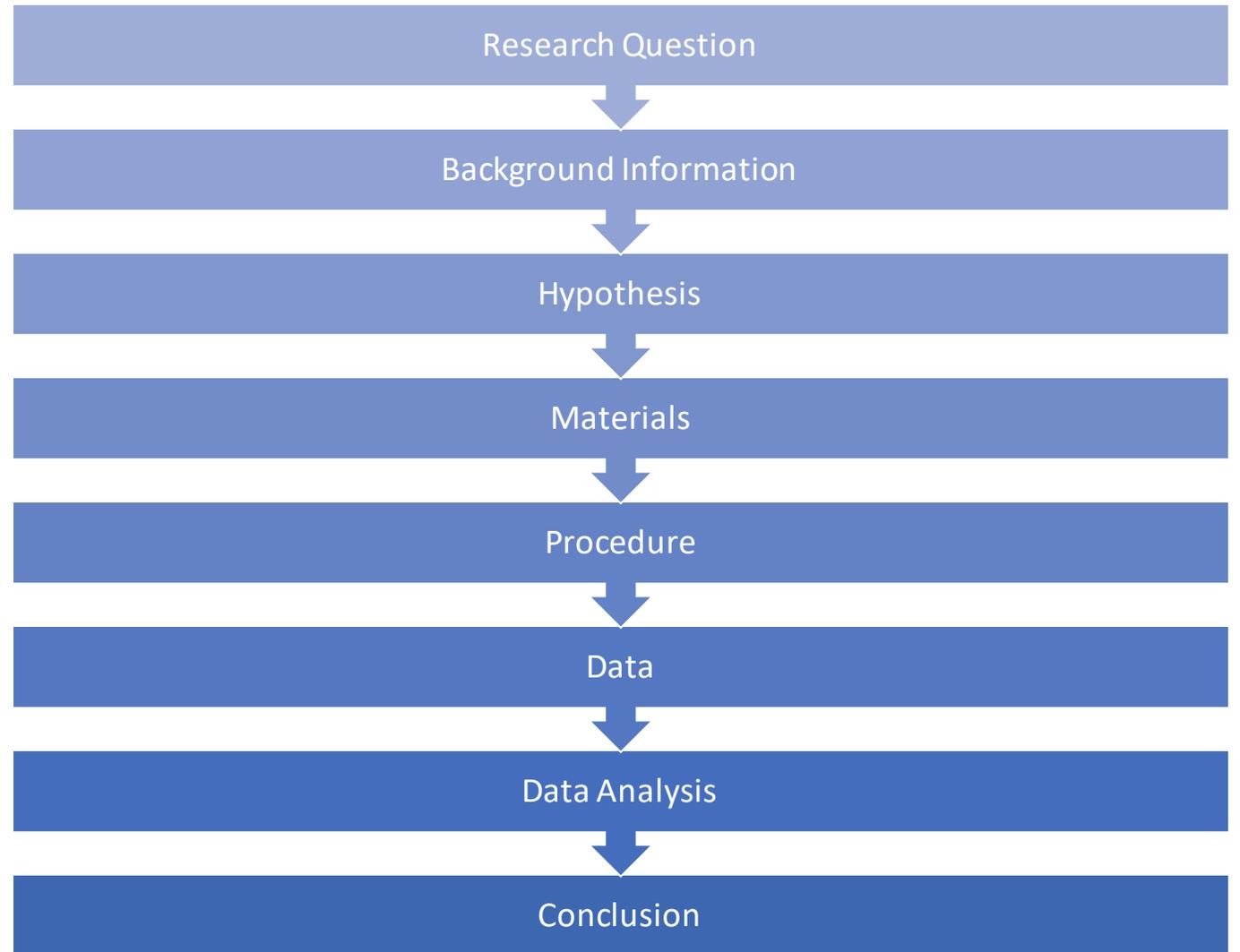


Behavioral and Physiological Study
on the Exposure of Various Vaping
Chemicals on Brown *Planaria* and
Blackworm *Lumbriculus*: Evaluation
of Internal Reactions and Physical
Changes

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Agenda





Research Question

What are the Effects of Propylene Glycol, Vegetable Glycerin, Nicotine, and Vape Liquid on Brown *Planaria* and Blackworm *Lumbriculus*?



