The Effects of Vitamin B\textsubscript{12} on Developing Zebrafish Embryos
Gabrielle Sherman and Jessica Heitke
Pardeeville High School

ABSTRACT
The zebrafish is a well-known model organism that is used in many scientific studies. In this study, zebrafish were used to determine the effects of Vitamin B\textsubscript{12} on embryological development. The independent variable was Vitamin B\textsubscript{12}, the dependent variable was the zebrafish embryos and the abnormalities that occurred due to the Vitamin B\textsubscript{12} concentrations. The key results were the high amounts of abnormalities and deaths. Vitamin B\textsubscript{12} is a widely used vitamin to which the side effects of it are not clearly known.

INTRODUCTION
The zebrafish (Danio rerio) is a small fish that develops in its embryonic stage very rapidly. The fish are fully grown after a brief three-to-five day period. Their development is strikingly similar to human development, making testing variables for reactions on them easier, safer, and reliable. Their clear body in these early stages allows for easy observations and diagnosis as well. Vitamin B\textsubscript{12} is used to boost the immune system, moods, energy and concentration. Studies have shown that mothers who took vitamin b\textsubscript{12} while pregnant did have children whose mental functions were affected by it. (What is Vitamin B\textsubscript{12}, 2016). Other studies show that having too much Vitamin B\textsubscript{12} while breastfeeding could cause an increased risk of disorders, anemia, and growth failure (Mayo Clinic, 2015). Based on previous research it can be hypothesized that taking vitamin b\textsubscript{12} during pregnancy will negatively impact the development of the zebrafish embryos.

MATERIALS AND METHODS
The materials needed to perform this experiment are: zebrafish embryos that were provided by the University of Wisconsin-Milwaukee, a well plate (3x4), one large beaker, one small beaker, two different sized disposable pipettes (1.5 mm. and 1 mL), instant ocean/embryo media solution, vitamin B\textsubscript{12} (0.5 mg/mL, 1.00 mg/mL, 2.00 mg/mL), a microscope, gloves, and goggles.

Day 1
- Obtained needed materials.
- Filled the well plates with the instant ocean/embryo media solution.
- Put approximately 10 zebrafish embryos in each well.
- Let the embryos sit overnight, no data was recorded.

Day 2
- A complete well change was made, removing all the dead embryos and debris rinsing them with a clean instant ocean/embryo media solution.
- Filled the wells with the corresponding concentrations of Vitamin B\textsubscript{12}.
- Let the embryos sit overnight, no data was recorded.

Day 3
- Data was taken and observations were recorded.
- A complete well change was made with new concentrations of Vitamin B\textsubscript{12}, and all the dead and debris were removed.
- Let the embryos sit overnight.

Day 4 and 5
- Methods used during day 3 were repeated on days 4 and 5.

RESULTS
Prior to the experiment a hypothesis was made, stating that the exposure of Vitamin B\textsubscript{12} to the zebrafish embryos would cause an increased amount of death, and developmental problems within the zebrafish. The independent variable in the experiment was the Vitamin B\textsubscript{12}. The dependent variables in the experiment were the developmental issues, hatch rate, and mortality that occurred due to the Vitamin B\textsubscript{12}. The control in the experiment was the instant ocean/embryo media solution.

Vitamin B\textsubscript{12} was chosen to be tested because of how commonly it is taken and used and to see if it could have any possible negative effects along with the positive effects that it can occur due to the vitamin B\textsubscript{12}. The results supported the hypothesis by showing developmental issues among the zebrafish. The effects of the vitamin B\textsubscript{12} was much more severe in the 1 mg/mL and 2 mg/mL solutions, showing that the higher concentrations of vitamin B\textsubscript{12} taken will greatly increase the impact of problems occurring within the zebrafish. The bubble like lumps that appeared on the zebrafish, the curved spines that developed, and the drastic increase in death as the solution were increased.

DISCUSSION
The hypothesis of this experiment stated that with more exposure to Vitamin B\textsubscript{12}, there would likely be more death and abnormalities. The experiment did support this hypothesis, however the most death occurred in the medium concentration of Vitamin B\textsubscript{12}, where it had previously been assumed that the most death would have happened in the highest exposure. Another observation was the large lumps that appeared on the zebrafish in the 2 mg/mL and 1 mg/mL solutions. Based off of the research done and the evidence gained pregnant women should avoid Vitamin B\textsubscript{12}. With the numerous developmental problems that arose and the research stating that the correct amount to take is not known, it is in the best interest of the mother to avoid taking Vitamin B\textsubscript{12}. Energy drinks also contain a very high amount of Vitamin B\textsubscript{12} and should be avoided.

Future studies would need to be done in order to support the observations made. Had the experiment been longer, the number of deaths in the high solution may have matched the medium solution, which would have made the data significant. By extending the length of the experiment, further knowledge and understanding of the effects of Vitamin B\textsubscript{12} could be discovered.

References