Effect of Colgate Total Toothpaste on Zebrafish Embryos

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Abstract

This experiment tested the effect that Colgate Total toothpaste has on the early growth and development of zebrafish. Colgate Total toothpaste was chosen as it contains the chemical triclosan, which has been found in other experiments to be harmful to human health. This lab was constructed by placing 10 live zebrafish embryos into 12 separate wells. The well tray contained one well with no toothpaste which served as the control and 11 wells of increasing toothpaste concentrations. Each day the zebrafish were observed under both a stereoscope and a compound microscope. The number of zebrafish alive and the whether they were hatched or unhatched was recorded every day in the lab notebook. The data showed that zebrafish exposed to toothpaste had higher rates of death and hatched slower as compared to the unexposed zebrafish. It was also found that the number of deaths increased as the toothpaste concentration increased. This is significant because it shows the harmful effects that the toothpaste had on the zebrafish, which were subject to represent humans. This chemical is of concern because as it remains in our toothpaste, it could potentially cause human health issues.

Introduction

The purpose of this experiment is to analyze the effects of various concentrations of toothpaste on the development and growth of zebrafish embryos. The chemical triclosan has been found to be detrimental to human health and has been consequently been removed from soaps by the FDA. However, it still remains in Colgate Total toothpaste. According to the New York Times, "carcinogenicity, reproductive and developmental toxicity, eye and skin irritation, and short term and long term toxicity" can be attributed to triclosan based on a comprehensive evaluation of human safety. Triclosan has been proven to be effective but at what cost? In this lab Colgate Total will be used because it is the only toothpaste that contains triclosan. Zebrafish embryos will be used in this experiment, being exposed to different levels of toothpaste concentration. It is hypothesized that if the

concentration of the toothpaste in a well is increased, then the embryos of that well will show more

deaths because the chemical triclosan is found to be detrimental to humans and will likely be

detrimental to fish as well. Zebrafish make a good test subject for a variety of reasons, including the

fact that zebrafish share 70% of their genes with humans. Also, zebrafish eggs are fertilized and

develop outside the mother's body, making it easier to analyze their development. Zebrafish embryos

are able to absorb chemicals that have been added to their water, allowing chemicals to be induced

into their systems. Lastly, zebrafish embryos are completely transparent, allowing for easy

observations to be made. The information on zebrafish was obtained on yourgenome.org and

nc3rs.org.uk.

Methods and Materials

This experiment did not contain anything that was a direct safety threat but the lab was still completed

with care and caution as the test subjects were living organisms. Many materials were needed to

complete this experiment: 120 zebrafish embryos, Colgate Total toothpaste, beakers, several pipets

(normal and wide-bore), graduated cylinders, Instant Ocean stock solution, dechlorinated water,

parafilm, black paper, fungicide, well-tray, incubator, stereoscope, compound microscope, stirring

rods, weigh boats, balance, labeling tape, lamp, and depression slides. This lab took place over the

course of five days and observations were recorded daily. The procedure started by first obtaining 1.4

liters of dechlorinated water then mixing in 7 mL of Instant Ocean stock solution to make the

appropriate solution necessary for the embryos. Next, 0.01 grams of Colgate Total toothpaste was

weighed out in 11 different weigh boats. The 0.01 grams of toothpaste were then dissolved with the

amount of Instant Ocean solution as follows:

	1	2	3	4
A	*N/A	150 mL $(6.7\times10^{-5}$ g/mL)	140 mL $(7.1\times10^{-5}$ g/mL)	130 mL $(7.7\times10^{-5}$ g/mL)
B	120 mL $(8.3\times10^{-5}$ g/mL)	110 mL $(9.1\times10^{-5}$ g/mL)	100 mL $(1\times10^{-4}$ g/mL)	90 mL $(1.1\times10^{-4}$ g/mL)
C	80 mL $(1.3\times10^{-4}$ g/mL)	70 mL $(1.4\times10^{-4}$ g/mL)	60 mL $(1.7\times10^{-4}$ g/mL)	50 mL $(2\times10^{-4}$ g/mL)

* A1 is the control so it does not contain any toothpaste.