*Background
Information*

Topic Selection

- **Reason for Vaping Study Topic Selection:**
 - Curiosity about effects of vaping on both life and the ecosystem
 - Curiosity of what element(s) of the vape liquid causes changes in the human body
- **Scientific Concepts**
 - Vaping toxins cause harm to the ecosystem and humans
 - Formula for Dilutions: $C_1V_1 = C_2V_2$
- **Benefit to Society**
 - Better understanding of effects of vaping
 - Allows for a novel understanding of the affects of vaping on the ecosystem
 - Data can help doctors and the Center for Disease Control and Prevention

Background Information

Definition of Terms

- **In Vivo** – Occurring within a living organism or setting
- **In Vitro** – Occurring surrounding an organism or setting within a vessel or control experiment
- **Vaping Liquid** – The liquid used in E-Cigarette that contain Propylene Glycol, Nicotine, Vegetable Glycerin, and flavoring created
- **Vaping** – The act of inhaling vapors charged from an electronic cigarette
- **Propylene Glycol** – A colorless liquid used as a lubricant, antifreeze, heat transfer fluid, and as solvents for resins and fats
- **Nicotine** – The addictive chemical in both e-cigarettes and cigarettes
- **Vegetable Glycerin** – Typically derived from soybean or coconut oils it is odorless with high viscosity and popular in the cosmetics fields.
- **Blackworm *Lumbriculus*** – Also known as *Lumbriculus variegates*, it is a worm inhabiting North America and Europe with dorsal blood vessels easily seen under a microscope in their tail
- **Brown *Planaria*** – A free-living flatworm found in both saltwater and freshwater ponds, *Planaria* is often used for scientific research
- **One Inch** – One inch is equal to 25.4 millimeters

Background Information

Variables

Experimental Control

- Worms in Baseline state will be compared with the worms in vitro with the toxins

Independent Variables

- Propylene Glycol
- Vegetable Glycerin
- Nicotine
- Vape Liquid
- Blackworm *Lumbriculus*
- Brown *Planaria*

Dependent Variables

- Movement rate of Brown *Planaria*
- Pulse rate of Blackworm *Lumbriculus*

Controlled Variables

- Media the worms live in
- Ambient Temperature

Hypothesis

If Propylene Glycol, Vegetable Glycerin, Nicotine, and Vape Liquids found in E-Cigarettes were tested on Brown *Planaria* and Blackworm *Lumbriculus* then, the **Propylene Glycol** will have the most effect on both the Brown *Planaria* and Blackworm *Lumbriculus* because it is the most abundant chemical in vape liquids and in other studies it is seen that Propylene Glycol had a negative impact on human health.

This was concluded using information found on:

<https://www.ncbi.nlm.nih.gov/books/NBK507184/>.

Procedure: Blackworm *Lumbriculus* BPM

To Test the Blackworm *Lumbriculus*:

1. Take a microscope slide and apply several strips of masking tape upon it
2. Create an rectangle shaped well the size of an average worm for where it will rest in the masking tape during the evaluation under the microscope
3. For a 100% dilution add 5 mL of the desired toxin, for a 25% dilution add five mL of the toxin and fifteen mL of water, and for 0% dilution add 5 mL water
4. Take a pipette and pick up a worm with it, dispose of any water collected with it when placing the worm inside of the notch of the slide created by the tape

****A mask and goggles are required
for both experiments****

Procedure: Blackworm *Lumbriculus* BPM

To Test the Blackworm *Lumbriculus*:

