The Effects of 100□M Nicotine Solution on Zebrafish Embryos

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Abstract:

Zebrafish embryos are commonly used today to test the effects of toxicants. Many of these chemicals are found in everyday life leading them to be consumed by humans. The intake of a mother has potential to greatly affect the unborn child inside of her. Based on previous research, the experimenter conducted this experiment to find if there would be an impact from nicotine. This experiment would run 100□M nicotine solution on zebrafish embryos at varying starting times. Embryos were placed in wells on a plate and either exposed to the nicotine or not. On day 2 another row of embryos on the plate was exposed, and so on for day 3. By 96 hours post fertilization many of the embryos were hatched because of the quick development many observations were able to be made. There was no significance found in the survival and hatching rates of the zebrafish embryos. Although in the physical observations it was found that some embryos had abnormalities, color changes, or curved tail or spine. Since zebrafish embryos have major similarities to a human fetus, they are good models for development. Because of this, human mothers should take note on the amount of nicotine they consume as their child may be directly affected.

Introduction:

Nicotine a drug most commonly found in cigarettes, is consumed by humans in everyday life. As a result, nicotine is one of the top causes of preventable cancer that takes around 5.4 millions deaths every year. Smoking is a general danger to human health, but pregnant mothers who continue to smoke can cause drastic effects on the child. Some effects that have been observed are stillbirth, miscarriages, mental retardation, respiratory problems, birth defects, and many more (Mishra et.al. 2015). George Streisinger, known as the founder of zebrafish research started the studies at the University of Oregon in 1972. Since then, zebrafish and their embryos are being predominantly used for research because of their size, color, similarities to human fetuses, and reproduction rates (Chuan et.al. 2013). The biggest advantage to using zebrafish embryos is the similar growth cycle to a human fetus and the fact that the cycle is relatively short in time. Research exposing zebrafish to nicotine resulted in reduced notochord length, eye diameter, and survival rates (Parker 2007). In another study their results showed that zebrafish exposed to nicotine lead long lasting effects on the nervous system (Svoboda 2002). A study previously conducted by the experimenter tested nicotine solutions on zebrafish, leading to large amounts of death. One abnormality that was observed was a curved spine and kinked tail in the 100□M exposed fish (Karweick 2014). This lead the experimenter to consider testing fish at this concentration to find if the results will be the same or similar. The purpose of this experiment is to find if there is a correlation between the timing of nicotine exposure at 100□M concentration and death rates or defects in zebrafish. It is hypothesized that exposing zebrafish embryos to a concentration of 100□M of nicotine over different periods of time will result in death or defects in all exposures.

Materials and Equipment:

Quantity	Item
1 bottle	Stock Solution of Nicotine 100□M
1	Beaker for dead embryos and liquid disposal
1	Sharpie
1	Tape
1 bottle	Instant Ocean/Embryo Media Solution
1	Disposable Pipette
1	Plate with wells
1	28.5°C Incubator
1	Depression slide with cover slip
1	Dissecting and Compound Microscope

This procedure is from SEPA-UW-Milwaukee.
Procedure:
Day 1

1. Obtain a well plate
2. Label the plate with name and class hour. Label the nicotine concentration of each well using the sharpie provided.
3. Fill one row of wells with 1 mL of Instant Ocean/Embryo Media solution using the disposable pipette. Fill the 2nd row of wells with the 100□M nicotine stock solution. Fill the third and fourth row of wells with 1 mL of Instant Ocean/Embryo Media solution using the disposable pipette. Divide the embryos so there are approximately 10 embryos in each well. Label the plate on the student data sheet.
4. Record exact numbers of live embryos on student data sheet.
5. Observe the embryos under the dissecting microscope. Record observations on the student data sheet.
6. Place each plate in the 28.5°C Incubator

Day 2

1. Remove the plate from the incubator.
2. Remove dead embryos from the plate using the disposable pipette. Squirt dead embryos into waste beaker.

3. Count the remaining embryos, hatched fish, and record in data table.
4. Remove nicotine stock solution from each well.
5. Replace the nicotine stock solution with the appropriate fresh nicotine stock solution using a clean pipette each time.
6. Remove the Instant Ocean/Embryo Media Solution from the control wells.
7. Replace the Instant Ocean/Embryo Media Solution with the appropriate amount using a pipette.
8. Replace the third row of wells with nicotine stock solution.
9. Place the plate under dissecting microscope and record observations on student data sheet. Note/describe any development markers and abnormalities. Repeat for all the wells.
10. Remove 1-2 embryos and place on the depression slide with cover slip. Observe the embryos using the compound microscope. Record observations on student data sheet. Repeat for all of the wells.
11. Return the embryos to their well in the plate.
12. Return the plate to the appropriate 28.5°C incubator.

Day 3

1. Repeat steps of Day 2 put replace row 3 with nicotine solution rather than Instant Ocean/Embryo Media Solution.

Day 4

1. Repeat steps of Day 3.

Day 5

1. Repeat steps of Day 4.
2. Place all embryos and fish in waste container. The teacher will properly dispose of the organisms.

