JOURNAL OF
EMERGING INVESTIGATORS

Article

The impact of Red 40 artificial food dye on the heart rate of *Daphnia magna*

Samay Inampudi, Megan Schwab, Annika Srivastava, Rao Inampudi
Oswego East High School, Oswego, Illinois

SUMMARY

Due to its relatively new appearance in foods, not many studies have been conducted on the effects of Red 40 food dye, therefore one aspect of its effects on the body should be explored. Red 40 is a chemical present in thousands of consumer products that poses potential health risks with one's heart rate and hemoglobin functioning. The purpose of this study was to examine the effects of Red 40 food dye on the cardiac activity of *Daphnia magna*, a 2 mm long freshwater crustacean. We hypothesized that a higher concentration of Red 40 dye would result in an increased heart rate and activity in the *Daphnia magna* because of a need for increased oxygen intake, due to Red 40's impact on hemoglobin molecules. *D. magna* were placed into Petri dishes containing Red 40 solutions ranging from 0% to 5% concentrations for five minutes. At the five-minute mark, the specimens were transferred to a glass slide and placed under a microscope to record their heart rates. The recorded video was slowed down to count the heart rate of each specimen, and the data was recorded. The results showed that as the concentration of the Red 40 solution increased, so did the heart rate and activity level of the specimen. We noticed the D. magna becoming more active and sporadic when concentrations increased as well.

INTRODUCTION

It is vital for the general public to understand the effects of Red 40 and its impacts on human health due to the relatively new uprising in artificial food dyes and its increased consumption around the world. The basic consumer appeal may increase economic value, but we know little to nothing about its adverse health effects and its impact on human health.

Red 40 is a chemical compound derived from coal tars that scientists produce to use as artificial dye (1). As an additive found in thousands of consumer items ranging from food to personal care, Red 40 can pose a threat to the health of people. Numerous studies have observed significant side effects of individuals exposed to Red 40 including allergic reactions, reduced reproductive success, and increased hyperactivity (1). The continued use of Red 40 presents an unnecessary risk to humans, especially among young children, to whom many Red 40-containing products are targeted.

Daphnia magna are clear, freshwater crustaceans that share the most genes with humans out of all sequenced invertebrate genomes, making them suitable candidates for a model organism (2). In this study, we researched the effects of Red 40 on the heart rates of *D. magna* to better understand the correlation between hyperactivity and exposure to Red 40. *Daphnia magna* are extremely sensitive to changes in their environment, and this can be monitored through their heartbeat (3). *Daphnia magna* consume particles up to 50 μm in size (4) and readily absorb toxins and other ions through chloride-absorbing glands, which makes them extremely susceptible to being poisoned in toxic environments. Therefore, this organism is extremely effective for visually seeing and testing the toxicity of various solutions.

To build off of the limited studies on the impact of Red 40 on organisms, we hypothesized that exposing *D. magna* to high concentrations of Red 40 would cause their heart to beat faster. After exposing them to this red 40 dye, we observed and recorded an elevated heart rate and a more active specimen as the concentrations became stronger.

RESULTS

To evaluate the impact of Red 40 dye on the heart rate of *D. magna*, we exposed the organisms to six different

Figure 1: Control specimen. Image of the Daphnia magna's internal anatomy. Based on the orientation of the image, the black dot on the right side of its body is the eye. The "hook" shape that spans horizontally across the D. magna represents its gut. While not clearly visible due to the inability to see movement in a picture, the heart is located above the right section of the gut in this image. As the concentration of Red 40 increased, we found that the translucency of the Daphnia magna decreased, and it appeared more red in color.

concentrations (0%-5%) of Red 40 solution. Ten *D. magna* were placed into reservoirs containing no Red 40 (0%) as our baseline control condition, or 1%, 2%, 3%, 4% and 5% Red 40. In each condition, they acclimated to the environment for five minutes before we began the heart rate measurements, which allowed the *D. magna* to absorb solution. This also reduced the possibility that their heart rate changes were caused by the new environment instead of the Red 40 solution. Immediately after acclimation, we moved the *D. magna* from the Red 40 solution to be observed under a microscope for one minute.