5. For the pure dilution take one drop of the toxin using the pipette and drip it upon the worm, for the dilution take a drop of the compound and drip it upon the worm, for the control allow a drop of the media water upon the worm
6. Turn on the microscope and place the worm underneath it on and adjusting the lens
7. Turn on a timer for one minute and count the pulsation rate, or beats per minute, in the dorsal blood vessel found at the end of the worm within the time frame and record
8. After the first minute immediately log the results and begin the second minute with the same worm
9. Repeat for five trials per toxin (20 in total)

****A mask and goggles are required
for both experiments****

Procedure: Brown *Planaria* Distance Rate

To Test the Brown *Planaria*'s Distance Rate:

1. Take a square of graph paper (3.175 mm squares) enough to cover the bottom of a Petri dish and tape it to the bottom of the dish
2. Using an pipette, place a Brown *Planaria* into the Petri Dish
3. Place the dish under the microscope and adjust it so that the Brown *Planaria* is in view along with the squares of the graph paper
4. Fill the Petri Dish with 1 mL of the liquid (Controlled media of 0% dilution or 1 ml-3ml toxin to water of 25% dilution)
5. Turn on a timer for one minute and count the number of squares the *Planaria* crosses
6. Using the conversion of one inch to 25.4 millimeters, multiply 25.4 mm by the number of squares crossed by the *Planaria* to find out the distance traveled in millimeters
7. Begin the second minute immediately after the first minute
8. Repeat each step above for all 5 trials per toxin (20 in total)

****All equipment must be cleaned using deionized water to rid of any remaining vaping substances for both worms****



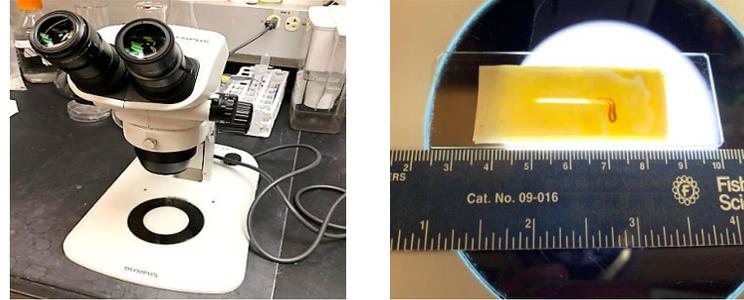
Step 1: Place the Worms in the Petri Dish



