Testing the Effects of Propylene Glycol and Vegetable Glycerin on Zebrafish Embryo Development

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Abstract

As vaping among teens and young adults becomes more common, there has been an increase in interest of the true health effects of its ingredients. The purpose of this experiment was to examine the effects of two main vape ingredients, propylene glycol and vegetable glycerin, on a developing human embryo. Zebrafish embryos are used in place of human embryos due to ethics and accessibility. Previous research indicates that exposure to these substances in the womb severely stunts development (Newsroom, 2018). Six groups were tested in this experiment, each containing a higher concentration of PG VG. Staring at a control with 0 mL, there are five experimental groups, with 1mL, 3 mL, 5 mL, 7.5 mL, and 10 mL of a propylene glycol vegetable glycerin mix. The experiment proved that higher concentrations of the solution negatively affected both the development and death rates of the zebrafish embryos.

Introduction

Propylene glycol (PG) and vegetable glycerine (VG) make up 95% of the liquid used in vapes and e-cigarettes. Usage of these devices has increased by 10% in 18-year-olds from 2017 to 2018 and has doubled in 16-year-olds (Stein, 2018). The rate of increase changes greatly from year to year as vaping becomes more widespread and usage rates, especially in young adults, continue to grow. Such significant increases in the relatively new product have researchers investigating possible long term health effects, but the main focus of their experiments is nicotine. As teenagers become adults and new situations occur, such as pregnancies, misinformation or ignored issues may lead to problems. Nicotine is an ingredient in vapes that has been highly researched and advertised as negative, especially to unborn babies, but nicotine-free products are available. Changes in the development of cells could also affect the developing brains and bodies of teenagers and young adults.

Propylene glycol and vegetable glycerin are the other two main ingredients in vapes, but little research has been done examining the health effects of inhalation for developing teens or embryo. One experiment, performed by the University of North Carolina School of Medicine, found that administering small amounts of propylene glycol and vegetable glycerin to test cells seriously stunted growth of test cells (Newsroom, 2018). The FDA has labeled as intake of PG

and VG orally is nontoxic, but when vaporized and inhaled, as done when vaping, the products become toxic. The biggest problem that may come from this misinformation may appear in pregnant women. The belief that non-nicotine vaping will have no effect on an embryo could lead to undevelopment of the child and possible miscarriage.

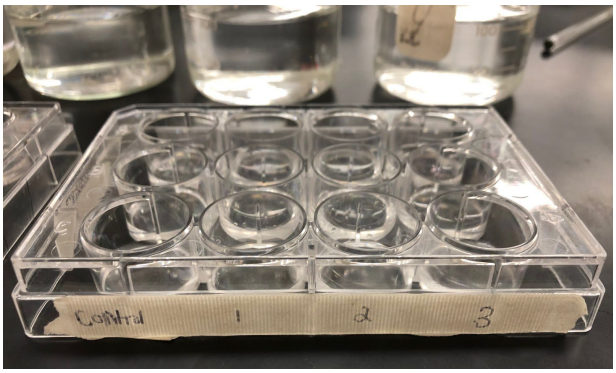
The purpose of the experiment comes from these concerns. A testable question was generated: what are the true effects of propylene glycol and vegetable glycerin on a developing fetus? Zebrafish embryos accurately mimic the development of human fetuses and react similarly when exposed to the same environmental factors. Exposing groups of normally spawned zebrafish embryo to different concentrations of the substances will result in an answer. The hypothesis comes from previous research on developing test cells. If zebrafish embryos are exposed to propylene glycol and vegetable glycerin, then development will be stunted. The higher the concentration, the higher the death rate and the slower the development.

Materials and Methods

The first step in setting up this experiment was gathering all the materials: 6 oz of propylene glycol, 6 oz of vegetable glycerin, 7 150 mL beakers, 1 1 liter beaker, 1 liter of distilled water, 5 mL of instant ocean, antifungal, pipettes, incubator, 2 trays with 12 wells each, 10 zebrafish embryo per well in tray, 100 mL beaker for disposal of used solution and dead embryos, tape, sharpie, dissecting and compound microscopes, depression slides for microscope. The second step was to label each beaker and tray, both rows and columns, their respective group number. The base solution was mixed together: 1 liter of distilled water, 5 mL of instant ocean, and one drop of antifungal in a liter beaker. Also, 50 mL of propylene glycol and 50 mL of vegetable glycerin were mixed in a 150 mL beaker. Then, 100 mL of the solution was added to each group beaker, Control through Group 5. The corresponding amount of PG VG was added to each group: Control is 0 mL, Group 1 is 1 mL, Group 2 is 3 mL, Group 3 is 5 mL, Group 4 is 7.5 mL, and Group 5 is 10 mL.

