The Effects of Nicotine on Hatch Rate and the Development of Zebrafish

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ABSTRACT:

The purpose of this experiment was to show the effects of nicotine on the development of zebrafish embryos. This is worthy of attention, especially for pregnant women who may vape while pregnant. The procedure was performed using a 12 well plate, in which a control group of fish were placed in embryo media solution and compared to treatment groups that were exposed to several different amounts of nicotine. In the experiment, there was one treatment solution, 0.1 mg/mL of nicotine, that showed a statistically significant negative effect on zebrafish survival. Using nicotine while pregnant could have a negative effect on a developing human embryo, as well.

INTRODUCTION:

Zebrafish are an ideal model organism to compare to humans because they have 70% of genes and biology as humans. Zebrafish develop outside of the mother's body and after they develop they live for about three years. Zebrafish are used in experiments because their embryos are almost transparent which makes them easy to observe. In the 1960's the zebrafish became increasingly important to scientific research; researchers use them for biology experiments instead of using actual humans (Yourgenome, 2014).

Vaping causes issues in today's society, specifically in the younger generation. Electronic-cigarettes, sometimes called vapes, run on batteries and heat up nicotine, flavorings, and other chemicals. They turn them into a vapor you can breathe in. Many chemicals that cause cancer are in this vapor. That includes formaldehyde, heavy metals, and particles that can get stuck in the deepest parts of your lungs.

This experiment aimed to test the effects of nicotine. The hypothesis of this experiment was that nicotine will stunt the zebrafish's growth and have an effect on the gills of the fish by not allowing water to pass through them and it will decrease their size, deform shape, and discoloration. Changes in color and overall size will also be examined. It is hypothesized that the hatching rate of zebrafish will decrease as the nicotine concentration in the solution increases. The independent variable is the amount of nicotine solutions added to the wells. The dependent variables that will be measured are the mortality rate, the growth, and the hatch rate in order to determine if the chemicals are making a difference. The control is no nicotine added to the embryos, but the embryos will be in the embryo media solution.

MATERIALS AND METHODS:

Procedure: On December 2, 2019 Mauston High School received zebrafish embryos from UW-Milwaukee's Science Education Partnership Award (SEPA) Program which is sponsored by the National Institutes of Health. Gloves and goggles were required to wear at all times due to the fact that the solutions could burn and soak through human skin. Immediately, 5 embryos were placed in each of the 12 wells. In wells A1, A2, A3, and A4 was the embryo media solution. And in wells C1, C2, C3, and C4 was 0.1 mg/mL nicotine solution. Each day the dead embryos were removed with large bore pipettes and disposed of into a beaker. Each night the well plate was placed into a 28.5 °C incubator. The next day embryos were observed under the dissecting/stereo microscope and the solutions were changed with small bore pipettes. Counting of living embryos took place after dead embryos were placed into a disposal beaker. For the next two days the procedure continued of counting the living embryos and replacing the solutions. The GraphPad website (https://www.graphpad.com/quickcalcs/ttest1.cfm) was used to determine the significance of the data (Table 1 Bottle

RESULTS:

After four days of collecting information, the data showed that the nicotine solution significantly affected the zebrafish's hatch rate plus how they developed at the 0.1 mg/mL concentration. This did support the hypothesis, which was that the nicotine would affect hatch hate and development such as discoloration, shape, and size. The solutions of 0.01 mg/mL or nicotine and 0.05 mg/mL did not have a statistically significant affect the zebrafish, as the averages were too close to the control. The only solution to be significant was the 0.1 mg/mL of nicotine and that is very important in data and results, as it relates to how nicotine exposure in an embryo can affect it if an embryo hatches or does not hatch, and how it develops.

