

How do the various dilutions of nicotine affect heart rate and behavior of Daphnia?

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Introduction

“In every puff there is a little taste of death,” said Richard Klein (Pringle, 1996). Most people who smoke don’t pay attention to the life- long consequences of smoking. People ignore the fact that every breath of smoke they take will reduce their lifespan. Nicotine is one of the substances in cigarettes that come from the plant *Nicotiana tabacum*. Nicotine is an organic compound that makes up 5% of a tobacco plant. One single cigarette contains 8 to 20 milligrams of nicotine, but a person actually takes up 1mg from that one cigarette. Most of the nicotine is degraded by heat. When people inhale nicotine through smoking, it is absorbed quickly into the bloodstream and reaches the brain in less than 10 seconds. It also stimulates the nervous system and causes relaxation (Pringle, 1996). This often leads to nicotine dependence (Chudler, 2007). Nicotine causes a short-term increase in blood pressure, heart rate and flow of blood (American Heart Organization, 2007). In this experiment, nicotine’s effect on the heart rate of a model specimen, *Daphnia Magna* is investigated. **Through the similarities *Daphnia* and humans’ share, it is expected that nicotine will increase the heart rate of the daphnia immediately. With increase in nicotine concentration, the heart rate will rise, putting the specimen at greater risk of high blood pressure and heart disease.**

Background Information

One of the leading causes of deaths in the United States today is from smoking. (Martin). Smoking accounts for around 20,000 deaths from Heart Disease (Napier, 1996). In 2004, The Centers for Disease Control and Prevention estimated that 44.5 million U.S. adults smoked. In 2004, CDC also found out that 22% of high school students and 8.1% of middle school students were smokers (American Heart Association, 2007).

One of the reasons why people smoke is because it stimulates the dopamine area of a person’s brain. Dopamine is a neurotransmitter that is responsible for pleasure and contentment.

This indulges people to smoke more. It is very interesting that a lot of people have ignored the life-threatening consequences of smoking. Each year, fewer than 1 out of 10 succeed in quitting smoking (Pringle, 1996).

Thus, this investigation evidently addresses an important health issue. Nicotine may be pleasurable, but causes fatal illnesses such as cancer, emphysema, heart disease, and stroke in humans. This experiment is significant because people tolerate different amounts of nicotine. Understanding the limitations of people's bodies can help predict possible diseases that people can be at risk for and initiate preventable measures. While it is simple to say that it's easier to make nicotine an illegal substance, the idea is much better said than done. The tobacco industry in the United States generates \$47 billion in profits a year (Pringle, 1996). This large amount of money spent creates a huge influence on society's people. The best way to address the dangers of the drug, is conducting sound scientific research that raises awareness of nicotine's various health effects. Speeches made against antismoking can only be achieved through specific information and multiple experiments that support the harmful effects of nicotine use.

Because multiple experiments are not brought up in speeches against smoking, the public has many misconceptions about nicotine consumption. Based on a telephone survey in the journal *Tobacco Control*, of the 6,000 people that participated, 1,245 of them being current smokers believe that exercise would undo the effects smoking has on the body. Thirty-six percent of the people believed that lung cancer development could only be inherited. Also 28% of people in the survey believe that vitamins would reduce the risk of lung cancer (American Cancer Society, 2005). From this data, Neil Weinstein, a professor in the Human Ecology Department at Rutgers concluded that, "Even though people know smoking is unhealthy, there isn't much they encounter that tells them how big the risk is" (American Cancer Society, 2005).

Therefore, people should not smoke because they do not know how vulnerable they are to diseases.

Though, nicotine can cause heart disease in the long run, the immediate reaction people get from nicotine is hypertension (high blood pressure). In nearly, 10 minutes of smoking, a person's heart rate increases by 30%. The blood vessels compress, causing the heart to work harder to transport oxygen successfully throughout the body (Napier, 1996). There is a difficulty in transporting blood to the arteries, which take blood away from the heart to other parts of the body. Thus, the force of blood pushes against the walls of the arteries. In addition, when a person smokes frequently, their heart rate elevates. The blood pressure of a person varies. High blood pressure can eventually lead to coronary heart disease such as arteriosclerosis. Arteriosclerosis is when the arteries become narrow, after the lining of the blood vessels is damage from nicotine. Fatty substances and platelets accumulate and clamp, forming blood clots in the damage area. The platelets release additional substances that lead to greater blockage in the heart flow towards the heart. Eventually, it will causes heart disease and/or strokes (Napier, 1996). Heart disease occurs when nicotine use overtime causes high levels of LDL cholesterol (harmful cholesterol) to clog up a person's arteries (Martin, 2004).

Nicotine also disrupts the homeostasis in other organs of the body. Nicotine plays a negative impact on the digestive system because the drug restrains the release of insulin from the pancreas. As a result, there is a sudden rise of sugar in the. Insulin is there to help in the digestion and absorption of sugar from the foods people eat into body cells. The sugar is then stored as fat and leads to having more weight.

Along with having a negative impact on the digestive system, nicotine can also increase heart rate and blood pressure through the nervous system. Nicotine use can lead to a discharge in

the epinephrine (adrenaline) of the adrenal cortex. This excites the central nervous system and endocrine glands, which helps release glucose from storage units in the body to the brain for stimulation. Epinephrine also makes the body store glucose into the blood stream (Volkow, 2006).

When nicotine is absorbed and gets to the brain, the acetylcholine receptors get active, creating a disruption in brain function. Acetylcholine receptors are neurotransmitters that carry messages between muscles and nerves. Acetylcholine receptors control respiration, heart rate, memory, and movement. Nicotine also affects the cholinergic receptors that are usually stimulated by acetylcholine receptors (Martin, 2004).

Ultimately, nicotine affects many parts of the brain that controls the heart rate and blood pressure. As a result, nicotine overdose leads to high blood pressure and an increase in heart rate.