For each concentration of Red 40, we recorded the heart rate for ten *D. magna*. As the concentration of Red 40 food dye increased, so did the heart rate of the *Daphnia magna* (**Table 1**). There is a positive correlation (r = 0.9926) between each concentration and the heart rate (**Figure 2**). As one can see there is a steady incline between the Red 40 concentration and the heart rate of the *D. magna*. Between the 0 and 1 percent solutions there is a 12.5% increase, the 1 and 2% solutions have a 3.9% increase, the 2 and 3% have a 6.6% increase, the 3 and 4% have a 7.2% increase, and the 4 and 5% have a 8.2% increase. This was tested through a chi-squared test, finding a significant impact of Red 40 on the heart rate of *D. magna*, X^2 (5, N = 60) = 112.7, p = 0.05.

DISCUSSION

This experiment intended to find the correlation between the concentration of Red 40 and heart rate of *D. magna* through exposing these organisms to various concentrations of Red 40, and then recording their heart rate. We hypothesized that if *D. magna* are exposed to Red 40, then their heart rate will increase. Our data supports our hypothesis by showing a consistent increase in the heart rate of *D. magna* with increasing percent concentration of Red 40. The results of this experiment show that Red 40 affects the heart rate of the specimen *D. magna*. The linear correlation between heart rate and the concentration of Red 40 signals a stress response in the organism, which contributes to additional negative health effects.

Our experiment used concentrations of Red 40 comparable to the amount of Red 40 that humans consume in their lifetime to make the results as biologically relevant as possible. According to Doell and colleagues, individuals with high exposure to Red 40 consume an average of 0.3 mg/day (5). Although it may be difficult to define levels of harmful exposure, any consumption of Red 40 contrasts with previous dietary practices. Given our experimental limitations, we were unable to measure how much Red 40 the *D. magna* absorbed from their aqueous environment, but we know some absorption occurred since the *D. magna* were dyed red after five minutes in the solution.

Table 1: Percent solution and Daphnia magna heart rate of each trial based on the percent solution. This chart shows the beats per minute of every Daphnia magna in their respective percent solution. There are 60 Daphnia magna specimens total and 10 Daphnia magna specimens were tested for each percent solution.

The impact that different concentrations of Red 40 Food dye have on the heart rates of *Daphnia magna*

Percent Solution	Trial 1 (Daphnia's BPM)	Trial 2 (Daphnia's BPM)	Trial 3 (Daphnia's BPM)	Trial 4 (Daphnia's BPM)	Trial 5 (Daphnia's BPM)	Trial 6 (Daphnia's BPM)	Trial 7 (Daphnia's BPM)	Trial 8 (Daphnia's BPM)	Trial 9 (Daphnia's BPM)	Trial 10 (Daphnia's BPM)	Average BPM
0%	254	235	232	262	243	240	236	237	242	249	243.0
1%	260	272	284	298	258	272	265	288	278	280	275.5
2%	279	294	283	286	281	301	292	275	283	291	286.5
3%	314	304	308	296	316	319	292	301	287	324	306.1
4%	334	325	335	329	331	328	331	329	322	327	329.1
5%	352	336	373	390	333	345	347	396	354	346	357.2

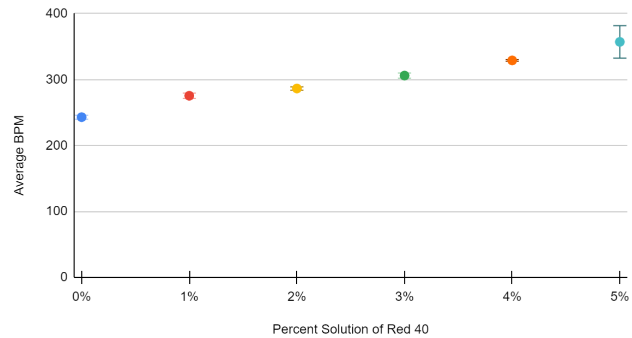

Figure 2: Average heart rate (beats per minute) as a function of percent Red 40. This is a visualization of the relationship between average beats per minute of the Daphnia magna and percent Red 40 solution with error bars (R^2 = 0.985).

There is a possibility that the increased heart rate of the *D. magna* may be due to their exposure to a new environment. Although this is a valid scenario, the five-minute acclimation period and the control group limits the likelihood of this possibility. Through the five-minute acclimation period, the *D. magna* have time to adjust to their environment and begin to carry out normal biological functions. For future experiments, researchers may want to incorporate a longer acclimation period to see if this impacts the heart rate of the *D. magna*.