Step 2:
Collect
the toxins



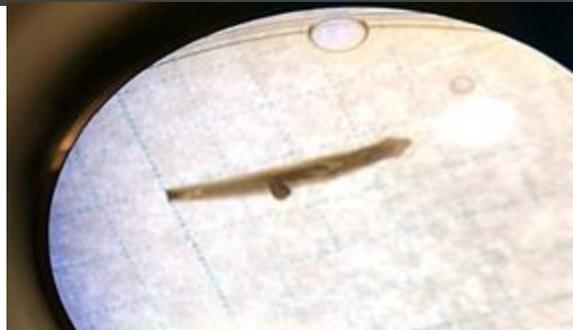
Step 3: Create the Diluted Substances



Step 4: Ready the microscope and
microscope slides for blackworm
lumbriculus



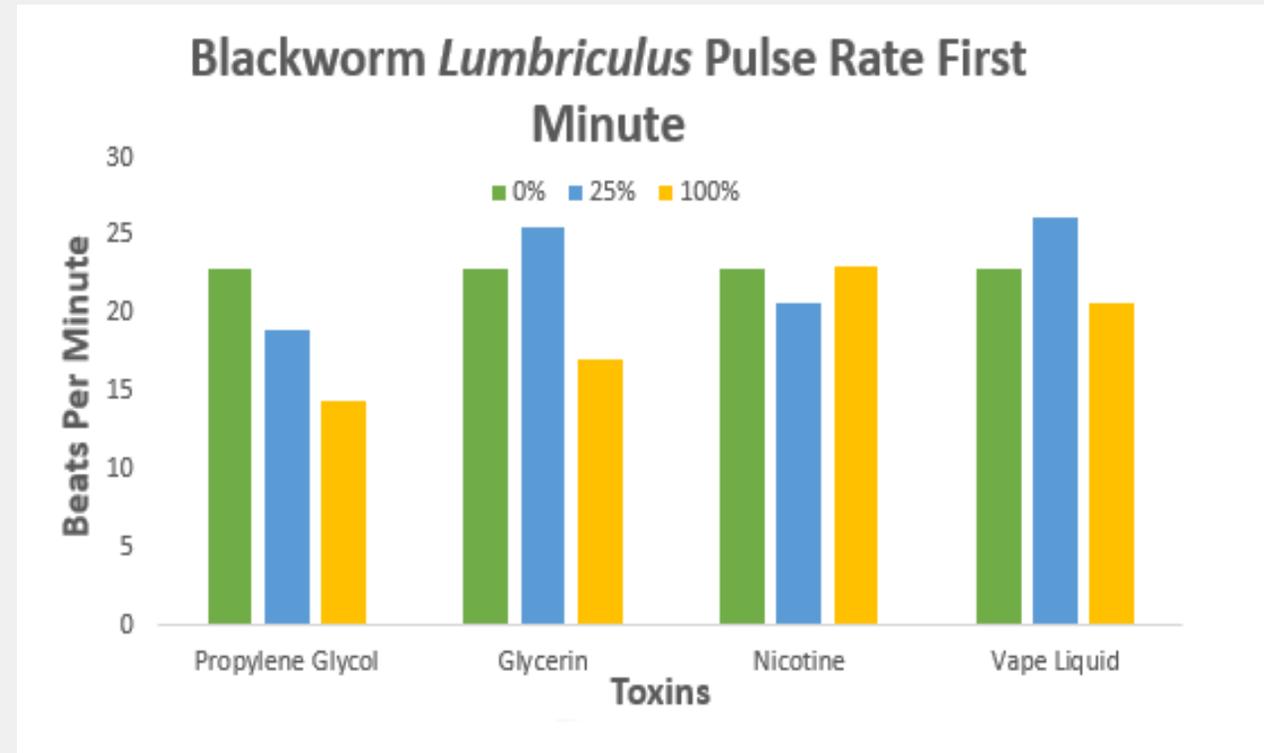
Step 5 (Blackworm Lumbriculus):
Count the BPM and record



Step 5 (Planaria): Count the
movement and record

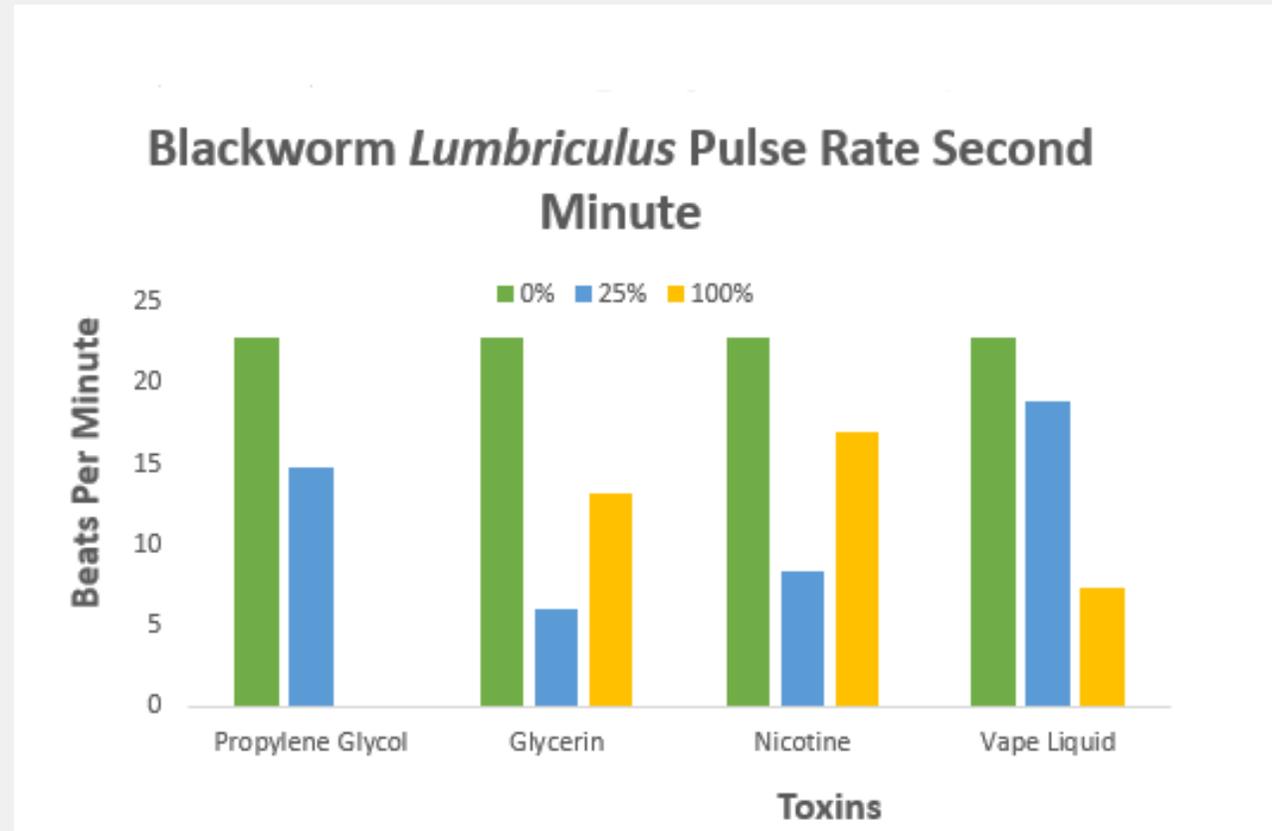
Procedure (Images)