After Day 1, Groups 4 and 5 were replaced with Groups 1 and 2 because every embryo had died in Groups 4 and 5. The results would not have produced any solid evidence without the

addition of these groups. To perform the exact experiment, clean out the 7.5 mL and 10 mL wells with all the dead embryo after Day 1 and replace them with solutions 1 mL and 3 mL.



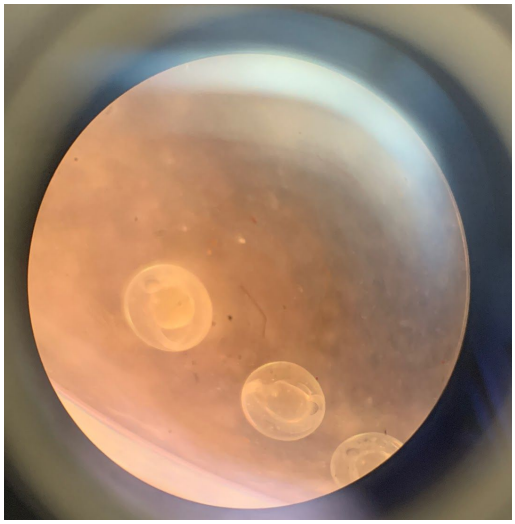
Picture 1 and Picture 2

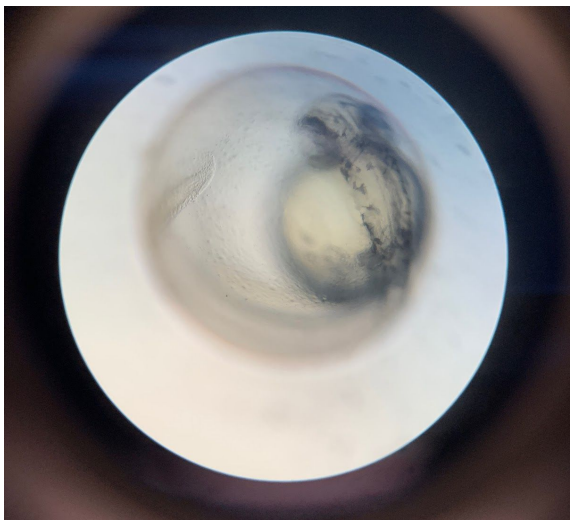
After the solutions were made, 10 embryos were carefully placed in each well, then any extra water was removed. When each well is filled, 1 mL of the corresponding solution was added to each well. The trays were placed in an incubator set to 28.5°C. For each experiment day following, the trays were taken out of the incubator, all dead embryos were removed with pipettes, and the solutions were discarded into the waste beaker. Solutions were replaced with previously made solutions. Amount alive and amount hatched were recorded and zebrafish were examined under microscopes. Trays were put back in the incubator when finished. Repeat for how many days the experiment is set to run. The experiment was done over one week (Days 1-5) and returned to on the following Monday (Day 8).

Results

The independent variable for this experiment were the solutions tested. Each group had a varying amount of propylene glycol and vegetable glycerin mixed with the ocean-mimicking solution. The dependent variables for this experiment were the number of deaths and hatchings over eight days. How many survived was influenced by the amount of PG VG they were exposed to. There were a number of controls in this experiment; including the instant ocean, distilled water and antifungal mix; incubation times and temperature; and the amount of liquid in each well.

Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Dead	0	21	6	5	2	4
Total Unhatched (Alive)	60	7	0	0	0	0
Total Hatched	0	32	36	31	29	25



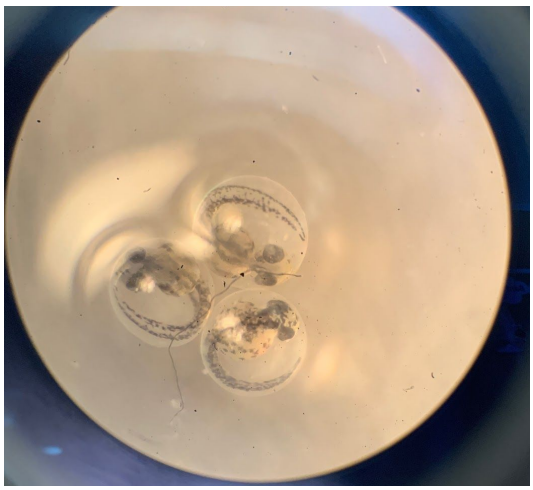
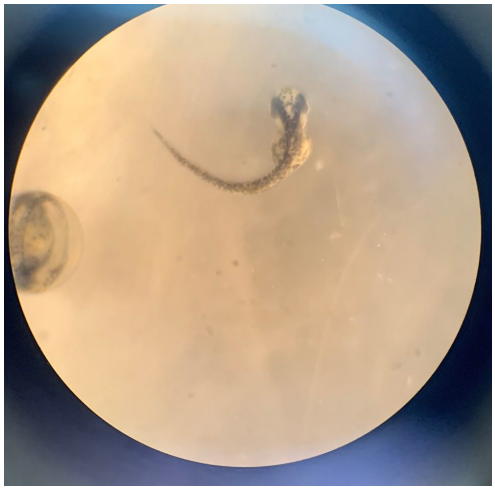


Picture 3 and Picture 4

Group 1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Dead	*no data	0	4	19	15	13
Total Unhatched (Alive)	*no data	60	50	8	0	0
Total Hatched	*no data	0	6	29	22	9

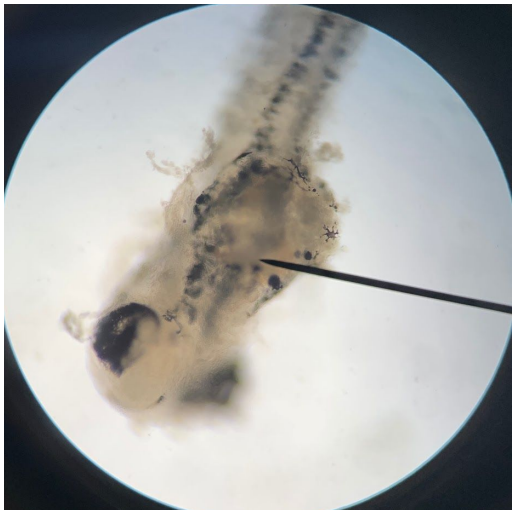
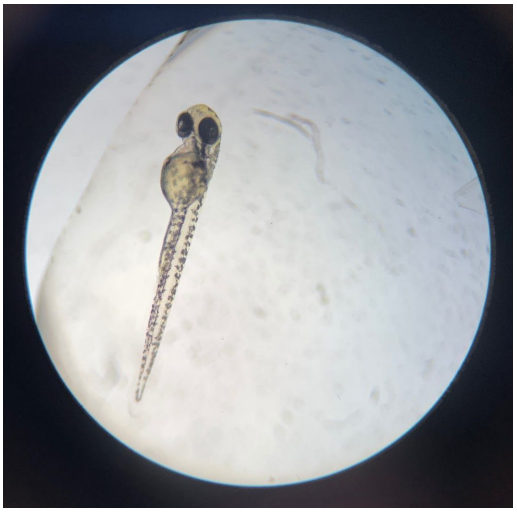
Note *no data = The zebrafish embryo used spent Day 1 in an environment that was equivalent to the control group. This was done due to the unexpected deaths of all zebrafish embryo in Groups 4 and 5. Additional groups (Groups 1 and 2) were added to provide more data.