Animal Studies On Nicotine and The Heart

At the Division of Cardiology at Cedars of Sinai Medical center, researchers have done studies on the effect of nicotine on canine hearts with myocardial infarction. Myocardial infarction is when the blood in the heart itself is cut off. The heart muscle receives an insufficient amount of oxygen. Scientists used canine hearts with a blocked left anterior descending coronary artery. An intravenous tube is a tube, exposing the nicotine directly into an organism's vein. Cardiac fibrosis is when fibrous connective tissue spreads and replaces smooth muscle on heart, lung or kidney (Van, 2005). The smooth muscle gives the heart, the tough and elastic structure to pump blood efficiently throughout the body. Connective tissue is usually loose and meshed for the function of supporting organs. Thus, the connective tissue does not have the strength and elastic structure to pump blood. Researchers found out that nicotine causes cardiac fibrosis through the study.

Another study investigated was the effect of nicotine on hearts with healed myocardial infarction. The approach of the study examined the hearts through surgery. In multiple studies, it was proven that smoking leads to coronary heart disease, myocardial infarction and a huge risk of chronic obstructive pulmonary disease. This also raised a possibility that nicotine and atrial flutter was link to patients with myocardial infarction. A person with myocardial infarction heart beats 400 beats per minute. The rhythm of the heart depends on the atria and ventricles working collaboratively with each other. In atrial flutter, the heart beats very quickly because the atria cannot fully transport blood to the ventricle. The blood left behind in the atria does not flow and can lead to heart stroke. The ventricles in a human heart are important because the right ventricle transports deoxygenated blood to the lungs, where it then becomes oxygenated. The oxygenated blood is transported to left ventricle, which gives blood to the body’s cells (Van, 2005). Van’s study had four groups in their investigation. In the lab there were four groups being tested.

Figure 1: Canine Hearts

MI and surgery	Nicotine, surgery and no MI	There is no Mi, no surgery and no nicotine	Mi, surgery and nicotine
∅ Flutter	∅ Flutter	∅ Flutter	√ Atrial flutter

(Van, 2005).

From the results, the researchers found out that nicotine increases atrial interstitial fibrosis. Atrial Interstitial fibrosis is a two-fold increase in the left atrium and a 10- fold in the right atrium with the MI group. An enlarged atrium is the heart’s attempt to try to maintain the amount of blood going into the ventricle. In the non MI group, the right atrium experienced was

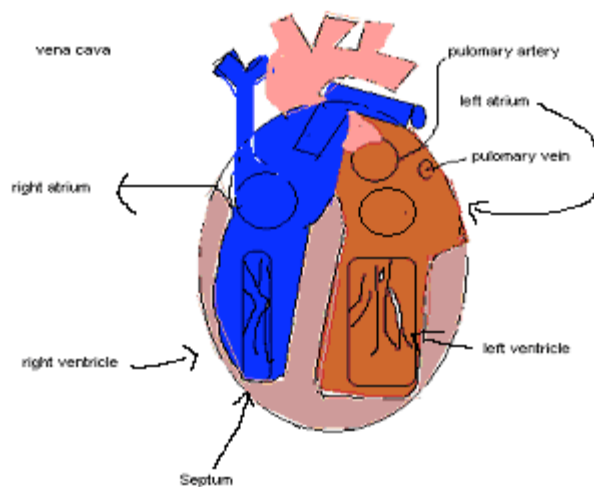
a 2-fold increase. Though the canine hearts didn't have Myocardial Infarction, they still had a decrease in the utility of the heart. Even those with MI surgery and consumed nicotine got atrial flutter, due to the fact that the heart was already affected. Furthermore, the Center For Disease Control and Prevention found out that smoking caused a two-to-three fold of atrial interstitial fibrosis. Through 1995 to 1999, approximately 442,398 Americans died from illnesses related to smoking. 33.5% were cardiovascular diseases. (Van, 2005). Therefore, the mechanism in which nicotine affects the heart is a complex process.

Another test done was on the effect of nicotine on the nervous system and blood pressure of frogs. Yan- Yi Peng, an assistant professor of pharmacology and Ying-Jun Cao, a postdoctoral researcher, did this experiment. They exposed nicotine and stimulated a neurotransmitter called utilizing hormone releasing hormone of the frog neurons (LHRH). LHRH triggers the release of other neurotransmitters such as norepinephrine, which restricts blood flow. Norepinephrine is the epinephrine in frogs.

This is what leads to high blood pressure. The scientists concluded that frogs and human nervous systems were similar. If a small amount of nicotine could caused a short-term blood pressure on frogs, then humans would react similarly and get hypertension (University of Chicago Medical Center, 1998). The scientists believed that the release of neurotransmitters is only due to calcium. They didn't know that nicotine stimulated LHRH. In this case, calcium was not needed to cause stimulation. Also, they found that nicotine affects the glial cells, which are located at the end of the axon. Glial cells controls signals and messages between neurons. Peng and Cao thought that fixing glial cells could regulate the messages passed through neurons. This could be an alternative to treating smokers who's brain was affected by nicotine use.

Using daphnia as a Model Specimen

Daphnia are similar to humans and would be an ideal specimen to observe the effect nicotine has on the heart. One example is that the daphnia and humans both have cellular respiratory systems where they take in oxygen to breathe and expel carbon dioxide as the waste product. The respiratory system relies on the circulatory system for blood. As well as sharing a commonality in the cellular respiratory system, Daphnia and humans have hemoglobin in their blood to transport oxygen molecules. While humans, have lungs for the exchange of gases, the daphnia have gills for this purpose (Biomedical associates 2).

