Ethanol on the heart rate

Effects of different concentrations of ethanol on the heart rate of the water flea

In this experiment I will be looking at the effects of different concentrations of ethanol on the heart rate of the water flea, Daphnia because using humans in a study to test the effects of Ethanol on heart rate would not be ethical. Daphnia, like many animals, are prone to alcohol intoxication, and make excellent subjects for studying the effects of the depressant on the nervous system – due to the translucent exoskeleton, and the visibly altered heart rate. Ethanol is a small molecule, RMM of 43, so it crosses cell membranes by simple diffusion. The exoskeleton of Daphnia is not waterproof, so there are no waterproof waxy layers to cross. The gills are particularly thin-walled and optimised for diffusion. They live in various aquatic environments ranging from acidic swamps to freshwater lakes, ponds, streams and rivers. They are tolerant of being observed live under a microscope and appear to suffer no harm when returned to open water.

The experiment consists in preparing 5 different environments to put the water flea in, and observe how the heart rate responds to each change; the change will be the increase of ethanol concentration %. This will be the independent variable. The dependent variable is the heart rate of the Daphnia.

Hypothesis : Ethanol will decrease the heart rate of the Daphnia.

Null Hypothesis : Ethanol will have no effect on the heart rate of the Daphnia.

I will change the concentration of my Ethanol solution by dilution and will therefore plot a graph. In addition, a correlation and ANOVA test will be

calculated to determine the relationship (If any) between the concentration of Ethanol and the heart rate of the Daphnia.

I have taken into consideration factors that will affect my overall conclusion

Factors:

Size of Daphnia – The size of the Daphnia will affect its absorption of Ethanol and also the metabolism of the drug in the liver. Different rates of metabolism will result in different heart rates. As a result I must ensure that I choose Daphnia which are the same size when conducting repeats. I will use a highly sensitive scale to confirm that both daphnia are of equal body mass.

Time kept in ethanol solution – The Daphnia must be kept in the Petri dish full of ethanol solution for a specific amount of time. If they are kept in the solution for too long they will become intoxicated which will result in abnormal heart beats that are hard to measure although they must be kept in the solution long enough for sufficient absorption of Ethanol. Each Daphnia will stay in the ethanol solution for exactly 3 minutes so that an equal volume of ethanol is absorbed.

Activity of Daphnia – Some Daphnia tend to be more active than others and these will have a higher heart rate compared to ones that are idle. After allowing the Daphnia to swim in a specific solution of ethanol I will place them on a cavity slide so that I can observe the heart rate with a microscope. As I will be using a pipette to transfer the Daphnia from the Petri dish to the cavity slide excess fluid will be found on the slide which must be removed with tissue so that all Daphnia remain idle/immobile and not active

I. e. swimming on the slide. This will also allow me to measure the heart rate with ease which reduces the likelihood of human error.

Time left under the microscope – If the Daphnia are left under the microscope for too long they will become stressed due to the heat of the microscope light and this will increase the heart rate of the Daphnia due to the secretion of adrenaline therefore I must ensure that the microscope is switched off when not in use. The cavity slides must be allowed to cool down before using them again as they tend to heat up.

Impurities on cavity slide – Traces of impurities including ethanol from a previous experiment may be left on the cavity slide which may slightly affect the heart rate of the Daphnia therefore the slide must be cleaned and dried thoroughly before each repeat. Alternatively, a new slide may be used for each repeat.

The materials needed to perform this experiment are the following:

- Normal size syringe
- 2 Small syringes (must have units of measurement)
- Open top pipette
- Ethanol of 1% concentration
- Various Daphnia to perform experiment on
- Microscope
- 6 Petri dishes
- Cavity slide
- Marker
- Kleenex tissue for absorbing excess liquid

- Scale
- Stopwatch

Method :

1. The first thing that has to be done is the preparation of the different solutions where the Daphnia will be placed. To do this you will need the small syringe, and 5 Petri dishes. It is very advisable to have labels. The first Petri dish will contain 0% Ethanol, in other words just water. With a small syringe, take 10 ml of distilled water (the use of distilled water is important as you will be removing any materials that may have an effect on the Daphnia heart rate) and place it in the Petri dish. The syringe you just used will only be used with water and not for the Ethanol. Put a 0% label on the Petri dish in order to keep track of the different concentrations you will be making. The next Petri dish will contain 0. 2% ethanol concentration, and you will make concentrations going up to 0. 8%, so:

- 0. 2 %: With the other small syringe (this one will only be used for ethanol), add 2 ml of the 1% Ethanol, to 8 ml of distilled water
- 0. 4 %: Add 4 ml of the 1% ethanol, to 6 ml of distilled water
- 0. 6%: Add 6 ml of the 1% ethanol, to 4 ml of distilled water
- 0. 8%: Add 8 ml of the 1% ethanol, to 2 ml of distilled water
- remember to label each concentration accordingly

2. Set your microscope up, put it on medium magnification. Do not turn it on yet because the light of the microscope can heat up the environment where you will be observing the Daphnia. It is important to try to keep the temperature of the experiment as stable as possible. Heat may modify the

Daphnia heart rate, and the effect of heat on the heart rate is not the purpose of this experiment.