Group 2	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Dead	*no data	0	8	12	18	22
Total Unhatched (Alive)	*no data	60	38	15	0	0
Total Hatched	*no data	0	14	25	22	0

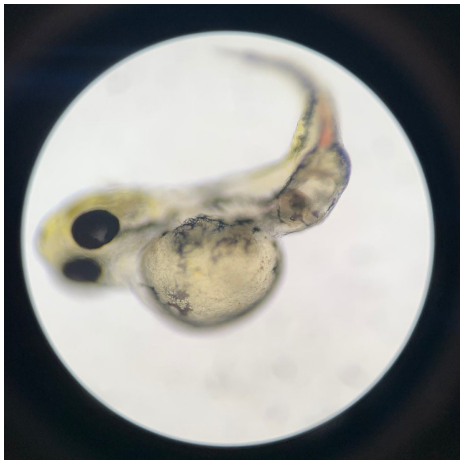
Picture 5 and Picture 6

Group 3	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Dead	0	51	0	0	0	0
Total Unhatched (Alive)	60	9	0	0	0	0
Total Hatched	0	0	0	0	0	0

Picture 7 and Picture 8

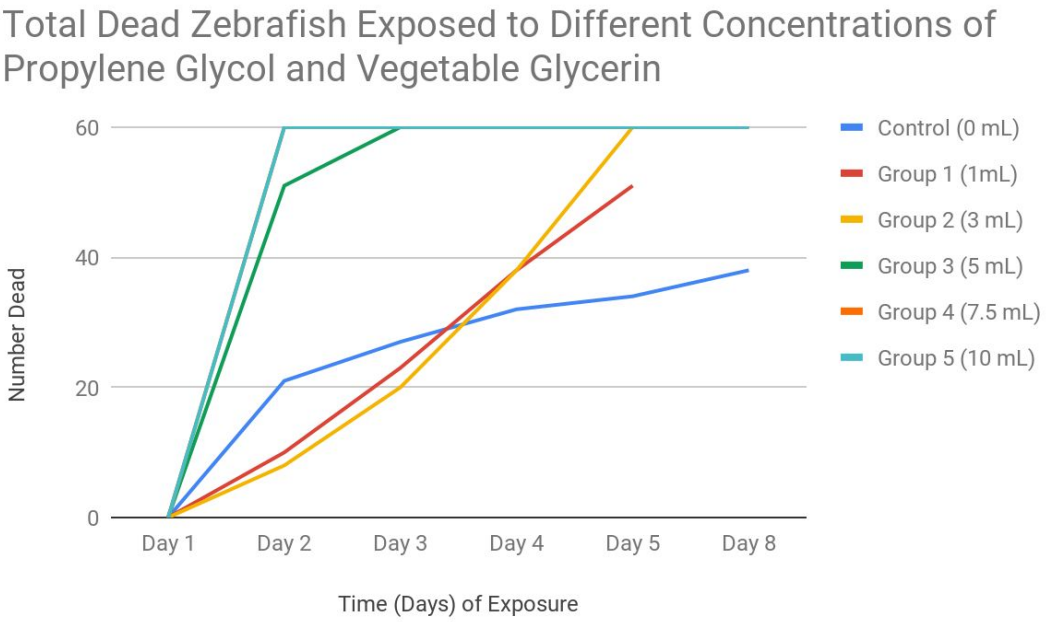
Group 4	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Dead	0	60	0	0	0	0
Total Unhatched (Alive)	60	0	0	0	0	0
Total Hatched	0	0	0	0	0	0




