The Effects of Carbaryl on the Development of Zebrafish

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Introduction

Danio rerio, commonly known as zebrafish, are tropical freshwater fish native to the Himalayan region. Once a zebrafish zygote is formed, the egg will typically hatch 72 hours post fertilization (hpf). Carbaryl is the active ingredient in many insecticides. Carbaryl is an acetylcholinesterase inhibitor (AChE) that prevents breakdown of acetylcholine (Ach) to acetate and choline at the synapse. If AChE cannot break down Ach, the muscular and nervous system will not function properly causing a low heart rate and inability to contract breathing muscles. The question of how insecticide runoff from farms affects aquatic life can be answered by testing the effects of carbaryl on the habitat of zebrafish embryos. It is hypothesized that if carbaryl is added to the environments of zebrafish embryos, then the development of the embryos will be delayed or defective. As the concentrations of carbaryl increase, the effects will also increase due to the toxicity present in the altered environments.

Materials and Methods

- 2 well plates
- Balance
- 5% carbaryl dust
- 5 pipettes
- Compound microscope
- Dissecting microscope
- Zebrfish embryos
- Instant ocean stock solution
- Graduated cylinders
- Dechlorinated water
- Deionized water
- 5 beakers

First, the well plates were prepared by washing them with deionized water and labeling 4 slots each for normal, 10 μg/mL, 20 μg/mL, and 30 μg/mL. Next, the control solution was made by mixing 5 mL of instant ocean stock solution with 1 L of dechlorinated water. Then, the carbaryl solutions were made by mixing 100 mg of carbaryl dust with 100 mL of instant ocean, and then diluting that solution to 10 μg/mL, 20 μg/mL, and 30 μg/mL. The zebrafish were then obtained and varying amounts were placed into each well. The yolk sac was deformed and the solution around the yolk sac was surrounded by paramecium.

Results

In this experiment, the independent variables were the three solutions with different concentrations of carbaryl, and the dependent variable was the amount of dead fish in each different solution. The control group was the group of zebrafish embryos placed in the solution of instant ocean with no carbaryl added. The constants were the temperature, the nutrition intake of the fish, and the size and space of their habitats. The aim of this experiment was to determine how carbaryl effects the development of zebrafish embryos, and the results obtained show the effects of this by showing the number of embryos that died in carbaryl solutions versus those that were in a non-carbaryl solution.

Results Continued

A statistical z-test examined whether or not the difference between two groups’ percents reflected a difference in the population from which the groups were sampled. The significance level of the test was .10. Calculating the z-statistic of the .01 mg/mL carbaryl solution came out to be 3.013 with a significance level of .0026. This made a claim in favor of carbaryl having an effect on the development of zebrafish. The z-statistic of the .02 mg/mL carbaryl solution came out to be 1.563 with a significance level of .1180. Because this number is greater than the .10 significance level, this does not provide significant evidence that carbaryl had an effect on the development of zebrafish at this carbaryl level. The final z-test for .03 mg/mL provided a z-statistic of 1.322 with a significance level of .10. Calculating the z-statistic of the .04 mg/mL carbaryl solution was .10. Calculating the z-statistic of the .05 mg/mL carbaryl solution was .10.

Discussion

The data supported the hypothesis because the development of the embryos was defective, a large portion of the carbaryl exposed embryos died, and it is not statistically significant. However, this experiment had many limitations and errors that could have contributed to this. If enough stress was obtained from the transporting process, the experiment would not have been completely controlled. If some dead embryos or empty egg shells were not cleaned out immediately, a chance of contamination and parasites were a risk. Naturally, all of the embryos would not survive even if they were all in the same environment. Carbaryl is illegal in other countries, but roughly 4-6 million pounds of Carbaryl is sold annually in America. Carbaryl harms insects, but in large amounts will harm humans and other organisms. Farmers need to take preventable measures to ensure safety to all living organisms, who are not the targeted species.

Works Cited


Image 3: pictured zebrafish on day three in the 30 μg/mL solution. The yolk sac was deformed and the solution around embryos contained broken embryos.

Image 1: Pictured zebrafish embryos on day two post fertilization in control solution.

Image 2: Pictured zebrafish embryos after death.

Image 5: pictured a hatched zebrafish with a zebrafish developing embryo surrounded by paramecium.

*Graph 1 Represents the percent of Zebrafish alive after each day in normal, 10 μg/mL, 20 μg/mL, and 30 μg/mL solutions.