on humans.

Vaping is when you take a pod or tank filled with an oily substance that could have nicotine, in addition to other unknown chemicals, and is sold as an alternative to smoking and is generally marketed as safer than smoking; however, from other research, it is showing signs of being worse. Because what happens is, the oily substance is vaporized by the coil and turned into a vapor that the user breaths in, this vapor has all those chemicals in the oily substance, but because it is heated the chemicals react with each other making more chemicals that scientists don't know about yet. This is bad because of the chemicals that are in the vape juice and it is bad because those chemicals made from the vaporization causing reaction could be so much worse.

The experiment tested the concentration of post vape with nicotine most likely a low concentration of 0.01mg/mL and a high concentration of 0.1mg/mL This experiment is to test what will happen when these chemicals are introduced to zebrafish embryos. The hypothesis is that there is probably going to be deformations that may include slower or faster heartbeat, their reaction time may

be slowed which means there nervous system is being affected in the developing zebrafish embryos, and that will also affect them if they are able to develop into a more mature stage of their life. In this particular experiment, the independent variable is the toxic vape juice, particularly the post-vaporized juice that contains nicotine which is an addictive stimulant. Nicotine is a substance that raises brain activity for a short time because nicotine is a stimulant. However, nicotine is not the only harmful substance in vape juice. The dependent variables were the hatch rate and survival rate of the fish. The control in this experiment was the embryos in the instant ocean water. The hypothesis is that in the end, all of the embryos exposed to the chemicals will die or if hatched will come out deformed.

Materials and Methods: First thing to experimenting with a 72 hour zebrafish experiment is having all the proper materials. The materials needed are chemical solutions (measured in mg/mL or mm) one bottle per group, a beaker for dead embryos and liquid disposal, breaker for clean embryos media solution, a dry erase marker, disposable 3mL pipettes with a large bore for daily solution changes, 12 well plates, 28.5 °C incubator, depression slide with coverslip, dissecting/stereo microscope, compound microscope, and goggles and gloves for each person in the group.

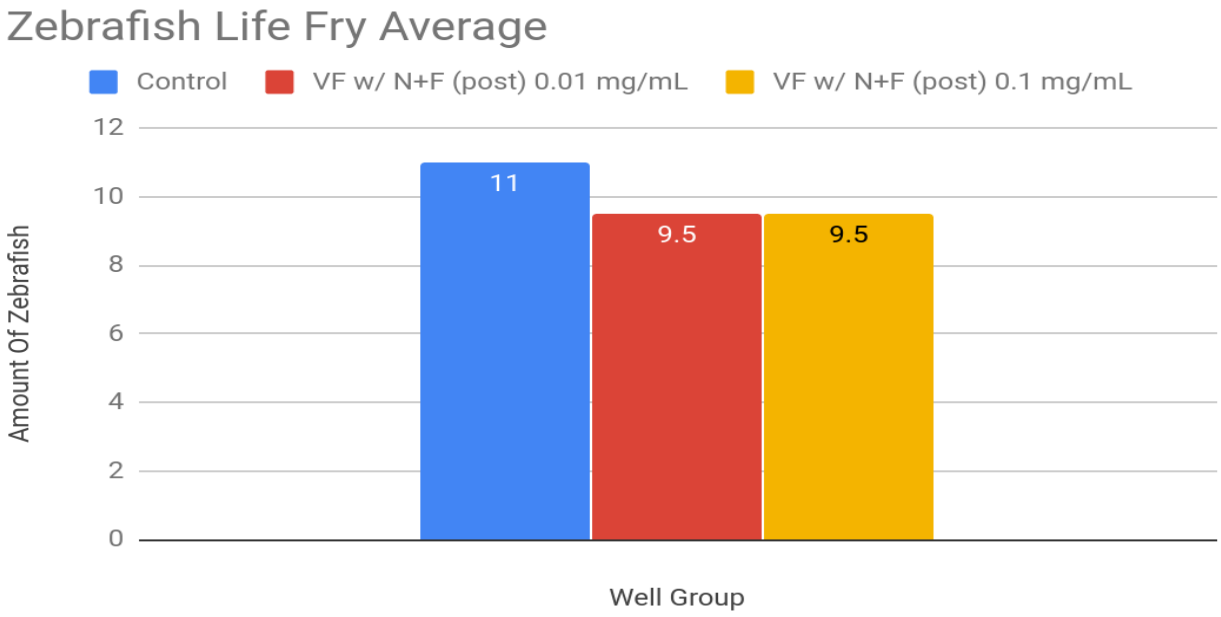
Setting up for the experiment, the first step is to obtain the embryos that were delivered from your advisor. After obtained the embryos take the well plates and fill them with several mL of embryos media solution. Then divide the embryos so that there are 10 embryos in each of the wells. (*Please note that if embryos are dead please discard of them). Then, place the embryos onto the well plates, label the well plates cover, and then label the diagram on the data sheet. Each person in the experiment should record the number of live embryos on a data sheet. Before proceeding on with the experiment, go to the advisor check over the plan and if the advisor says the plan is good, replace the media solution in wells with the chosen chemical solution. Observe the embryos in the well plate under a dissecting microscope, record observations, and record on the data sheet. Before putting the experiment away for the day, place each plate into the 28.5 C incubator. Finally, to finish the first day, clean up the lab area properly.