3. Now it is time to pick out one Daphnia from the glass or container where you put all of them in. It is important to use only one throughout this whole experiment because different animals may present variations in their response to different environments. With the open top pipette, try to pick out a Daphnia which is not too small, as it will be harder to observe the heart rate if it is small. Once you have managed to take one out, place it in the remaining empty Petri dish. Take the normal size syringe and very carefully suck the Daphnia in with as least water possible. The objective is to have the Daphnia right at the tip of the syringe. “ Squirt” the Daphnia out into the cavity slide. It is very important to put the Daphnia in with the least water possible, in order to prevent it from moving too much. It is recommended to try to “ squirt” it out with only one drop of water, as this will keep it alive, but immobile. Use tissue to remove excess fluid. Put the slide under the microscope. Turn the microscope on.

4. Make sure you can see the Daphnia clearly under the microscope, once you are happy with the image, look for the heart:

7 is the heart.

If you can see the heart, and can keep track of its beating, put the Daphnia, with the normal sized, syringe into the 0% labelled Petri dish. Wash the microscope slide with water and dry it. Turn the microscope off.

5. Keep the Daphnia in the Petri dish for 3 minutes, this lets it “ adapt” do the conditions and also increases the probability of it surviving the whole experiment. With the normal size syringe take it out of the Petri dish and put it onto the microscope slide, make sure that it is practically immobile (by making sure that you squirted the least amount of water possible) and put it under the microscope. Turn the microscope on.

6. Get the paper and marker ready. Look into the microscope and make sure you can count the heart beat. Get someone to count 15 seconds with the stop watch. During 15 seconds, tap the paper with the marker each time the heart beats, after this, count the number of dots on the paper. Multiply this number by four; this gives you the heart rate per minute. Record the result. Do this process 3 times in order to get 3 heart rates. Add the 3 heart rates and then divide the result by 3; this will give you the average of the Daphnia heart rate under those conditions. Keep the Daphnia under the microscope for a maximum of 2 minutes, because the heat of the light in the microscope could have effects on the experiment. Turn the microscope off after the count to prevent further heating caused by the light.

7. Remove the slide from the microscope, and with the normal size syringe put the Daphnia into the 0. 2% labelled Petri dish (wash the slide with water and dry it). Leave the Daphnia in the Petri dish for 3 minutes once again. After 3 minutes, use the normal sized syringe to put it onto the microscope slide. Repeat step 6 and record results.

8. Count the heart rate of the Daphnia when placed in all the concentrations. Work your way up from 0% to 0. 2% to 0. 4%, 0. 6%, 0. 8%. Make sure you

rinse and dry the slide with the distilled water after each time. You must start from the lowest concentration up to the highest concentration because the Daphnia has to gradually adapt to the changes, you will be reducing the probability of it dying. Another reason for this is that if you start at the highest concentration, the impact on the Daphnia will be too dramatic and you will not see any trends once you try a lower concentration, it will have an effect of " intoxication". Remember to repeat each count 3 times to obtain an average of the heart rate. Keep the Daphnia under the microscope during the same amount of time for each concentration, this will ensure that if there was any type of effect from the light under the microscope, all tests will be fair because they were under the exact same conditions. It is possible for the Daphnia to die during these tests; this is why you must have acquired a fair amount of Daphnia, in order to have back-ups.

9. Repeat this experiment once or twice with different Daphnia, in this way you will be able to analyse any trends present in the experiment more accurately.

I will use the following tables to record the results of this experiment:

- Averages will be calculated for both experiments and the results will be organised in a separate table. My conclusion will be based on these averages because they are more representative.

- **Modifications made to method:**

- The Daphnia used in the first experiment died after being placed on the cavity slide from the 0. 6% ethanol solution probably due to the

lack of fluid on the slide so I had to restart the experiment using another Daphnia.

- No one was available to count 15 seconds with a stopwatch so I had to use my mobile phone which beeped after 15 seconds prompting me stop counting the number of heart beats.

Results