Data Tables:

Data Table 1: Number of Living Zebrafish Embryos Over a Period of Time

			24 Hours Post Fertilization		48 Hours Post Fertilization		72 Hours Post Fertilization		96 Hours Post Fertilization	
Treatment	Well #	# of Starting Fish	# Hatched	# Live	# Hatched	# Live	# Hatched	# Live	# Hatched	# Live
Control	1	10	0	9	0	9	3	9	8	9
	2	10	0	9	0	9	7	9	9	9
	3	10	0	10	0	10	5	10	8	10

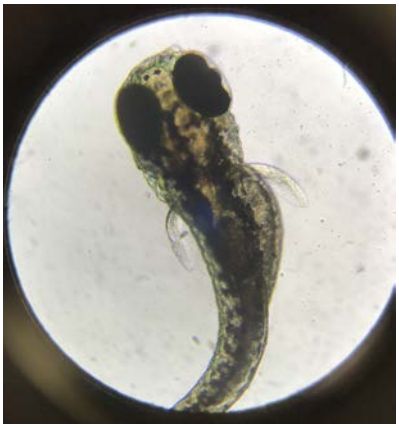
100m□M Exposure on Day 1	1	10	0	7	2	7	6	6	6	6
	2	10	0	8	1	8	5	8	8	8
	3	10	0	10	0	10	5	10	10	10
100□M Exposure on Day 2	1	10	0	10	0	10	2	10	10	10
	2	10	0	9	0	9	7	8	1	1
	3	10	0	9	0	9	6	9	9	9
100□M Exposure on Day 3	1	10	0	8	0	8	7	8	8	8
	2	10	0	10	0	10	8	8	7	7
	3	10	0	10	0	8	2	2	1	1

This data table shows the number of living and hatched zebrafish embryos in varying concentrations over 24 hour periods.

Data Table 2: Observations				
Treatment	Day 2	Day 3	Day 4	Day 5
Control				
100□M Exposure on Day 1		Cell 1 weird body shape and light colored body	Cell 2 curved spine	Cell 3 curved spine
100□M Exposure on Day 2		Cell 1 Some have light and some have dark spine		Cell 1 curved spine Cell 3 abnormal growth under the belly
100□M Exposure on Day 3		Weird shaped spine		

This data table shows the visual observation of the zebrafish throughout their growth.

Figure 1



Fish with crooked tail

Figure 2

Fish with abnormal underside

Figure 3

Fish with curved spine

Figure 4

Full view of fish with crooked tail

Figure 5

Full view of fish with abnormal underside

Figure 6

Full view of fish with curved spine

Graphs: Figure 7

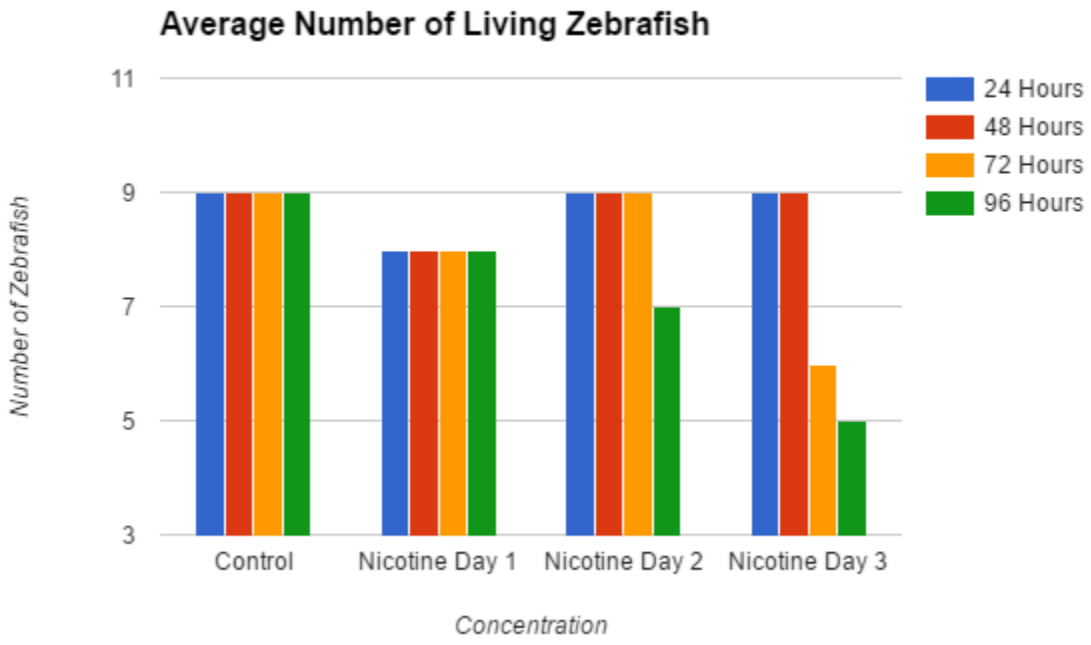
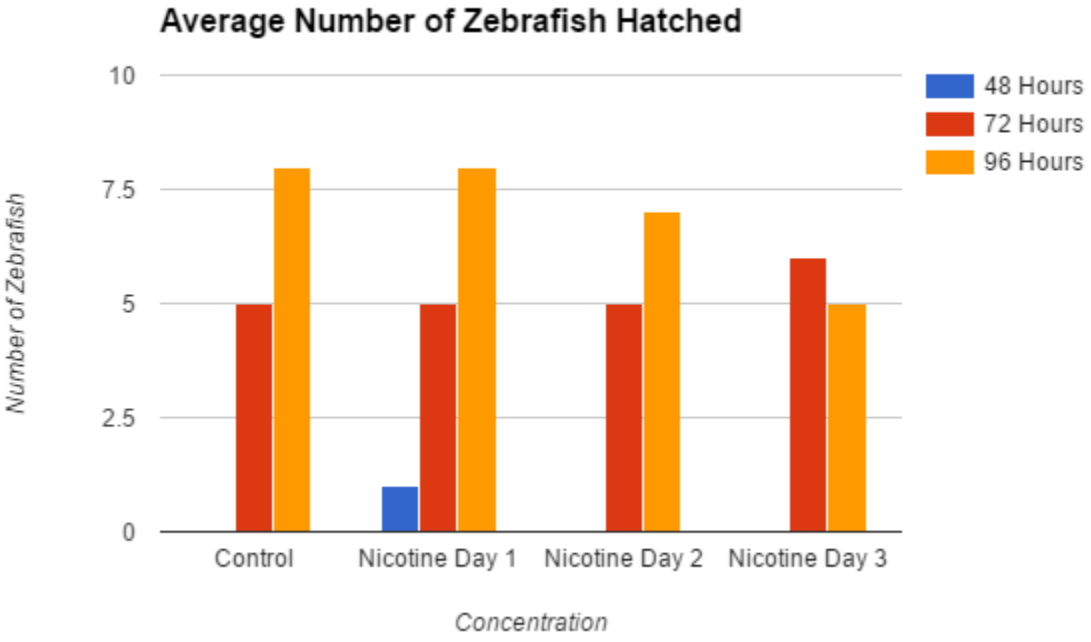