Now to start the experiment for the first day after 24 hours of treatment, take the well plates from the incubator and take a look at the embryos. When looking at the embryos, if there are any dead embryos in the plates, remove them from the plate using the disposable pipette. Place the dead embryos into the waste beaker. Remember to be careful and only remove the dead ones. After the removal of the dead embryos, count the remaining embryos or hatched fish, and record it in the data table. When finished counting the embryos, remove the old solution from each well plate and replace the old solution with the appropriate fresh solution using a clean pipette each time. For close viewing of the zebrafish, remove 1-2 embryos and place them on a depression slide and cover with a coverslip. Then, observe using the compound microscope and remember to record observations on the data sheet. Once finished, place the embryos back into the proper well plate and then return the plate back into the incubator and clean up lab area. After 48 hour of treatment, repeat day 1 and observe and record. After 72 hours repeat day 1, observe and record. 72 hours will be the last day so after that day the advisor will properly dispose of the organism, so all you have to do that day is clean up the lab station.

Results: The purpose for testing this experiment is to test if flavored vaping fluid with nicotine post-vaporization would affect the zebrafish embryo. The hypothesis was that all the embryos that were exposed to the chemicals would die or come out deformed.
The control group was the group with nothing being tested; it just had regular instant ocean water, which was group A on the well plate. The independent variable was the vaping fluid with nicotine and flavoring post vape. In the experiment, the dependent variable was the zebrafish hatch rate, survival rate, and developmental changes.

The results show that the vaping fluid did not affect the hatch rate or mortality rate of the zebrafish or have the zebrafish come out deformed. The data showed that the vaping fluid mostly affect the nervous system and the discoloration of the zebrafish. During the experiment if one would touch the zebrafish with the pipette in the controlled group, the zebrafish would move away, but then, if the zebrafishes in the low concentration of post-vape (0.01 mg/mL) were touched they would also move, but only more slowly. The zebrafish move slower the higher the concentration of vaping fluid they were exposed to.

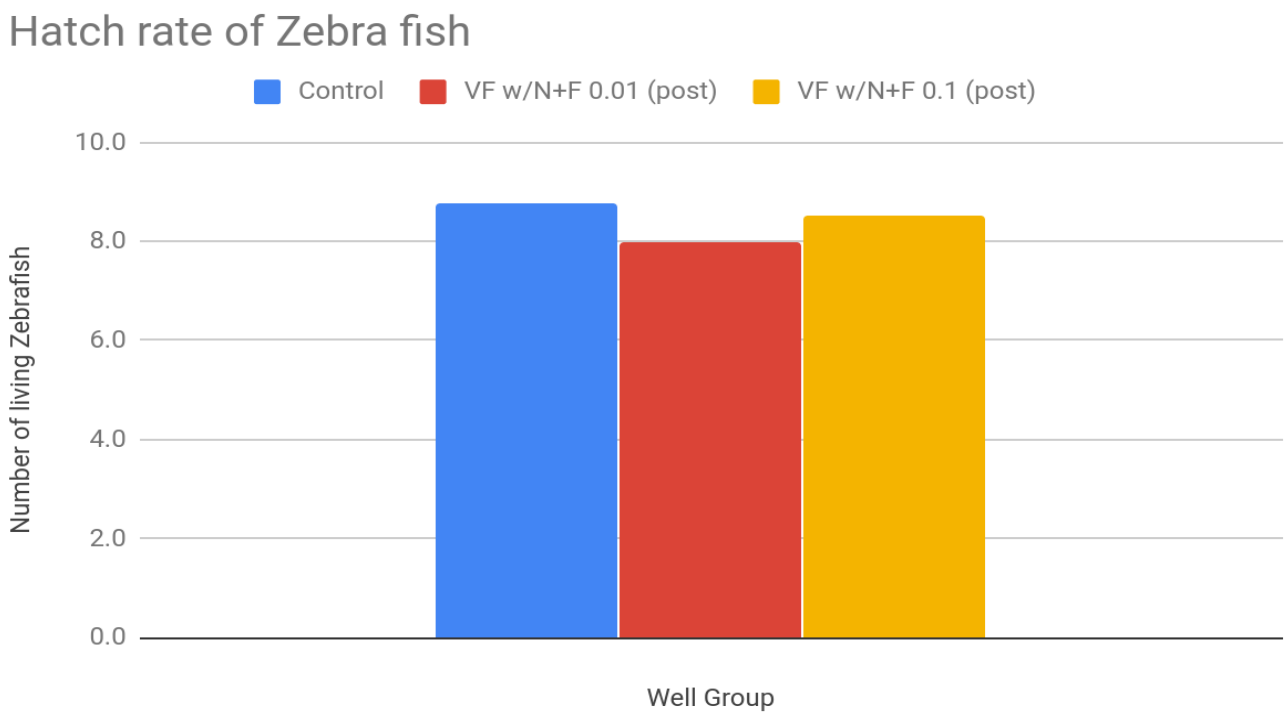
The experiment also showed that the higher concentration of vape fluid the zebrafishes were exposed to, the more colorful the zebrafish would be. When looking at the zebrafish, the zebrafish in the control group were more brown (see Photo 2) and then the zebrafish in the vaping fluid appeared bluer, green and red (see Photo 1). The hatch rate of the zebrafish and the final number of living fry averages were not statistically significant.