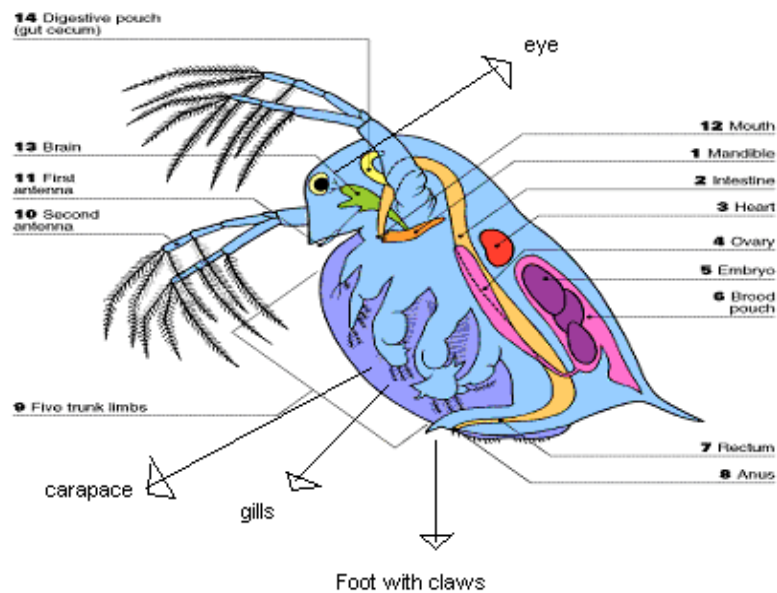


Furthermore, the hearts of the daphnia and humans are alike. Humans have a circulatory system where their heart distributes blood to the other parts of the body. The veins are blood vessels that bring blood to the heart. The heart rate of an average human being is 60 to 80 times a minute (Bruun, 1982).

While the human heart is structured as chambers, the daphnia heart is just one big sack of muscle tissue. Daphnia has a simple heart filled with blood. It is a muscle that forces blood to the

whole body through contractions. It is open at both ends. At one end, it takes in deoxygenated oxygen, while the other end dispels carbon dioxide. The daphnia's heart is located on the back behind its head (Stein, 2007).

Additionally, the daphnia is a crustacean with a clear exoskeleton that is transparent, which is helpful because scientists can view the body processes going on. Because, it is transparent its easy to look at the heart rate of daphnia. Because of the similarities between humans and daphnia, testing the effect nicotine has on daphnia can be useful because if nicotine creates dangerous effects, humans will most likely face the same issues. Moreover, smokers will see the severe damages nicotine can cause and possibly stop smoking to become healthier.



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(Laurence Hall Of Science, 2006)

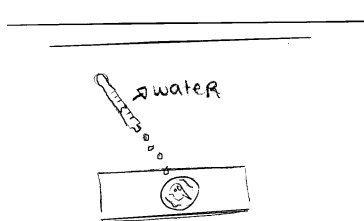
Methods:

In this experiment, bioassays were done to see what amounts of dilutions affect the daphnia's heart rate greatly. There were dilutions of 20%(20 μ l), 40%(40 μ l), 60%(60 μ l), 80%(80 μ l) and 100%(100 μ l) of nicotine. Water was a solvent used. Small dilution differences were used to account for the daphnia's smaller size compared to that of a human. The original solutions were made in a total of 100 μ l.

A tiny portion of petroleum jelly was used to slow daphnia movement, so that it was easier to test the heart rate of the daphnia in 10 seconds. Videos were taken to view the daphnia without the nicotine solution and with nicotine. The independent variable is the amount of nicotine in microliters (μ l) and the dependent variable is the total amount of daphnia heartbeats per 10 seconds. The constant is the temperature daphnia is in. The temperature is 22°. Another constant is the amount of food. I gave 1/8 a teaspoon of algae to daphnia every other day.

Procedure:

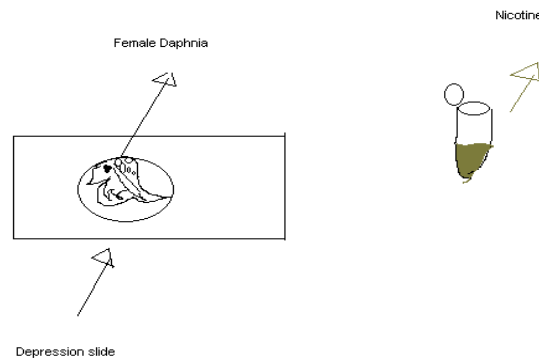
- 1) First gather all the materials needed.
- 2) Make a hypothesis of the essential question because it is based on your investigation.
- 3) Make a chart where data can be recorded. Organized data limits confusion.
- 4) First, get the female Daphnia required. It's important to see what kind of daphnia it is because males and females react to harmful substances in different ways.



In the depression slide, there is a daphnia.

5) Then record the temperature it's living in. The variables in the experiment must be kept constant. If this isn't done, the results will be misinterpreted

6) Apply the daphnia onto the depression slide and view the daphnia under low power objective. The knob of the microscope must be set at that moment the daphnia is put under the light.



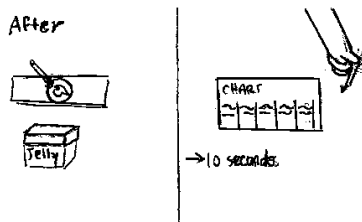
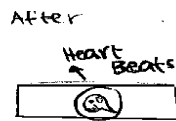
7) Put jelly on the depression slide next to daphnia and record the video of the daphnia moving for 10 seconds. This is important because one can compare a healthy daphnia with daphnia that consumed nicotine. One can understand the effects nicotine has on daphnia.

8) Apply nicotine solution on the daphnia with a micropipette. Put 20 μl (micro liters) of nicotine and 80 μl (microliters) of water. Take 40 μl of that concentration on to the depression slide with the daphnia. It's important to use a micropipette to keep the measurements accurate. This makes the conclusions more accurate because your results are based on certain measurements. Using separate pipette tips is important because mixing substances together will mess up the results.