Figure 8



Data Table 3: Statistical Analysis of Live Embryos				
Concentrations	Control 24 hours	Control 48 hours	Control 72 hours	Control 96 hours
100□M Nicotine on Day 1	The two-tailed P value equals 0.3486 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.3486 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.3295 This difference is considered to be not statistically significant	The two-tailed P value equals 0.3295 This difference is considered to be not statistically significant
100□M Nicotine on Day 2	The two-tailed P value equals 1.0000 This difference is considered to be not statistically significant.	The two-tailed P value equals 1.0000 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.6433 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.4050 This difference is considered to be not statistically significant.
100□M Nicotine on Day 3	The two-tailed P value equals 1.0000 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.4216 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.1755 This difference is considered to be not statistically significant.	The two-tailed P value equals 0.1447 This difference is considered to be not statistically significant.

The statistical test used to calculate the above data was an unpaired T-test. The T-test compares the means of two groups that are independent of one another. This test is able to tell if the data sets are significant by calculating to find if the p-value is less than 0.05.

Results:

The experiment was set up to test how zebrafish embryos treated with 100□M nicotine solution at varying times would react compared to zebrafish embryos not exposed to nicotine. The independent variable was the time of exposure for 100□M nicotine solution. The dependent variables were the effects of the solution on the zebrafish embryos. The dependent variable relies on the independent variable for results. The control was the zebrafish embryos only exposed to Instant Ocean Solution. By statistical analysis it can be determined that nicotine did not have an effect on the death rate of zebrafish embryos. All of the data run through the T-test came back as not statistically significant.

Discussion:

In the experiment it is shown that zebrafish are a valuable method of research. As discussed in the results the statistical data showed no significance in the entire experiment. Although the statistical data may not have shown to be significant, the physical observations showed to have importance. Figure 1 and 4 show the same fish from different views. The fish has a crooked tail similar to fish in the previous experiment conducted by the experimenter.This fish was in the 100□M exposure on Day 1. Figure 2 and 5 show a fish with an abnormal belly. This fish was in the 100□M nicotine Day 2 concentration. Figure 3 and 6 show a zebrafish with a curved spine, this fish was in 100□M nicotine on Day 3. It can be observed from Figure 7 that zebrafish exposed to nicotine on Day 2 or 3 have a higher death rate. Although it was found not to be significantly significant it can be observed that the amount of fish dying out in the more developed fish was more common. This trend is seen in the fish exposed to nicotine on Day 2 and 3. From this trend it can be predicted that if the experiment was run for a longer period of time that the fish would have eventually died off. Figure 8 also shows that zebrafish exposed to nicotine on Day 1 hatched earlier than the fish in the control. The data and results reported above support the hypothesis "It is hypothesized that exposing zebrafish embryos to a concentration of 100□M of nicotine over different periods of time will result in death or defects in all concentrations," because there was observable deaths and defects. A limitation of this experiment is that the dosage amount of nicotine the zebrafish received may be much too high or low compared to what would be affecting a child. This experiment is extremely useful in studying the possible effects nicotine could have on an unborn child. In the future this experiment could have variables modified to further study nicotine and its effects on embryos.

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