Experiment 1A



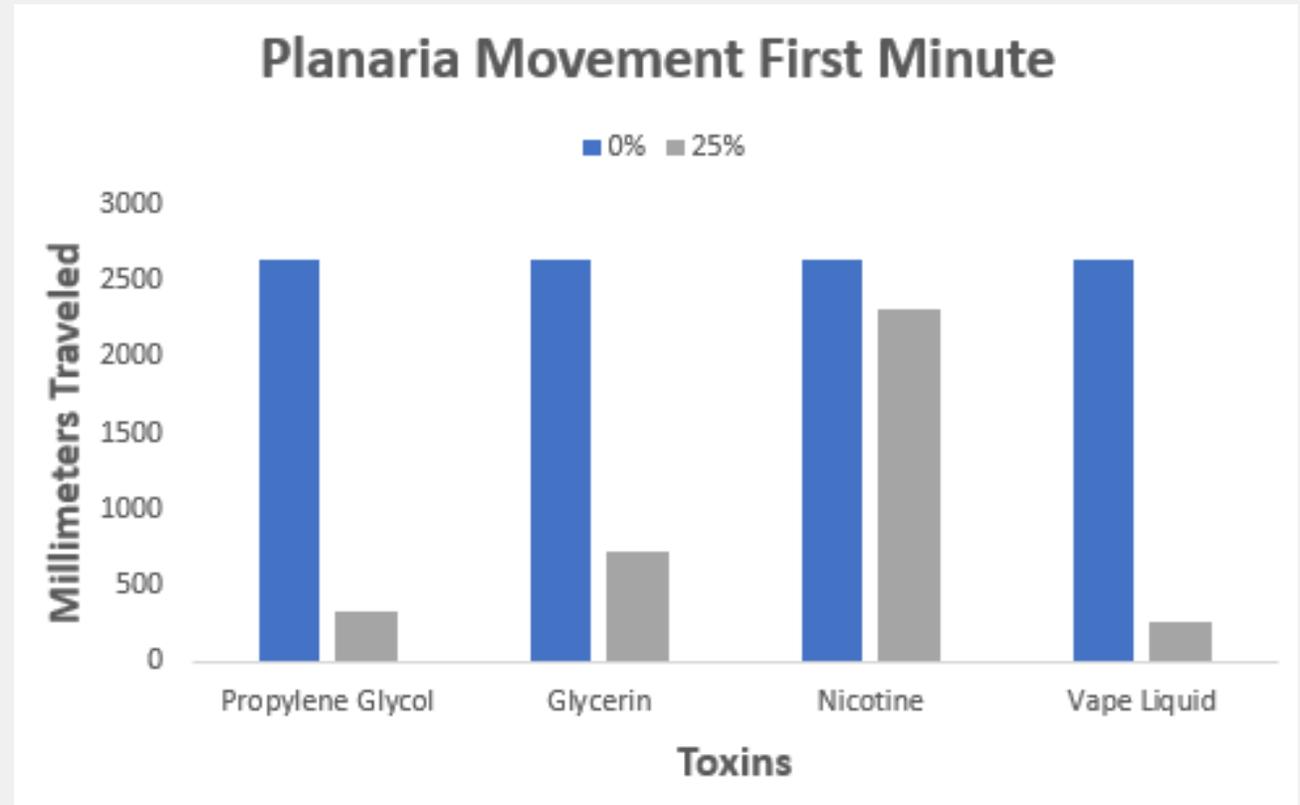
****0% is the control of media water, 25% is the dilution of 1-3 toxin to water, and 100% is pure toxin****