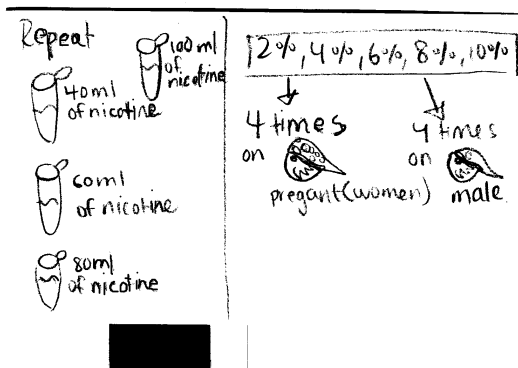
9) After, put a dab of petroleum jelly to slow the daphnia movement. Then wait 10 seconds for the daphnia to get ready and record the number of heartbeats in 10 seconds. If

petroleum jelly is not added, the daphnia will not stay still. Putting a tiny portion of jelly allows one to view the heartbeats the daphnia makes visibly.

10) Record observations on the daphnia's activity. One can tell if the daphnia is a male or female because male daphnia magna are 2mm, while female daphnia magna is 3mm- 5mm. The daphnia was actually 3mm, but on the computer screen its 5 inches. This proportional was used to find out the length of the daphnia. Also record the number of heartbeats it makes in 10 seconds. Here, one would view the videos and record notes on data.



11) Repeat these steps for 4%, 6%, 8% and 10% of nicotine.



12) Do this for 5 trials. Then do 5 trials on the effect of nicotine on male daphnia. Doing more trials is an accurate way of getting close to the conclusion of the investigation.

13) Afterwards, clean all the materials and put them away.

Results:

Figure 1-

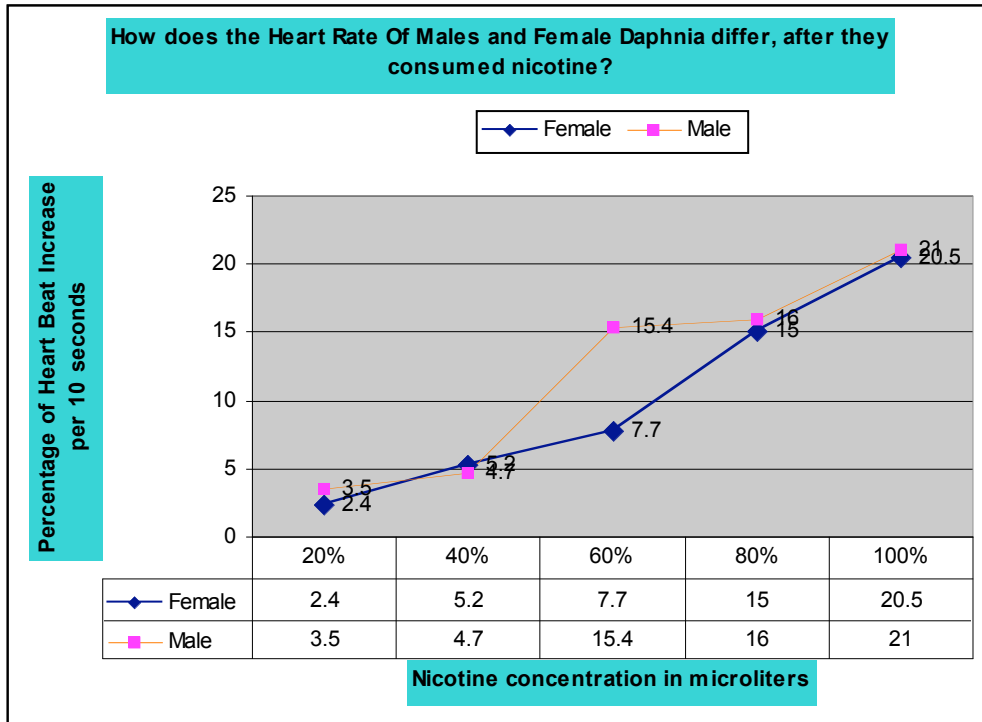
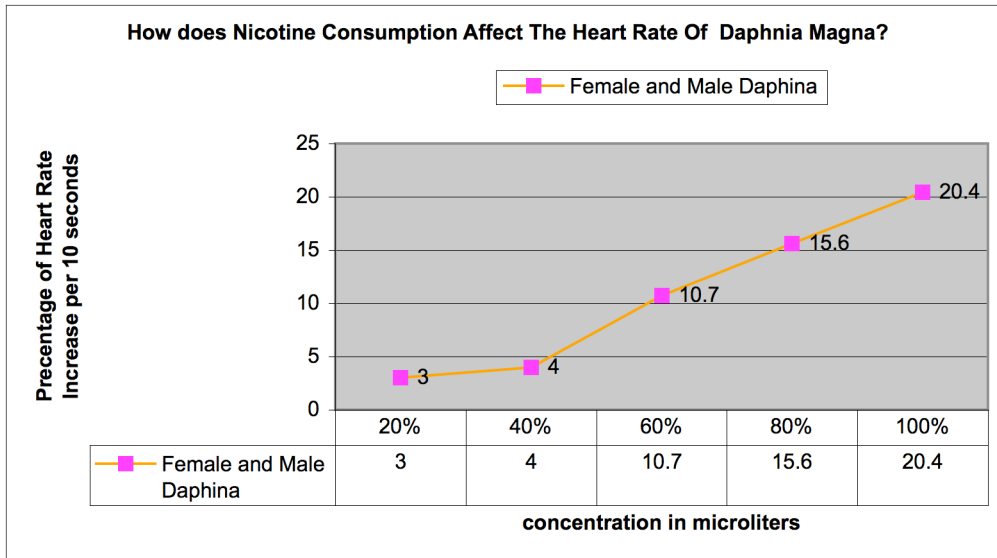


Figure 2-



Conclusion:

The hypothesis states that the number of heart beats the male and female makes, acclivities when higher doses of nicotine are utilized. In Figure 1, the difference in percentage of heart beat increased among the 100 microliters μ l dosed daphnia and 20 μ l-dosed daphnia was around a 17.6% increase. This demonstrates that the quantity of nicotine use affected the functioning heart.