The number in parenthesis is the relative amount of toothpaste in each well. The concentrations had to

be extremely dilute due to the opaque nature of toothpaste. If they were not this dilute, the embryos

would not have been visible. Since there was no background information on the amount of toothpaste

that provides a practical range of exposure for the zebrafish, the concentrations were chosen by

opacity. Once all solutions were made, a trace amount of fungicide was added to each beaker. This

was done by dipping a pipet into the fungicide then dipping it into the solution. No fungicide was

actually sucked up into the pipet. Then all beakers were labeled with the correct concentrations. This

step completed the process of making the various concentrations that were used in the wells

throughout the entire course of this experiment. Parafilm was kept over the beakers to prevent any

evaporation or contamination. Once all concentrations were made, 3 mL of each were put into the

coinciding wells. Ten zebrafish embryos were added to each of the 12 wells using a wide-bore pipette

to reduce any harm the transfer would cause the embryos. Once all zebrafish were placed in the wells

they were examined under the stereoscope to make sure they were all alive. A lamp was used next to

the stereoscope to give better lighting while viewing the embryos. Black paper was placed underneath

the well tray for a more contrasting background. The well tray was then placed in an incubator at 28.5

degrees celsius each night. Every day the zebrafish were examined and counted under a stereoscope.

Any dead embryos were removed using a pipet and disposed into a waste beaker. A microscope was then used to further examine the living zebrafish. To view the zebrafish under the microscope they had to be carefully transferred from the wells to a depression slide using a wide-bore pipet. All deaths and observations, such as hatched or not hatched, were physically counted and recorded in the lab notebook. The solution in each of the 12 wells was replaced daily. This was done by removing the liquid with a pipet, being extremely careful as to not suck up any of the zebrafish, then quickly adding 3 mL of new solution of the correct concentration back to each well. Only one trial was completed for each of the concentration levels. The measurement taken was the number of living embryos in each well and whether they were hatched or not over the course of the five days. The data found for each well was then analyzed, graphed, and compared to each other. Comparisons were made between the number of living, hatched, or unhatched zebrafish in the various concentrations. Once all data was compared, a t-test was completed between the control group and the A2 well to test the significance of the results.

Results

The experiment tested the effect of Colgate Total toothpaste on zebrafish embryo growth and development. This was done by placing the zebrafish into wells of various toothpaste concentrations. The control group was the well A1 as it had no toothpaste in it. It represented the natural environment of the zebrafish and is what the toothpaste-containing wells were compared to. The experimental group consisted of the other 11 toothpaste-containing wells of different concentrations. Constants are the things that remain the same throughout the whole experiment.

The volume of the solution in each well, the temperature of the incubator, and the starting quantity of embryos in each well were all constants. The independent variable was the toothpaste concentration in each well and the dependent variable were the number of deaths and growth rate in the zebrafish in

each well. The dependent variable depends on the independent variable; the number of zebrafish

deaths depends on the toothpaste concentration. The toothpaste was found to have harmful effects on

the zebrafish, and that statement can be supported by analyzing the data. It can be seen that the

toothpaste had caused slowed rates of development in the embryos. It can also be seen that the

zebrafish exposed to toothpaste had higher rates of death than those that were not it exposed. The data

show a general trend of increasing deaths as the toothpaste concentration increased.

Data Presentation

The A1 well serves as the control as it contains no toothpaste. The concentration of toothpaste

increases from left to right down the rows.

Data Table 1: Number of Embryos Alive in Each Well on Day 2

	1	2	3	4
A	10	8	7	10
B	9	10	9	10
C	5	7	3	4

Data Table 2: Number of Alive Hatched, Half-Hatched, and Unhatched Embryos in Each Well on Day 3

	1	2	3	4
A	3 hatched 7 unhatched ------------------- 10 alive	0 hatched 7 unhatched ------------------- 7 alive	0 hatched 6 unhatched ------------------- 6 alive	1 hatched 9 unhatched ------------------- 10 alive
B	1 hatched 8 unhatched ------------------- 9 alive	0 hatched 7 unhatched ------------------- 7 alive	0 hatched 1 half-hatched 7 unhatched ------------------- 8 alive	1 hatched 9 unhatched ------------------- 10 alive
C	0 hatched 4 unhatched ------------------- 4 alive	0 hatched 6 unhatched ------------------- 6 alive	0 hatched 1 half-hatched 2 unhatched ------------------- 3 alive	0 hatched 1 half-hatched 3 unhatched ------------------- 4 alive

Data Table 3: Number of Alive Hatched, Half-Hatched, and Unhatched Embryos in Each Well on Day 4