Picture 9 and Picture 10

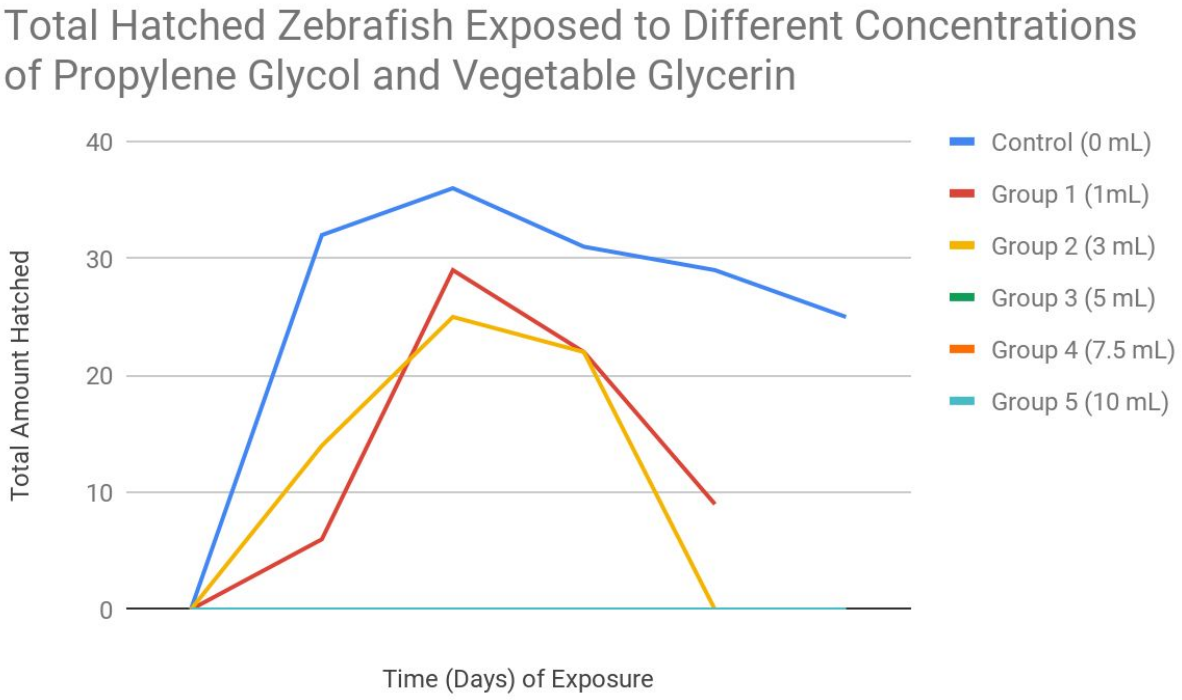
Group 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Dead	0	60	0	0	0	0
Total Unhatched (Alive)	60	0	0	0	0	0
Total Hatched	0	0	0	0	0	0

Data Analysis



Graph 1

The graph shows the total deaths of zebrafish that were exposed to different concentration of propylene glycol and vegetable glycerin. There was a significant difference in deaths between groups 1-4 and the control group. Groups 1 and 2 results were not complete representations of exposure because the first day of development the embryo spent in a control group like environment. In the end, the control group had the least amount of deaths when compared to any group exposed to the stimuli.



Graph 2

This graph shows the relationship of hatched embryo between the groups. The experiment aimed to examine the effects of a developing human embryo when exposed to different levels of PG VG. This comparison shows the stunted development of the zebrafish due to exposure to propylene glycol and vegetable glycerin. Between 3 and 5 mL of PG VG per 100mL of water was the cut off for the complete destruction of development.

Conclusion

This data proved the hypothesis that the higher concentration of propylene glycol the zebrafish embryos were exposed to, the higher the death rate and the slower the development. There is a consistent increase in hatching rates as less PG VG was used in the groups. The results produced by this experiment back up the results from the study performed by the University of North Carolina School of Medicine (Newsroom, 2018).

A very impactful change was made to this experiment that was not planned for. The solutions in Groups 4 and 5 were too concentrated and every embryo placed in those well died. This may be caused by suffocation or an intense example of how propylene glycol and vegetable

glycerin affected developing embryo. Due to this mishap, Groups 1 and 2 are not totally accurate in showing the effects at those concentrations. The embryo had a full 24 hours to develop with no stimuli. In graph 1, the low death rate for the first days was a direct cause of this error. Graph 2 may also be affected as the true data should show less hatching in the first few days.

A notable observation made during this experiment was the number of deformities in the surviving fish in groups 1 and 2. Compared to the hatched fish in the control groups, there were an odd number of bent of shrunken fish in groups 1 and 2. A few documented deformities can be seen in pictures 6 and 10 compared to the normally developed zebrafish shown in picture 8. These deformities caused strange swimming behavior as well, rapid circles or swimming into the wall. This shows that using non-nicotine vapes while pregnant could harm the fetus. The overall research shows that the main ingredients in vapes, besides nicotine, can seriously harm a developing fetus. Widespread acknowledgment of this correlation is vital to the prevention of serious issues in pregnant women and their children.

Work Cited

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