One expected conclusion was that the heart beats of male and female daphnia differed dramatically. This conclusion was supported through the results. In one case, it was found on Figure 1 that after 60ml of nicotine use, the female daphnia’s heart beat rise approximately 7.7% in 10 seconds, while the male daphnia’s heart beat rise by around 15.4% in 10 seconds. It was found that the male daphnia couldn’t tolerate the same amount of nicotine as female daphnia. The male daphnia’s heart beat faster than the female daphnia’s heart. Ideally, a person who is heavier can tolerant more than a person who is small in size. At first, the male daphnia’s heart

beat faster than the female daphnia's heart. Moreover, the male's body was smaller and more sensitive to the nicotine due to the size and surface area for absorption.

When the female daphnia consumed 20 μ l of nicotine, the percentage increase of heartbeat per 10 seconds was around 2.4 %. When 100 μ l was applied, the female daphnia's heart rate went up by approximately 20.5%. The outcome is that the female daphnia's heart rate rose greatly when higher doses of nicotine were given.

The male daphnia's heart beats increased by roughly 3.5% in 10 seconds when 20 μ l of nicotine was applied. The application of 60 μ l of nicotine on the male daphnia, resulted in an accretion in the heart rate of daphnia. The male daphnia's heart rate rose to approximately 15.4% in just 10 seconds. Finally, 100 μ l (100%) created a tremendous increase in the daphnia's heart rate after 10 seconds. It increased the heart rate of the male daphnia by approximately 21%.

Though the heartbeats differ from males and females, the effect of nicotine on male and female daphnia was also looked at as a whole. In other words, the percentage of increase in heart rate were averaged out for both male and female. On Figure 2, the data was expressed as linear progression graphs that show the heart rate of daphnia according to the amount of concentration consumed. The graph illustrates how the heart rate of daphnia increased at a steady rate with higher nicotine concentration. From the results, it was observed that with increased nicotine concentration, the heart rate of male and female daphnia magna rose greatly, but not drastically.

Once males and females consumed 20 μ l of nicotine, there was approximately a 3% increase per 10 seconds (p10s) from the heart rate before nicotine consumption and the heart rate after nicotine use. But, the daphnia's heart rate did not ascend drastically. This meant that 20 μ l was a dose that daphnia could tolerate because their heart rate increase by a little and/or stayed the same.

Though, 20 μ l doesn't play such a huge increase in the heart rate of most of the daphnia tested, some daphnia's heart rate augmented more than others. This is because everybody is different and has a dissimilar tolerance. For this reason, it's difficult to observe how much nicotine can affect a person in a short amount of time. This relates to smokers where some face harsher symptoms than others because everyone's body is different. This was supported through the results where all the daphnia's heart rate didn't increase by the same amount, however the numbers were relatively close. Though individuals react differently to the drug, nicotine is shown to affect the heart rate of daphnia overall in this research. However, through multiple trials done, it is concluded that nicotine does in fact increase the heart rate of daphnia.

Another possible experiment that could be done is using dilutions out of 100 μ l. The possible dilutions could be 1%, 2%, 3%, 4% and 5%. If 20 μ l of nicotine was used, the daphnia will die because it's a lot for it to handle. This can be done in a beaker where the daphnia can be placed back to see the heart rate of daphnia after every 10 seconds. There won't be a problem with evaporation that can inflict with the results.

This could be a significant experiment in figuring out how people become addicted to smoking. Researchers know that people who smoke for a few months, develop a tolerance for nicotine and need a certain level of nicotine in their body (Pringle, 1996). When a person finishes a cigarette, the nicotine concentration in a person's bloodstream is cleansed out gradually in the liver. As a result, people crave for nicotine. As smoking continues overtime, it has a lesser stimulation on people's nerves because they are used to smoking and their bodies dispose the nicotine out of the bloodstream faster. So, when smoking continues, the process of removing the drug out of the blood stream amplifies. This brings up another investigation. If the level of

nicotine in a person's blood level drops and people smoke more, does their heart pump blood faster? To see the effect of nicotine overtime, dilutions smaller than micro liters should be done.

It was also found on Figure 2, that 60 μ l of nicotine created an estimated 10.7% increase per 10 seconds in the daphnia's heart rate. From the daphnia's heart rate before and after 100 μ l of nicotine dosage, the daphnia's heart rate raised approximately 20.4% increase in 10 seconds. From the data, any percentage of heart beat increase that is about 10.7% to around 20.4% reveals a very significant change in the daphnia's heart rate.

One dependent variable that was not measured was the activity rate of the daphnia. It was observed that with increased nicotine consumption, the daphnia's activity increased. The activity rate differed between male and female daphnia. This was proven correct through the observations on the affect of nicotine on both female and male daphnia's heart. On figure 1, when 20 μ l of nicotine was added, the female daphnia's movement changed. Normally, the daphnia's antennas moved in a neutral state. The antennas of the daphnia moved at times back and forth. After 20 μ l of nicotine, the daphnia's antennas move a little bit faster, but it didn't move in a frantic way. However, when the male daphnia consumed 20 μ l of nicotine, the legs and antennas velocity was faster than the female daphnia's antennas. The daphnia rotated in circles.

Due to the consumption of 60 μ l of nicotine, the nicotine played a huge role on the female daphnia's heart. At first, the daphnia move around a lot and its antennas, legs and heart motion in a hasty manner. After nicotine utilization, the daphnia's foot with claw moved in and out of the exoskeleton quickly and the daphnia's antennas moved in a rapid pace. This movement continued on for a long period of time, but end in an incredibly small amount of time.