	1	2	3	4
A	10 hatched 0 unhatched ------------------- 10 alive	0 hatched 5 unhatched ------------------- 5 alive	0 hatched 0 unhatched ------------------- 0 alive	1 hatched 1 unhatched ------------------- 2 alive
B	1 hatched 8 unhatched ------------------- 9 alive	2 hatched 5 unhatched ------------------- 7 alive	1 hatched 1 unhatched ------------------- 2 alive	2 hatched 7 unhatched ------------------- 9 alive
C	0 hatched 1 unhatched ------------------- 1 alive	0 hatched 0 unhatched ------------------- 0 alive	0 hatched 1 half-hatched 2 unhatched ------------------- 3 alive	0 hatched 0 unhatched ------------------- 0 alive

Data Table 4: Number of Alive Hatched, Half- Hatched, and Unhatched Embryos in Each Well on Day 5

|	1	2	3	4
A	10 hatched 0 unhatched -------------------- 10 alive	0 hatched 0 unhatched -------------------- 0 alive	0 hatched 0 unhatched -------------------- 0 alive	2 hatched 0 unhatched -------------------- 2 alive
B	7 hatched 0 unhatched -------------------- 7 alive	2 hatched 0 unhatched -------------------- 2 alive	0 hatched 1unhatched -------------------- 1 alive	4 hatched 2 unhatched -------------------- 6 alive
C	0 hatched 1 unhatched -------------------- 1 alive	0 hatched 0 unhatched -------------------- 0 alive	0 hatched 0 unhatched -------------------- 0 alive	0 hatched 0 unhatched -------------------- 0 alive