Final Average Embryos Hatched
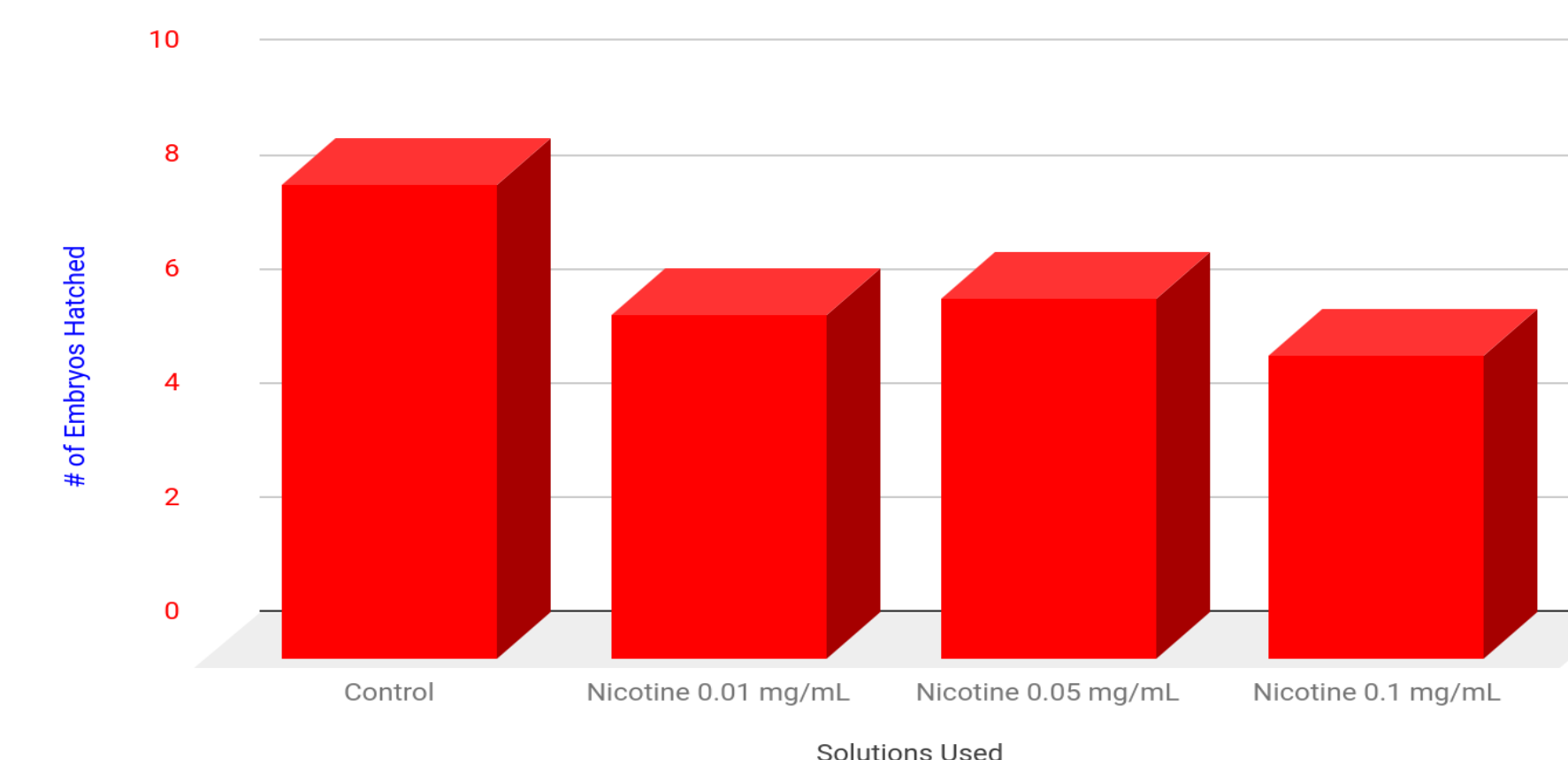
Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	9	9	7	8.3	-	-
Nicotine 0.01 mg/mL	2	6	10	6.0	p = 0.3867	not statistically significant.
Nicotine 0.05 mg/mL	4	6	9	6.3	p = 0.1276	not statistically significant.
Nicotine 0.1 mg/mL	3	6	7	5.3	p =0.0178	statistically significant.

p < 0.05 (result is significant)
p > 0.05 (result is not significant)

DISCUSSION:

After reviewing the results, the hypothesis stated that nicotine will stunt the zebrafish's growth, decrease the hatch rate, and will decrease their size, deform shape, and discoloration. This was found to be supported by the 0.1 mg/mL solution and the figures taken. In the experiment, the zebrafish embryos did not get killed, but were affected by 0.1 mg/mL of nicotine. The pictures show that the embryos seemed to be deformed in the ways of pigmentation. The pigmentation was affected by a difference of color from the control compared to 0.1 mg/mL the color appeared to be cloudy in the nicotine solution and clear in the control. Size and shape were another deformity in the results as well.

This experiment could have been more accurately performed with a larger variety of nicotine of solutions, to further analyze and confirm or deny how zebrafish's hatch rate and development react to nicotine. Also a longer time frame would allow an adult stage which would improve an accurate conclusion on the hypothesis.

REFERENCES:

References:

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Figure 1 (Control taken 12/16 12:00 PM) Seems to be healthy compared to 0.1 mg/mL

Figure 2 (Solution 0.1 mg/mL, taken 12/20 12:10PM) Significantly deformed