**Toxicity Lab**

Purpose: Students will investigate how various concentrations of nicotine affect the behavior of Lumbriculus variegatus (California blackworms).

Background information: Students will need to initially observe the worms to establish what they will consider their “baseline measurement”, or “normal behavior”. This “baseline behavior” activity level will be used in comparing worm behavior when nicotine is introduced into the test environment. Several nicotine concentrations will be used, with lab groups observing and recording worm behavior during exposure.

Normal behavior includes clinging activity in which multiple worms clump into a mass. Gently probing the worms will cause separation of clumped worms. Worms exposed to nicotine may twitch. A loss of response may be illustrated with tail-curling.

*Acute and Chronic Toxicity*: Acute toxicity refers to a relatively high dose of a given toxin over a short time period. Chronic toxicity refers to relatively small doses of a toxin over a longer time period. Lethal Dose 50, or LD50, is commonly used in measuring acute toxicity. LD50 is the dose of a given substance which is lethal to 50% of the test organisms. The information below displays the LD50 for humans for five selected chemicals:

|  |  |
| --- | --- |
| **Substance** | **LD50 (measured for 150 lb person)** |
| Botulinum toxin (Botox) | 75 units (75 ng), avg. is 20 ng treatments |
| Alcohol (40%) | 13 shots (1 shot = 45 mL) |
| Water | 6 L |
| Caffeine | 118 coffees (1 coffee = 240 mL) |
| Nicotine | 75 cigarettes (1 cigarette = 1.0 mg nicotine) |

Procedure:

1. Label 8 Petri dishes in the following manner:
* “Water Control”
* “Water 1”
* “Water 2”
* “Water 3”
* “Control”
* “Low Concentration”
* “Medium Concentration”
* “High Concentration”
1. Using a beaker, obtain approximately 50 mL of the nicotine stock solution. Decide what concentrations to place in your petri dishes. Remember that each petri dish will contain about 10 mL of liquid. Plan accordingly.
2. Fill each of the water/control petri dishes with approximately 10 mL of water using the small graduated cylinder.
3. Place 10 worms in each of the following Petri dishes:

Water Control, Water 1, Water 2, and Water 3

1. Observe the worms “normal” behavior, with and without probing.
2. Move the worms from the Petri dishes indicated in the “Left Column” to the Petri dishes in the “Right Column” below:

|  |  |
| --- | --- |
| **Left Column** | **Right Column** |
| Water Control | Control |
| Water 1 | Low Concentration |
| Water 2 | Med. Concentration |
| Water 3 | High Concentration |

1. Observe and note worm behavior at the following time intervals:
* Time 0 (immediately after the 10 worms per petri dish have been moved.)
* 4 minutes after the transfer
* 8 minutes after the transfer
* 12 minutes after the transfer
1. Move the worms back into the petri dishes in the “Left Column” (original dishes) for the “recovery” period.
2. Again observe and note worm behavior at Time 0, 4, 8, and 12 minutes.
3. Worms will be left in their respective “recovery” Petri dishes until the next class meeting (24 hours later), when final observations/notes will be made.

**Toxicity Lab Report**

Purpose: Students will investigate how various concentrations of nicotine affect the behavior of Lumbriculus variegatus (California blackworms).

Materials:

40 blackworms

Nicotine solution

8 Petri dishes

Water

Plastic Spoon

Graduated cylinder

Beaker(s)

Hypothesis: Construct an appropriate hypothesis for the given experiment. Additionally, construct a null hypothesis (a statement that refutes or counters your original hypothesis).

Data: Attach the data sheet into your lab report. For each petri dish tested (the control, and three concentrations of nicotine), include notes on activity level, behavior and any abnormalities for each of the 4 minute intervals. You are collecting information for minutes 0, 4, 8 and 12 after transference into the testable solution.

Discussion: In this section, you are to explain the process by which you came up with your experiment; explain your control(s) as well as your independent and dependent variables. Additionally you will explain your hypothesis in more detail (how you think the experiment will turn out). Briefly describe the course of your experiment, noting anything that supports or counters your hypothesis, and finally (most importantly), discuss any errors witnessed in the experiment that would improve next time you were to conduct a similar study.

Post-Lab Questions: answer these questions following the laboratory experiment.

1. Acute toxicity tests are a relatively high, one-time exposure for a brief duration. Chronic toxicity tests typically involve a persistent and generally longer exposure, although this can vary greatly due to variation in life span for different organisms. Which type of toxicity test did you carry out in this lab?
2. Explain a possible benefit of using an acute toxicity test.
3. Is this lab investigation a controlled experiment? Explain how it is or is not:
4. Using the data from this lab, design a chronic toxicity test that could be conducted on blackworms.
5. Describe three intrinsic factors which could affect toxicity and discuss how each might influence the impact(s) of nicotine or another toxin.
6. Identify an extrinsic factor which might influence the severity of a toxin in a given organism and briefly explain how this factor could be involved in how adversely the organism is impacted by a toxin.
7. Briefly discuss how lifestyles/lifestyle choices are involved in assessing risk in terms of human health and the potential toxins which humans may be exposed to:
8. Discuss whether the effects of nicotine exposure were irreversible or reversible at each concentration used.
9. Can your test results in this lab be applied to humans or to other vertebrates?

Please explain:

 **Toxicity Lab** **Data Sheet**

**Attach this to your completed lab**

Behavior/Activity Rating: Rate the worm activity at each time interval.

* No Apparent Activity = 0
* Not as Active as Normal = 1
* Normal Activity = 2
* More Active than Normal = 3
* Extremely Active = 4

Notes: When prompted, make notes regarding the behavior of the worms. Remember, normal behavior means they pack together in clumps and curl, whereas sick activity means they are isolated and straight

 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**Exposure**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

CONCENTRATION: 0% \_\_\_\_\_% \_\_\_\_\_% \_\_\_\_%

  Control Low Nicotine Medium Nicotine High Nicotine

 **0 minute 0 minute 0 minute 0 minute**

 BEHAVIOR 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4

 NOTES \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **4 minutes 4 minutes 4 minutes 4 minutes**

 BEHAVIOR 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4

 NOTES \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **8 minutes 8 minutes 8 minutes 8 minutes**

 BEHAVIOR 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4

 NOTES \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **12 minutes 12 minutes 12 minutes 12 minutes**

 BEHAVIOR 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4

 NOTES \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Recovery**: Record the most appropriate letters applicable to the majority of worms in each Petri dish at each time interval.

 \_Behavior Control Low Nicotine Medium Nicotine High Nicotine

###  CR = Completely 4 min 4 min 4 min 4 min

 Recovered \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 PR = Partially 8 min 8 min 8 min 8 min

 Recovered \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 NR = Not 12 min 12 min 12 min 12 min

 Recovered \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_