Graph1: Stacked bar graph showing the amount of live zebrafish each day in every toothpaste concentration
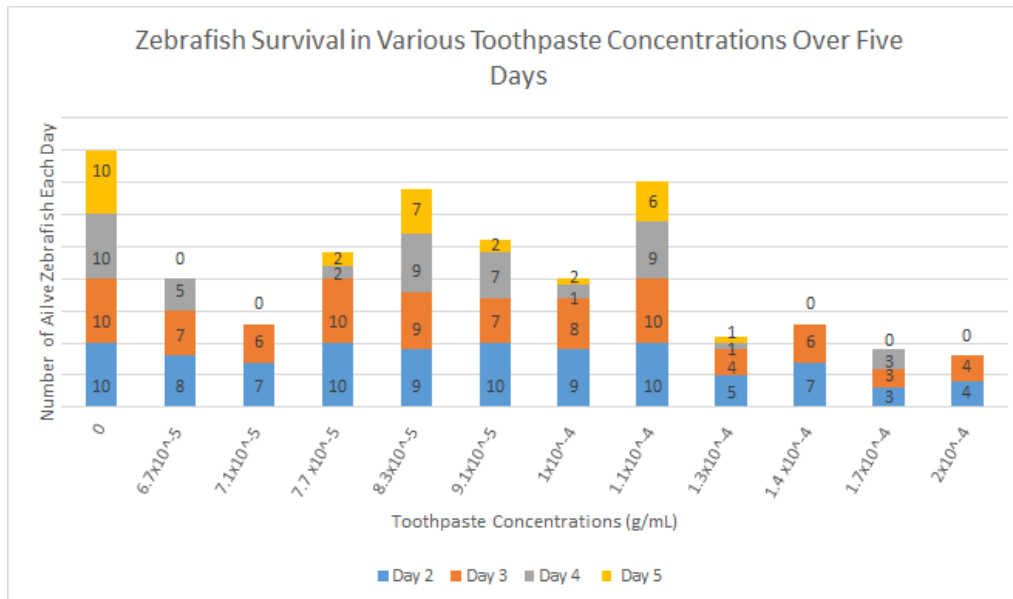
Graph 2: Multi bar graph showing the amount of zebrafish hatched and not hatched in every toothpaste concentration on Day 3
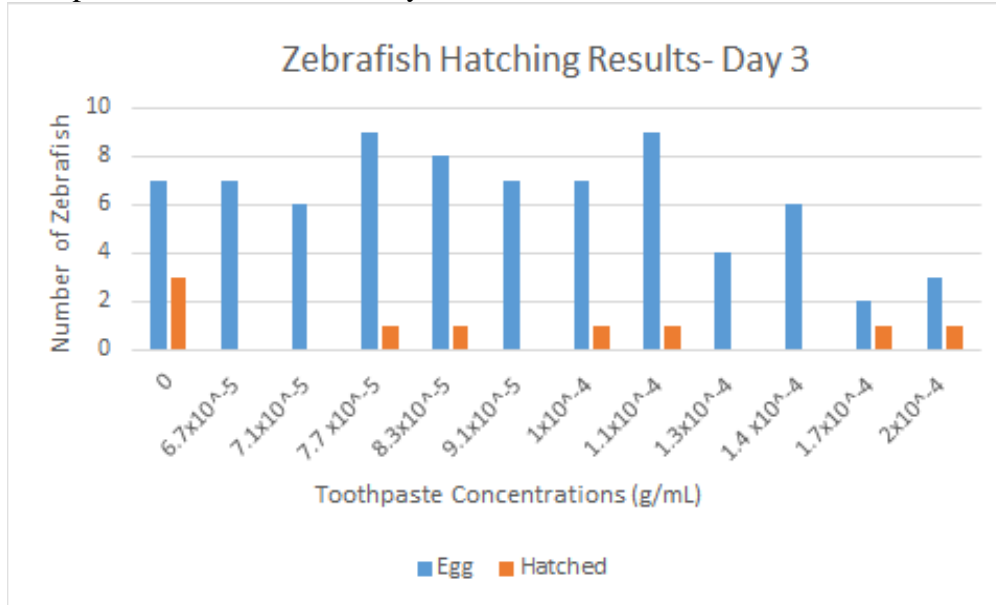
Graph 3: Multi bar graph showing the amount of zebrafish hatched and not hatched in every toothpaste concentration on Day 4
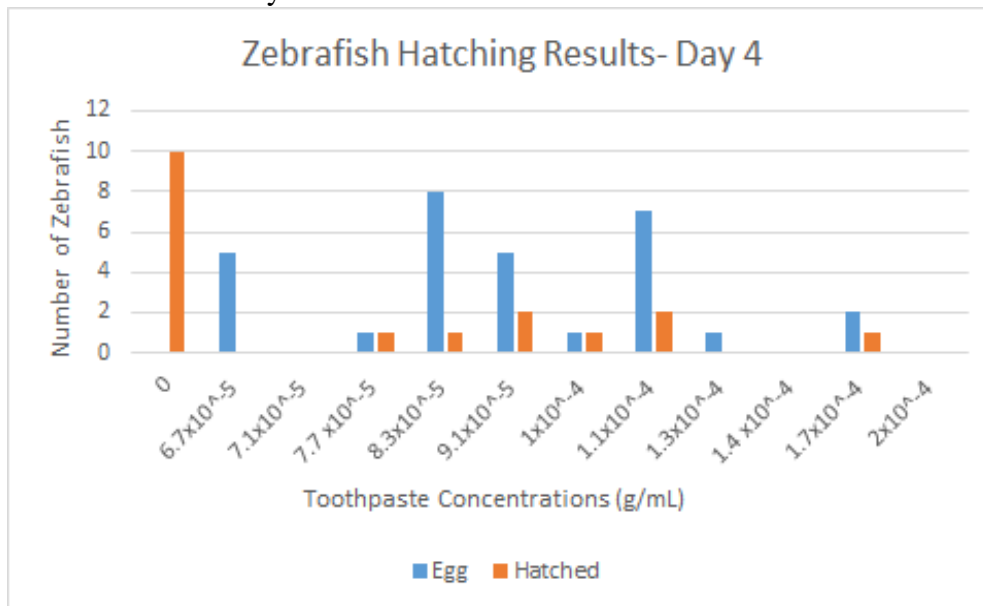
Graph 4: Multi bar graph showing the amount of zebrafish hatched and not hatched in every toothpaste concentration on Day 5
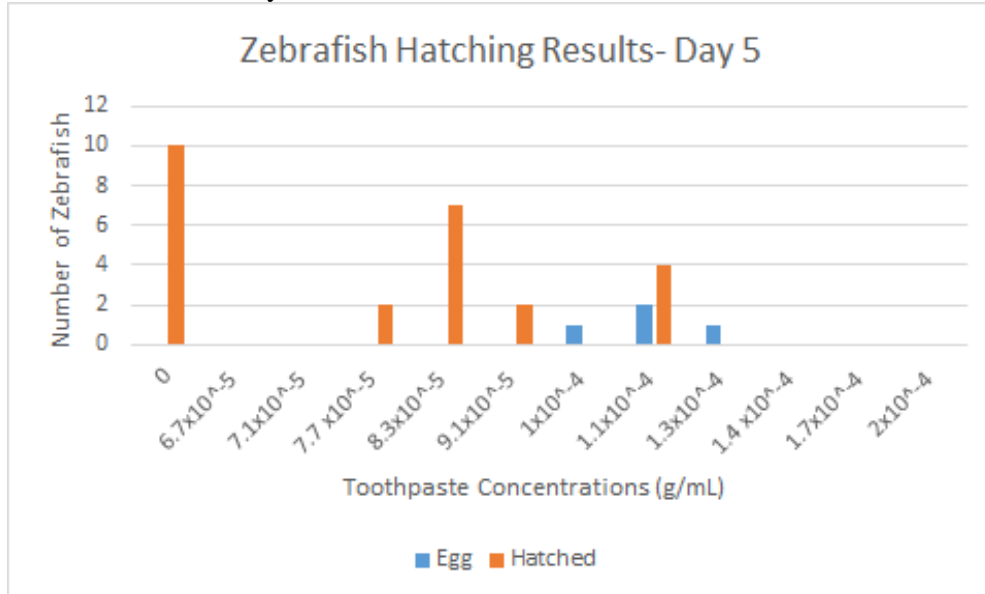
Data tables 1-4 consist of all the raw data that come directly from the observations written down in the lab notebook during the experiment. The data tables present the number of zebrafish that were alive each day and whether they were hatched, half-hatched, or unhatched. Graph 1 shows the number of zebrafish alive each day in the various concentrations. However, this graph only shows days 2-5 because on day 1 it was ensured that all zebrafish were alive. Graphs 2-4 show the number of living zebrafish that were hatched or unhatched in the different concentrations. Any zebrafish that were recorded as half-hatched were graphed as hatched since they had already partly broken through the egg. These graphs only show days 3-5 because all zebrafish were in the egg stage for the first two days of the experiment.

