# 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. Each well had an increasing concentration of toothpaste except for the control well which contained none. The number of zebrafish alive, dead, hatched, and unhatched were 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. The number of deaths also 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. In this lab Colgate Total will be used because it is the only toothpaste that contains triclosan. Zebrafish embryos will be 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 the fish as well. Zebrafish make a good test subject because they share 70% of their genes with humans, they are completely transparent, they develop outside of the mother's body, and absorb chemicals in their water.

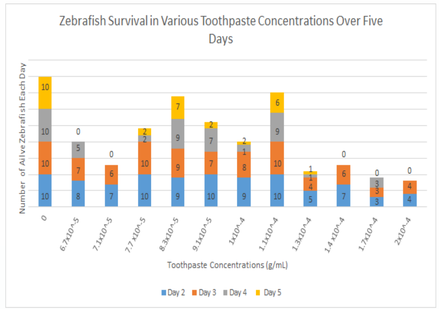
## Methods and Materials:

This lab took place over the course of five days and observations were recorded daily. The procedure started by mixing 1.4 liters of dechlorinated water with 7 mL of Instant Ocean stock solution. Next, 0.01 grams of Colgate Total toothpaste was weighed out in 11 different weigh boats and then dissolved with the amount of Instant Ocean solution as follows:

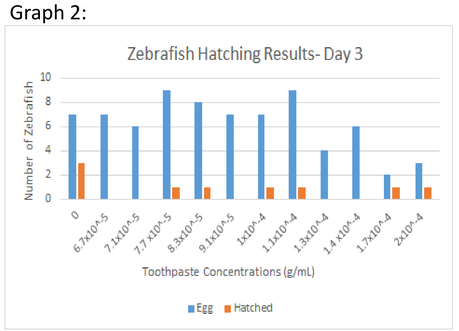
| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A | *N/A | 150 mL ($6.7x10^{-5}$ g/mL) | 140 mL ($7.1x10^{-5}$ g/mL) | 130 mL ($7.7x10^{-5}$ g/mL) |
| B | 120 mL ($8.3x10^{-5}$ g/mL) | 110 mL ($9.1x10^{-5}$ g/mL) | 100 mL ($1x10^{-4}$ g/mL) | 90 mL ($1.1x10^{-4}$ g/mL) |
| C | 80 mL ($1.3x10^{-4}$ g/mL) | 70 mL ($1.4x10^{-4}$ g/mL) | 60 mL ($1.7x10^{-4}$ g/mL) | 50 mL ($2x10^{-4}$ 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, this was labeled on the beakers. A trace amount of fungicide was added to each beaker by dipping a pipet into the fungicide then dipping it into the solution. 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 pipet. Once all zebrafish were placed in the wells they were examined under the stereoscope to make sure they were all alive. 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 and disposed into a waste beaker. A microscope was then used to further examine the living zebrafish on depression slides. 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 by removing the liquid with a pipet then quickly adding 3 mL of new solution of the correct concentration back to each well. The data found for each well was then analyzed, graphed, and compared. Comparisons were made between the number of living, hatched, or unhatched zebrafish in the various concentrations. A t-test was completed between the control group and the A2 well to test the significance of the results.

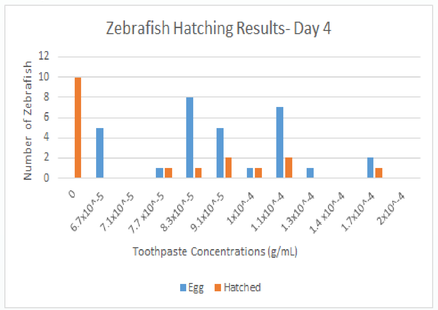
Graph1:



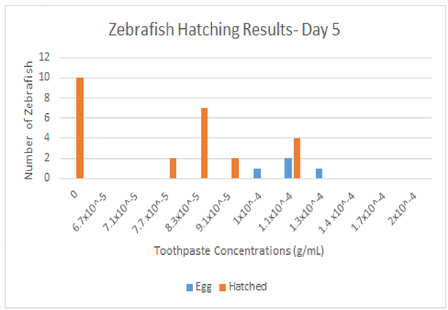

Graph 2:

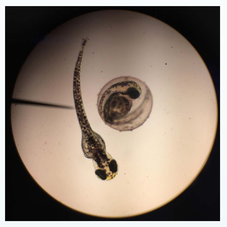



Graph 3:




Graph 4:



Well A1, Day 3

Well A4, Day 3

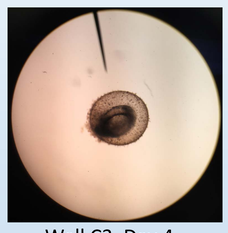
Well B1, Day 3

Well B2, Day 4

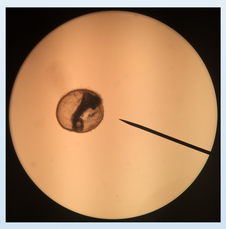
Well B3, Day 4

Well B4, Day 4

Well B4, Day 4



Well C3, Day 4



Well C3, Day 4

## Results:

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.

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. 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 number of zebrafish deaths depends on the toothpaste concentration. The toothpaste was found to have harmful effects on the zebrafish. 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 exposed. The data show a general trend of increasing deaths as the toothpaste concentration increased.

A t-test was used because it shows whether the difference found between two groups was 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. 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, triclosan could have been obtained in a pure form and tested in a similar experiment. Due to time and embryo quantity restraints this experiment is limited to completing only one trial. This lab relates back to the safety of human consumer products. The FDA has already removed triclosan 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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