On the other hand, when male daphnia consumed 60 μ l of nicotine the daphnia's movement was a shift from moving steady, to moving very frantic. At first, the non-dosed

nicotine daphnia's antennas were moving back and forth in a calm way. The daphnia's foot with claw moved slightly back and forth. But, after nicotine the daphnia's antennas and foot with claw moved up and down constantly and rapidly. The male daphnia foot with claw and antennas was moving quicker than when the daphnia consumed 60ml of nicotine.

100 μ l was another concentration given to the female daphnia. Before the daphnia consumed 100 μ l of nicotine, the daphnia's antennas moved inward and outward in a moderate pace and its foot with claw sway in small distances. After nicotine consumption, the daphnia's foot with claw moved back and forth in greater distances and its antennas were stimulated. The movement of the antennas was nonstop, but in a moderate pace. The daphnia's antennas were shaking.

Compared to female daphnia, before 100 μ l of nicotine consumption, the male's heart beat faster and its antennas moved more speedily. But, this movement was not constant. After nicotine was utilized, the daphnia's antennas were shaking, and the daphnia's foot with claw continually moves back and forth. The daphnia's whole body and tail was shaking to the extreme. After, the antennas moved more frantically and nonstop. When 100 μ l of nicotine was exposed to the male daphnia, its whole body shook. This did not happen when the female daphnia consumed 100 μ l of nicotine.

Observations were also made on the male and female daphnia's activity as a whole. Through the observations on figure 2, it was noticed that when both female and male daphnia consumed 20 μ l of nicotine, it made the daphnia's antennas shake in a moderate pace, whereas some of the other daphnia's antennas didn't react to the nicotine at all. Also the antennas didn't move constantly back and forth, but paused sometimes. Normally, the daphnia's legs and antennas moved in a moderate pace back and forth sometimes. Overall, after 20 μ l of nicotine

was consumed, the daphnia's heart was normal and its antennas and legs motion the same speed compared to the non- dosed daphnia.

Through the consumption of 60 μ l of nicotine, the daphnia antennas move more swiftly than before nicotine consumption. It moved back and forth for longer periods, but not in a constant way. After, the antennas of the daphnia moved quickly and constantly. Finally, when the daphnia consumed 100 μ l of nicotine, its antennas move more uncontrollably and frenetically than when 60ml of nicotine was consumed.

It was concluded from Figure 1 and 2 that when the daphnia took 20 μ l, the daphnia's heart rate didn't elevate that much. Yet, when the daphnia took in 60 μ l and 100 μ l of nicotine, their heart rate mounted a lot in a short period of time. Hence, the daphnia's activity from 60 μ l and 100 μ l was much worse, than when it consume 20 μ l of nicotine. This demonstrates that daphnia could not handle high amounts of nicotine.

Furthermore, Figure 1 and Figure 2 show that smoking gives rise to high blood pressure gradually, not drastically. For this reason, people continue to smoke because they don't feel any instant health effects. This relates to humans because when people start smoking, there is a small increase in heart rate at first, but because it's a small gradual increase, the effect is not felt within a short amount of time. But, as people smoke daily, their heart rate will slowly escalate overtime and in the long run lead to heart disease. 37,000 to 40,000 people die each year from heart and blood vessel disease. High blood pressure leads to a risk of cardiovascular disease and kills 437,900 Americans each year. Thus, smokers have a very high risk of getting heart disease (Children's Hospital Boston, 2007).

Even though, the daphnia's antennas and foot with claw shook, a definite conclusion can't be made that the daphnia's nervous system was affected by nicotine. However, it is

possible that an experiment can be done to test the effect of nicotine on the nervous systems of daphnia. A person can take a fluid sample out of daphnia and examine the neurotransmitters. If a person counts the number of neurotransmitters before and after nicotine dosage, it can be found if nicotine releases a lot of neurotransmitters. What kinds of neurotransmitters can be observed, when it's very difficult to detect the different types of neurotransmitter on the daphnia's brain? All neurotransmitters have a different purpose.

Also in Figure 2, there was a large amplification in heart rate of daphnia from 80 μ l to 100 μ l nicotine use. The difference was around a 4.8% increase in heart rate, since 80 μ l cause the daphnia to increase around 5.6% in heart rate and 100 μ l cause around a 20.4% increase in heart rate. As a result, the nicotine actuated the daphnia which heart pump faster, due to the dangerous effects of nicotine. This could be the fact that when daphnia consumed 100 μ l, there was no solvent used, because the dilutions were out of 100 μ l. Thus, undiluted nicotine caused the daphnia's heart to beat quicker. If its undiluted, then the nicotine will play a larger impact on the number of heartbeats the daphnia has in 10 seconds. With an increase in heart rate, the daphnia was at a risk of getting high blood pressure because more nicotine use can lead to a greater constriction in the blood vessels. This connects to the studies done on frogs where their blood pressure went up in a short amount of time. Thus, nicotine triggers the daphnia to beat its heart faster to allow blood to travel through the whole body efficiently.

There was room for improvement in this experiment. The first method tried was testing the effect of nicotine on daphnia without the camera because it was difficult to take accurate data. Another dependent variable that was looked at was the number of rotations the daphnia made per minute. Rotations were looked at because the daphnia rotated in a circular motion. This happened when a few trials were done. But, when multiple trials were completed, rotation wasn't

a consistent dependent variable to look at because not all of the daphnia rotate due to nicotine consumption. It was concluded that all daphnia behave differently. While rotations wasn't an accurate variable to use, heart rate was accurate to look at because jelly could be use to slow down the daphnia. Looking at the effect of nicotine on daphnia overtime was difficult because a few trials were done to see the effect of nicotine over a day, but the daphnia died. When the daphnia was looked at over 10 minutes, the nicotine solution already evaporated.