Graph 1: This graph shows how the survival rate of the zebrafish differs from the control to the treatments. But both treatments have the same survival rate.

Treatment	Well 1	Well 2	Well 3	Wells 4	Average	Probability	Result
Control	11	11	13.0	9.0	11.0	-	-
VF w/ N+F (post) 0.01 mg/mL	11	9	7.0	11.0	9.5	0.2782	Not statistically significant.
VF w/ N+F (post) 0.1 mg/mL	11	6	10.0	11.0	9.5	0.3388	Not statistically significant.

Table 1: This is the table for the survival rate of the Zebrafish. The information that is displayed is the types of treatments, the amount of living fish in each well by the end of the experiment, the average, and the significance of the difference



Graph 2: This graph shows the zebrafish hatch rate, all of the values are around the same so that means that the difference between the two treatments is not significant.

Treatment	Well 1	Well 2	Well 3	Well 4	Average	Probability	Result
Control	10	9	10	6.0	8.8	-	-
VF w/N+F 0.01 (post)	8	9	7	8.0	8.0	p = 0.6540	not statistically significant.
VF w/N+F 0.1 (post)	11	6	10	7.0	8.5	p = 0.8748	not statistically significant.

Table 2: This table is for the hatch rate and shows the type of treatments, the amount of fish hatched by the end of the experiment, the average at the end of the end of the experiment, and the significance of the difference

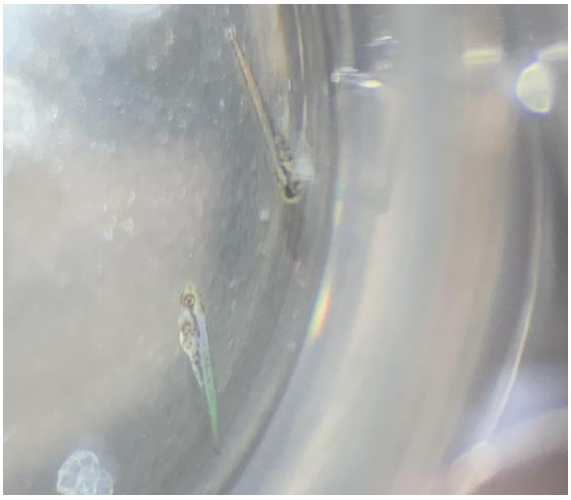


Photo 1: If you look closely at the bottom Zebrafish you will see that this fish as a green blue tail and a blue looking head Zebrafish are not supposed to look like this. This fish was in our 3rd treatment row which was the vaping fluid with nicotine and flavoring that was put in the waster post vape.

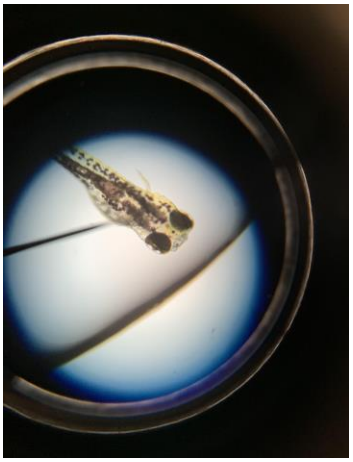


Photo 2: This Zebrafish was in the control row of the wells this picture was taken to show what the Zebrafish are supposed to look like so if you compare the two images you can tell that the color is very different. But it is also just showing the Zebrafish under a more focused micro-scope

Discussion: The results of the experiment showed that vaping chemical at two different concentrations with nicotine were not statistically significant in killing the fish or affecting their hatch rate. However, this experiment shows that vaping while pregnant may affect the reactions of the developing embryo. It may affect the nervous system of the embryo after it hatches; there also may be a chance that the embryo may hatch and come out deformed. In the end of this experiment it shows that vaping while pregnant is dangerous and it may cause some damage, which may either be noticeable right at birth or future on in life.

During the experiment there could have been some errors that may have affected the results. An example of something that could have been messed up is that some of the embryo could have been sucked up by accident or thrown away a living embryo. Another possible thing is the vaping fluid could have been mixed with each other and cause an inaccurate result.

For future experiments of this type, it should not only be done with developing zebrafish, but also with adult zebrafish. It would let the developing zebrafish that were exposed to the vape fluid survive to grow into adults and another test is to let the zebrafish grow up in a healthy environment until adulthood, and then expose them to the vaping chemicals with flavoring and nicotine post-vaporization. Doing this would show how the vaping fluid might affect an adult versus an embryo, and show if it is more dangerous or less dangerous. If this experiment were to be done again, another suggestion would be to experiment with more than 120 embryos.

Reference:

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NIH, Published in October 2017, Why we use Zebrafish in research. https://www.nigms.nih.gov/Education/Pages/modelorg_factsheet.aspx