To apply the findings with the *D. magna* to humans, we can look at the similarities with their circulatory systems, specifically with hemoglobin. The structure of the hemoglobin protein in humans is extremely similar to the structure of the hemoglobin protein in the *Daphnia magna,* so these organisms may exhibit similar interactions with Red 40 dye. Looking first at the *D. magna,* within the specimen's hemolymph, there are hemoglobin proteins that carry oxygen throughout the hemocoel. However, this process is interfered with when exposed to concentrations of Red 40. If the hemoglobin is functioning correctly, this protein will interact with other compounds within the hemolymph. However, Red 40 interferes with these protein interactions (6), as the dye binds to the central cavity of hemoglobin. The binding of Red 40 to hemoglobin weakens its polypeptide bonds and results in conformational changes, which impacts its ability to bind with oxygen and carbon dioxide. With higher concentrations of Red 40, the amount of Red 40 bound to hemoglobin molecules increases as well, causing more hemoglobin molecules to lose function. Due to this change in the functional hemoglobin content, less oxygen is circulated around the hemocoel of the *Daphnia magna*, causing the specimen to increase its heart rate so more oxygen can enter the body (6). Considering the similar structure of hemoglobin proteins between humans and *D. magna,* it is possible that this same problem with the hemoglobin occurs with humans as well.

Further research is also needed to establish the effects these food dyes may have on neurological disorders such as attention-deficit hyperactivity disorder (ADHD). ADHD is a disorder characterized by an abnormal state of hyperactivity that results in irritability, impulsiveness, forgetfulness, and rapidly repeated behaviors and actions (7). Many studies have shown a clear relation between hyperactivity in children and Red 40 consumption (1). For example, previous findings have shown that limiting Red 40 in the diets of those with ADHD reduces its common symptoms (8). Findings such as these suggest that Red 40 may be a contributing factor to ADHD symptoms, including the possibility that it could be a cause of smaller brain volume (1). In an article by Lofthouse and colleagues, the researchers claimed that artificial food dyes are not the primary cause of ADHD but could be significant enough to push some people over the diagnostic threshold. In addition, the authors stated that food dyes could have effects on the brain without crossing the blood-brain barrier (8). Red 40 has been identified in several neuromuscular junctions, which is the synapse between a motor neuron and a skeletal muscle fiber. Presynaptically, the Red 40 food dye causes increases in the frequency and amplitude of end-plate potentials (9). End-plate potentials are the depolarization of muscle fibers caused by motor neurons releasing a neurotransmitter onto those muscle fibers, and they can lead to muscle contraction. By increasing the amplitude and frequency, Red 40 causes a muscle to contract more frequently with more vigor. Postsynaptically, Red 40 and related artificial food dyes increase the potassium conductance in muscle fibers, which allows for increased muscle activity (9). In the brain, dopamine uptake is a presynaptic mechanism in which the neuron terminates the action of the neurotransmitter dopamine in the synapse. Red 40 has been recognized to decrease dopamine uptake, which can lead to hyperactivity (9). Lastly, Red 40 and other artificial dyes also decrease the incidence of non-specific binding, which means that a ligand can bind to receptors other than high affinity partners. Normally, this can allow for action potentials to be terminated faster, but since it is decreased in the presence of Red 40, the inability to stop a certain task comes back into play (9). This is because the Red 40 interferes with this process through competitive inhibition. Through end-plate potentials, dopamine uptake, and non-saturable binding, it can be understood how and why Red 40 and other similar synthetic food dyes, including but not limited to erythrosin B, Blue #1, Yellow #5, and Yellow #6, affect the body, including hyperactivity. Although food dyes have become a part of everyday life, people should attempt to be very conscious when selecting foods and other products with such dyes due to their potential adverse health consequences.

MATERIALS AND METHODS

To create the control Red 40 solution, we filled two Petri dishes with 40 mL of spring water and put five *Daphnia magna* in each. After this, we created the 1-5% Red 40 solutions. Prior to creating the other solutions, we created a 10% Red 40 solution by filling a Petri dish with 36 mL of water and 4 mL of Red 40 food dye. This 10% solution was then diluted to create 1%, 2%, 3%, 4%, and 5% concentrations, with 2 petri dishes of solution per concentration. Following this, we added 5 *Daphnia magna* to each of the Petri dishes excluding the 10%.