Some of the flaws made in the newest experiment is that some petroleum jelly was put on daphnia because some moved around a lot. Petroleum jelly wasn't added on all of the daphnia tested because some daphnia were more active than others. Another error is that the effect of nicotine on daphnia could be looked over every 10 seconds. This could be done three times. Because daphnia was looked at through a microscope, the light could have played an effect on the movement of daphnia. Jelly, which was added, could also have affected the movement of daphnia because the daphnia might have moved to be free from the jelly. Lastly, when dilutions were made, distilled water and tap water was used. This could have effect the results because the daphnia thrive the best in fresh water

So, what can people take away from this experiment? This experiment adds on one more reason on why addiction is so difficult to overcome. Because people can not see the immediate health effects of nicotine consumption (in this case, the linear increase of heart rate over time), it is easy to ignore and be unconvinced by the long term outcomes that health officials warn often to the public. Perhaps if this study is made widely to the public, more people can be more aware of the short-term effects of nicotine. Then maybe, more people are willing to at least try to commit to quitting smoking.

Appendix 1

Daphnia belong to a group called Daphnidae. Daphnia population is found mostly in vegetation in lakes and ponds (Miller, 1995). The conditions it best tolerant is 72 degrees or below (Carolina Biological Supply company). Daphnia has many qualities. They have one eye, two branchlike antennas and leaf like limbs inside the carapace, which helps move oxygen and food towards the mouth of the daphnia. Their antennas help them swim (<http://fossweb.com>). The black substance in the gut is the food that the daphnia is eating (Carolina Biological supply company). Daphnia has a light-sensing organ (Miller, 1995). The digestive gland is connected to the gut (Biomedics associates).

Appendix 2

In the bottom of the carapace, body, is a foot with claws that allow daphnia to take debris out of the carapace (Clare, 2002). When daphnia swim, it pauses after each stroke. The 5 legs inside its body, carapace, help them get food and breathe at the same time. Daphnia feeds of vegetation or organic material (Clare, 2002). Daphnia moves around the bottom of the water for food. Daphnia's legs help it to clean out food particles. Daphnia is a cleaner that can clear fish tanks with algae bloom because their main source of food is algae. Algae bloom is when there is a lack of oxygen in water, killing off the organisms that need oxygen. (Miller, 1995) When predators come, daphnia change in size, depending on the predator. This course of action is called cyclomorphosis. When there are adult fish the Daphnia will decrease in size, making it hard for the predators to see them. When the predators are smaller fishes, daphnia will increase in size to scare them off (Miller, 1995). Daphnia females have a brood chamber that is used to carry babies (Miller, 1995). When the babies hatch, they are release into the water. Like daphnia, humans also carry babies, but in their uterus.

Appendix 3

Males are smaller in size than females. *Daphnia magna* males are 2 mm or less. *Daphnia magna* female is 3mm to 5mm(Clare, 2002). Males have longer antennules and changed post abdomen (Miller, 1995).

Appendix 4:

Heart rate of male and female daphnia:

Gender	Dilutions	Observations
Male	40 μ l	<p>1st trial: <i>Daphnia</i>'s legs and heart moved in a slow pace. After nicotine consumption, the <i>daphnia</i>'s antennas moved back and forth in a short distance and motion in a moderate pace.</p> <p>2nd trial: Before nicotine dosage, the <i>daphnia</i>'s legs moved very rapidly. This behavior stayed the same after nicotine consumption.</p> <p>3rd trial: At first, the <i>daphnia</i>'s legs and antennas motion in a fast way. After, it was exposed to nicotine; the <i>daphnia</i>'s body was shaking a little and its foot with claw shifted back and forth.</p> <p>Through, the <i>daphnia</i>'s behavior was similar; their behavior was not entirely the same.</p>
Female	40 μ l	<p>Before nicotine consumption, the legs were moving in a mild pace.</p> <p>After nicotine dosage, the <i>daphnia</i>'s antennas trembled a tiny bit and its legs moved in a fast pace.</p>
Female	80 μ l	<p>When the female <i>daphnia</i> was immersed in 80μl of nicotine, its antennas were moving rapidly and the <i>daphnia</i>'s whole body was shaking a lot. The</p>

		daphnia has no self-control and the amount of nicotine was not bearable. Before nicotine was given, the daphnia's antennas moved in a quick pace.
Male	80µl	Unlike the female daphnia, the male daphnia's antennas moved back and forth in huge distances. Also the daphnia's foot with claw kept moving back and forth for a long period of time. One commonality male and female daphnia shared was that after nicotine consumption, the daphnia's body shook frantically and the antennas moved extremely quick. Before the daphnia was exposed to nicotine, their heart beat in a calm pace. Its antennas and foot with claw moved sometimes back and forth.

Observations on the Overall Heart Beat of Female and Male Daphnia:

<u>Daphnia</u>	<u>Dilutions</u>	<u>Observations</u>
<u>Female and male daphnia together</u>	<u>40µl</u>	But, when the female and male daphnia consumed 40µl, both female and male daphnia's heart generally beat a little faster. Their antennas were moving faster back and forth. However, when the daphnia didn't consume nicotine, their antennas were actually moving in a normal pace. After looking at the effect of nicotine on the daphnia for 10 seconds, the daphnia's foot with claw moved in and out of its exoskeleton occasionally.