Experiment 1B

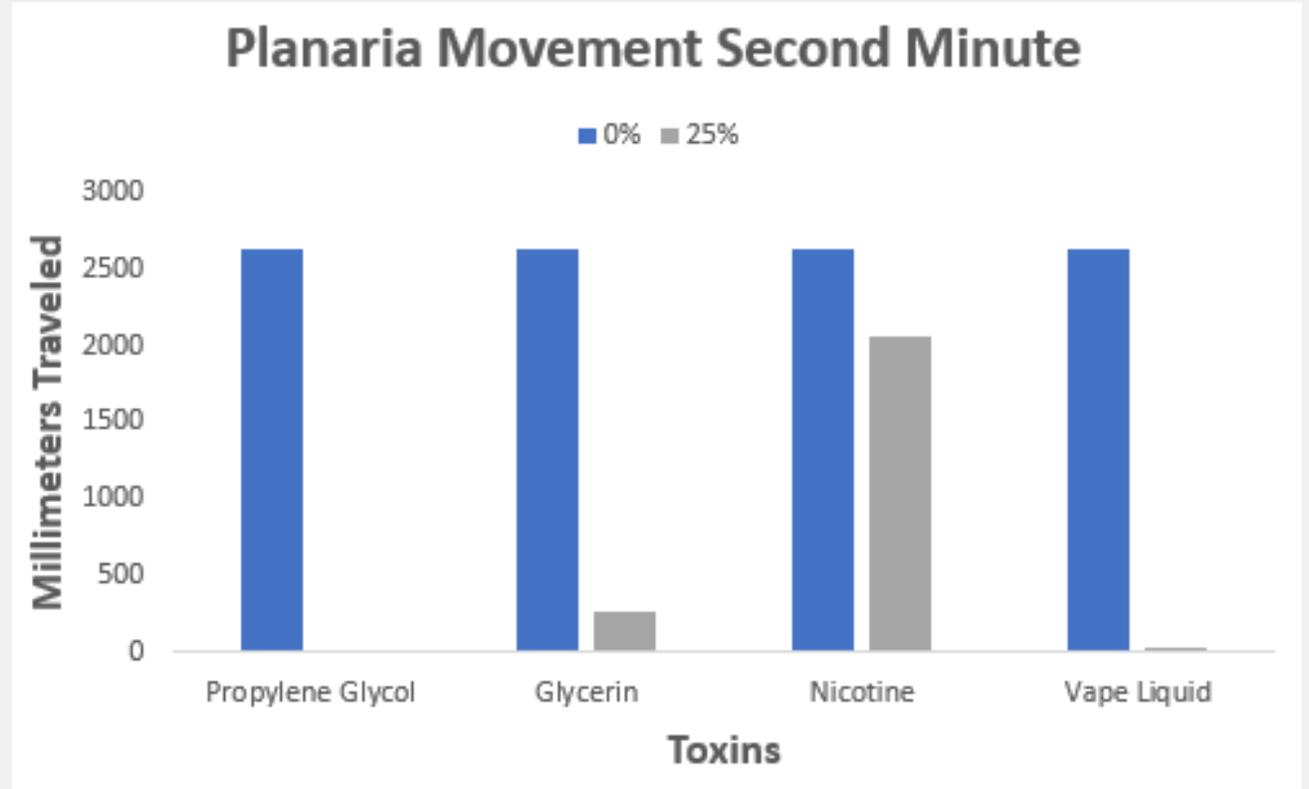


****0% is the control of media water, 25% is the dilution of 1-3 toxin to water, and 100% is pure toxin****

Experiment 2A



Experiment 2B



Data Average

Brown <i>Planaria</i> Movement Average		
DILUTION AMOUNT	0%	25%
Propylene Glycol	2629.027	177.419
Glycerin	2629.027	500.026
Nicotine	2629.027	2188.706
Vape Liquid	2629.027	145.161

Blackworm <i>Lumbriculus</i> Pulsation Average			
DILUTION AMOUNT	0%	25%	100%
Propylene Glycol	22.8	16.875	7.2
Glycerin	22.8	15.8	15.1
Nicotine	22.8	14.5	20
Vape Liquid	22.8	22.5	14

****0% is the control of media water, 25% is the dilution of 1-3 toxin to water, and 100% is pure toxin****

Data Analysis

Per the progression of the experiment over two minutes:

- At 25% dilution **Vegetable Glycerin** lowered the pulse rate of Blackworms *Lumbriculus* more in the second minute than **Propylene Glycol**
- At 100% concentration **Vape Liquid** came second in lowering the pulse rate of Blackworms *Lumbriculus*
- At 25% concentration **Propylene Glycol** had the most lowering effect in the movement of Brown *Planaria* in the first minute
- At 25% concentration **Vape Liquid** came second in lowering the movement of Brown *Planaria*
- **Vegetable Glycerin** held marginal effect on both the Blackworm *Lumbriculus* and Brown *Planaria* in their dilutions

Conclusion

Per the Hypothesis:

Propylene Glycol was expected to have the most severe effect on both the worms

- Based on the data the experiment seems to accept the hypothesis, but more statistical analysis is required to reach a conclusion
- **Propylene Glycol** had the highest effects on Blackworm *Lumbriculus* and Brown *Planaria* as a pure toxin
- **Nicotine** had the highest effect on Blackworm *Lumbriculus* as a 25% dilution
- **Vape Liquid** had the highest effect on Brown *Planaria* as a 25% dilution
- **Vegetable Glycerin** held marginal effect on both the Blackworm *Lumbriculus* and Brown *Planaria* in their dilutions

Conclusion

➤ **Observations:**

- Propylene Glycol caused external bleeding in the Blackworm *Lumbriculus*
- Vape Liquid caused internal bleeding of the Blackworm *Lumbriculus*
- Vape Liquid caused the Brown *Planaria* to evert its Pharynx

➤ **Some possible experimental errors were:**

- Diurnal variation in when the worms were tested
- Picking up the Blackworm *Lumbriculus* and Brown *Planaria* with the pipette may have injured them
- A small amount of water may have stayed with the worm when being moved from its container to the Petri Dish which may have diluted the substance more

Conclusion

➤ **Future Research Help:**

- Understanding how circulation and the heart is affected by vape liquids
- Understanding of how creatures and the ecosystem are affected by Vape Liquids