Data Analysis

A t-test was completed to further analyze the data acquired from the experiment. A t-test was used because it shows whether the difference found between two groups were statistically significant or not. The two groups compared were the control well (A1) and the lowest concentration well (A2). The control well represents the natural environment of the fish so it was used as a comparison in the t-test.

The lowest concentration well was used in the t-test because if it was found to be significant that would mean that all the concentration levels higher than it should also be statistically significant. The t-test between wells A1 and A2 resulted in a two-tailed P-value of .0308. Since this P-value is less than 5%, it is considered to be statistically significant. This means that there is a significant difference in the development of zebrafish exposed to toothpaste and ones that are not.

Discussion

The hypothesis stated that as the toothpaste concentration increased in a well, there would be more zebrafish deaths in that well due to the detrimental effects of the chemical triclosan contained in Colgate Total toothpaste. This hypothesis is supported by the results of the experiment. The incidence rate of deaths increased as the toothpaste concentration increased. This general trend can be observed in the data, but the concentrations were chosen without any background knowledge on appropriate toothpaste concentrations for zebrafish and may not have covered an effective range. It may be more conclusive to observe the effects of toothpaste exposure as compared to no toothpaste exposure. The data displayed that exposed zebrafish show more deaths and hatch slower than zebrafish that were not exposed. One major source of error arises from the chemicals used in the test. Originally, triclosan was the chemical of interest, but as it makes up only 0.3% of Colgate Total toothpaste, it cannot be the only chemical taken into consideration when analyzing the results. Consequently, the experiment analyzed the effects of toothpaste, not just triclosan, on zebrafish embryo growth and development. In order to fix this problem and analyze only the effects of triclosan, triclosan could have been obtained in a pure form or aqueous solution and tested in a similar experiment. Due to time and embryo quantity restraints this experiment is limited to completing only one trial at 12 different concentration levels, including the control group. This lab relates back to the safety of human consumer products. It was found that the triclosan containing toothpaste had detrimental effects on the development of the

zebrafish. The FDA has already removed this chemical from soaps, but perhaps they should further

analyze the effect the chemical can have if it continues to remain in toothpaste. The results from this

experiment give reason to believe that the triclosan containing toothpaste could potentially have

detrimental effects on its users.

References

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