To collect the data, we allowed each of the *Daphnia magna* to acclimate in their Petri dishes for 5 minutes before beginning data collection. The order in which we collected data went from the control group up to the 5% Red 40 group. We did not add the specimens in the next condition to the Petri dishes until finished with the previous condition to minimize time discrepancies. Then, after acclimation and using a pipette, we transported individual *Daphnia magna* while minimizing the water taken. The organisms were then placed onto a microscope slide (with no slide cover), and we used a paper towel to soak up excess water to minimize the movement. We recorded the *Daphnia magna* through the Optical Glass Lens Student Biological Field Microscope for one minute and then placed the *Daphnia magna* into a jar filled with 180 mL of spring water. We repeated this step for all of the organisms. We then slowed down the videos to accurately count the heart rate and record data. A heartbeat was defined as each time the heart contracted to the pericardium. Then, we counted the amount of contractions in one minute to calculate the beats per minute.

To mathematically justify our conclusions, a chi-squared analysis was done. The null hypothesis was that the higher concentration of solutions does not impact the heart rate of the *Daphnia magna*. This Chi-squared was done with a significance level of 0.05 and 5 degrees of freedom. The expected value was the mean of the control group because the null is that the food dye is not affecting the organisms. The following equation was used to calculate the Chi-squared value:

$$X^2 = \frac{(243-243)^2}{243} + \frac{(275.5-243)^2}{243} + \frac{(286.5-243)^2}{243} + \frac{(306.1-243)^2}{243} + \frac{(329.1-243)^2}{243} + \frac{(357.2-243)^2}{243}.$$

In order to calculate the error bars as seen in Figure 2 we had to calculate several components. First, one must find the standard deviation of each different concentration. All the error bars refer to the standard error of the mean.

Received: October 29, 2020
Accepted: April 1, 2021
Published: July 23, 2021

REFERENCES

1. "Red 40 Side Effects." *Institute for Integrative Nutrition*, 2020. www.integrativenutrition.com/blog/2016/09/red-40-side-effects
2. "The Most Genes in an Animal? Tiny Crustacean Holds the Record." *National Science Foundation*, 2011. www.nsf.gov/news/news_summ.jsp?cntn_id=118530
3. Deken, Anne. "SEEING RED: Daphnia and Hemoglobin." *Summer Research Fellowship for Science Teachers*, 2005. www.nslc.wustl.edu/elgin/genomics/srf/Daphnia6-8.pdf
4. Ebert, Dieter. *Introduction to Daphnia Biology*. 1 Jan. 1970. www.ncbi.nlm.nih.gov/books/NBK2042/
5. Doell, Diana, *et al*. "Exposure estimate for FD&C colour additives for the US population." *Food Additives and Contaminants*, vol. 33, no. 5, 2016, pp. 782-797. doi:10.1080/19440049.2016.1179536
6. Saurabh, Bansal, *et al*. "A Study of the Interaction of Bovine Hemoglobin with Synthetic Dyes Using Spectroscopic Techniques and Molecular Docking." *Frontiers in Chemistry*, vol. 4, 2017, pp. 50. doi:10.3389/fchem.2016.00050
7. "Attention-deficit/hyperactivity disorder (ADHD) in children." *Mayo Clinic*, 2019. www.mayoclinic.org/diseases-conditions/adhd/symptoms-causes/

syc-20350889?utm_source=Google&utm_medium=abstract&utm_content=Attention-deficit-hyperactivity-disorder&utm_campaign=Knowledge-panel

8. Lofthouse, Nicholas, *et al*. "Artificial Food Colors and Attention-Deficit/Hyperactivity Symptoms: Conclusions to Dye for." *Neurotherapeutics*, vol. 9, no. 3, 2012, pp. 599-609. doi:10.1007/s13311-012-0133-x
9. Lafferman, Jeffrey, *et al*. "Erythrosin B Inhibits Dopamine Transport in Rat Caudate Synaptosomes." *American Association for the Advancement of Science*, vol. 275, no. 4404, 1979, pp. 410-412. doi:10.1126/science.451609