➤ **Create a New Future Project for:**

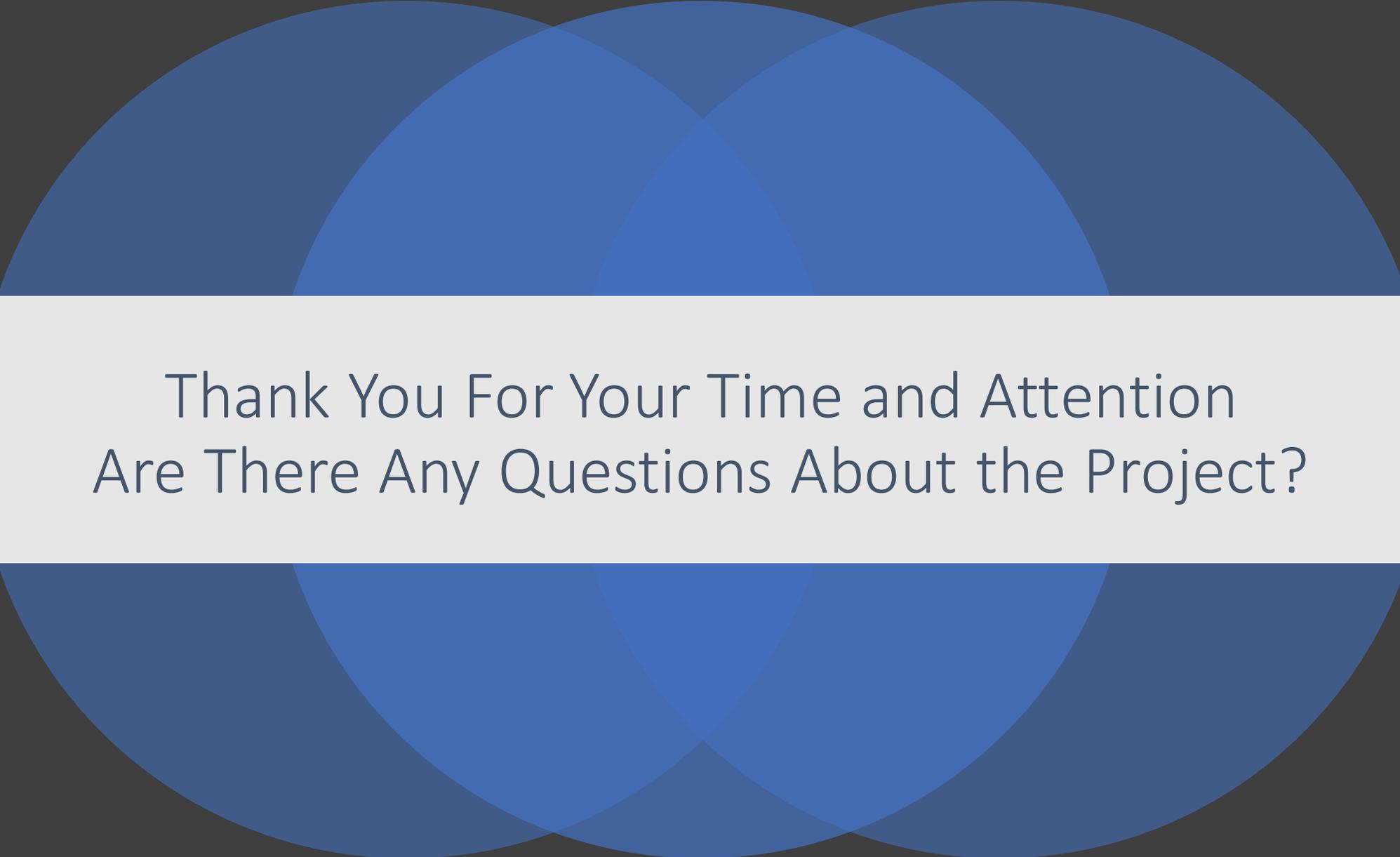
- Researching the Effect of Vaping Liquid Chemicals on the Environment
- Helpful because it figures out how human usages of vape not only harm humans but also the ecosystem around them

A SPECIAL THANKS TO...

**Dr. Christopher Sacchi PhD,
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and

Dr. J.V. Roth MD



Thank You For Your Time and Attention
Are There Any Questions About the Project?