Daphnia	Dilutions	Percentage of Heart Beat Increase per 10 seconds At roughly...
Female	40µl	5.2%
Female	80µl	15%
Male	40µl	4.7%
Male	80µl	16%

Both female and male daphnia	Dilutions	Percentage of Heart Beat Increase Per 10 seconds
Both male and female daphnia	40 µl	4%
Both male and female daphnia	80 µl	15.6%

Appendix 5

Gender Name	Solution	Heart rate before nicotine	After Nicotine
Female 20ml 3/14/07 CD 2	20µl	36	37
Female steph 2 of CD 5: 20ml before #1	20µl	29	29
Female stephanie 4 of CD 5: 20ml 3/27 before #2	20µl	42	42
Female stephanie 4 of CD 5: 20ml 3/27 before #3	Steph 4 of cd 5: 20µl 3/27 #3	33	34
Female 20ml 3/27 before #4: CD 5	20µl	36	37
Female 20ml 3/24 before #5: steph 5 of cd 5	20µl	27	30
Female 20ml before #6 steph 5 of CD 7	20µl	32	32
Female 20ml before #7 steph 5 of CD 5	20µl	35	32
Female 20ml before #8 steph 5 of CD 5	20µl	35	36
Female 20ml before #9 Steph 5: CD 5	20µl	30	32
Female daphnia 20ml#2 before 4/21	20µl	30	30
Female daphnia 20ml #3 before 4/21	20µl	29	31
Female 20ml #4 before My key	20µl	31	31
Female 20ml daphnia #5 before- my key	20µl	26	30
Female 20ml before #6 my key	20µl	32	34

Female daphnia 20ml #7 before	20 μ l	25	28
Female #8 20ml before my key	20 μ l	30	34
Female # 9 20ml before- my key	20 μ l	24	27
Female #5 before 20ml 3mm: CD 7	20 μ l	46	54
Female daphnia 20ml 3/27 before #4: steph 4 of CD 5	20 μ l	36	36

Gender Name	Solution nicotine	Heart rate	
		before	After
Male daphnia 20ml #3 before: my key	20 μ l	29	31
Male #8 20ml before: my key	20 μ l	30	34
Male #1 20ml before 2mm : cd 7	20 μ l	42	43
Male 2mm before 20ml	20 μ l	41	44
Male #4 20ml before	20 μ l	44	47
male #6 before 20ml 2mm: cd 7	20 μ l	40	40
male #7 before 20ml	20 μ l	46	48
male #8 before 20ml 2mm	20 μ l	43	43
male #9 before 20ml 1mm	20 μ l	39	42

Gender Name	solution nicotine	Hear rate	
		before	After
Female 2nd trial nico on daphnia 40ml- Ms. Chien's key	40 μ l	32	33
female 40ml of nico before and after: CD 2	40 μ l	25	28
female daphnia 40 3/21 CD 4	40 μ l	26	27
female 40ml before #1	40 μ l	27	29
female #2 before 40ml: CD 9	40 μ l	43	44
female #2 before 40ml	40 μ l	53	53

Gender Name	solution nicotine	Hear rate	
		before	After
Female 2nd trial nico on daphnia 40ml- Ms. Chien's key	40 μ l	32	33
female 40ml of nico before and after: CD 2	40 μ l	25	28
female daphnia 40 3/21 CD 4	40 μ l	26	27

female 40ml before #1	40µl	27	29
female #2 before 40ml: CD 9	40µl	43	44
female #2 before 40ml	40µl	53	53

Gender Name	solution	Hear rate	
		before nicotine	After nicotine
Female daphnia after 60ml only heart beats: CD 1	60µl	29	32
Female 60ml 3/21 before nico: CD 4	60µl	34	36
Female #4 60ml before: CD 6	60µl	36	37
Female 60ml #2 before: CD 8	60µl	45	51
Female 60ml #3 before female: CD 8	60µl	46	51
Female #5 before 60ml: CD8	60µl	44	45
Female 60ml #6 after	60µl	45	47
Female #1 before 60ml: CD11	60µl	55	58
Female #2 before 60ml: CD 11	60µl	57	60
Female #3 before 60ml: CD 11	60µl	55	58

Gender Name	Solution	Before After	
		nicotine	nicotine
Male Daphnia after 60ml and heart beats: CD 1	60µl	30	35
Male #1 60ml after: CD 6	60µl	26	31
Male #2 60ml before: CD 6	60µl	32	36
Male 60ml #3 before: cd 6	60µl	42	44
Male #5 60MLbefore CD 6	60µl	33	39
Male #6 before 60ml: CD 6	60µl	41	46
Male #7 60ml before: CD 6	60µl	43	46
Male #8 before 60ml	60µl	35	41
Male 60ml before#4	60µl	48	51

Gender Name	Solution	Heart rate	
		before nicotine	After Nicotine
Female 80ml before 3/21: CD4	80µl	32	39
Female #6 80ml before: CD 9	80µl	40	47
Female #2 before 80ml CD10	80µl	53	59
Female #4 before 80ml CD11	80µl	52	60
Female 80ml before 3/21 CD 12	80µl	44	49

Gender Name	Solution	Heart rate	
		before Nicotine	After Nicotine

		nicotine	
Male	#6 80 after CD 9 80µl	54	64
Male	#1 80ml before CD 11 80µl	51	60
Male	#2 before 80ml Cd 11 80µl	59	66
Male	#3 80ml before CD 11 80µl	52	61
Male	#4 before 80ml CD 11 80µl	52	60

		Heart rate	
		before	After
Gender Name	Solution nicotine	Nicotine	Nicotine
Female	100ml before and after CD 2 100µl	28	37
Female	#3 before 100ml 100µl	48	56
Female	#5 before 100ml CD 10 100µl	52	62
Female	#7 before 100ml 100µl	44	52
Female	#1 100ml before my key 100µl	42	51

		Hear rate	
		before	After
Gender Name	Solution nicotine	Nicotine	Nicotine
Male	100ml #1 before CD 10 100µl	55	66
Male	#2 before 100mlCd 10 100µl	54	66
Male	#4 100ml before CD 10 100µl	62	74
Male	#6 before 100ml Cd 10 100µl	65	78
Male	#8 before 100ml CD 10 100µl	57	73

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