

### CALIFORNIA STATE SCIENCE FAIR 2005 PROJECT SUMMARY

Name(s)

Michelle K. Reed

**Project Number** 

J0412

#### **Project Title**

# Glutathione Antioxidants Protect Living Organisms from Fenton Oxygen Free Radical Damage

## Objectives/Goals

#### **Abstract**

When antioxidants are advertised, they claim that they can work miracles- but are they telling the truth? The goal of my project is to test sulfur-based antioxidants to see if they could actually protect planarian worms from oxidation. I tested GSH (glutathione; gamma glutamyl-cysteine), Reduced GSH (also in capsule form), Oxidized GSH, and R-DHLA (a reduced form of Lipoic acid). This is my 3rd year of experiments and I am using a new assay to show the oxidation getting into the Planarians.

#### Methods/Materials

The overall procedure is to generate free radicals (Copper Sulfate plus Hydrogen Peroxide) and see if the antioxidants can protect the worms from the oxidation damage. Planarians are placed in wells of a 12 or 96 well dish with water. Next, antioxidants are added, then the copper sulfate and hydrogen peroxide. In the 12 well assay I observed the worm between 4 to 24 hours and assigned them a health score. The 96 well assay is called Image-iT. A chemical called Carboxyl-H2DCFDA is added to the wells and turns green in a biochemical reaction when there are free radicals present in the worm cells. The worms that are harmed by the free radicals take in the dye and with a special wave length of light the green dye is turned into numbers by a machine called a fluorometer. I used a fluorescent microscope with a digital camera and I also used a special Intel microscope to collect time lapsed pictures of the free radical attack on Planarians.

#### Results

R-DHLA was too toxic, so I dropped it from the rest of my experiments. So, now my experiments became a comparison of Red. GSH capsules, Red. GSH, and Oxidized GSH. In my visual score experiments the Red. GSH worked best, followed by the Red. GSH capsules and then Oxidized GSH. In the Image-iT experiments, the Red. GSH and capsules were very similar. However, the Oxidized GSH worked best.

#### **Conclusions/Discussion**

I had hypothesized that the Red. GSH would protect better then the capsules. In one set of assays that is the case, but in the other they tied. I expected the oxidized GSH wouldn#t work at all since it is already oxidized. However, my Image-iT results led me to research more about the biochemistry of oxidized GSH. I realized that it makes perfect sense, the worm must have actually taken the oxidized form and reduced it in its tiny body so it could scavenge free radicals again and again. This also helps prove the antioxidants are getting into the planarian too.

#### **Summary Statement**

I am determining which sulfur-based antioxidants protect planarian worms the best from free radicals created in a Fenton Reaction.

#### **Help Received**

My mom and dad both helped me get supplies and supervised my experiments. My dad helped me with the LIVE-iT dye and chemical information. I used equipment at my mom and dad#s work.