TOXI-LAB

SYNTHETIC TOXICITY EDUCATIONAL KIT

STREAM

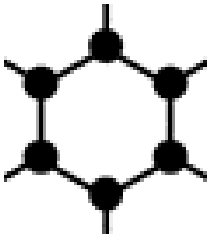


TABLE OF CONTENTS

Educator Version

Student Lab Report

Introduction

EBPI's synthetic Toxi-Lab™ kit gives students the opportunity to have a hands-on experience on how science is incorporated into technology and to relate scientific concepts as well as principles to their environment. Students can determine environmental toxicity through a simulated experiment with unknown samples to demonstrate a simulation of chemical toxicity to bacteria.

Purpose

The study of the adverse effects of a chemical on living organisms, whether human, animal, plant, or microorganism is called toxicology. An "adverse effect" can range from a life-threatening incident to an event that is barely noticed. Toxicology is an inter-disciplinary science that integrates principles and methods from many fields: chemistry, biology, biochemistry, pharmacology, molecular biology, physiology and medicine. The synthetic Toxi-Lab™ kit is able to simulate a test to different compounds to determine toxicity levels. EBPI designed these kits to give reproducible results that are similar to the bacterial based experiment. This experiment involves a simulation of a test that includes four unknown samples and three control samples: one positive sample (standard toxicant), one negative control, and one blank column.

WARRANTY

EBPI warrants that, at the time of shipment, the synthetic Toxi-Lab™ kit is free of defects in material and workmanship, and complies with the company specifications. Since actual experimental conditions prevailing at user's laboratory are beyond the control of EBPI or its representatives, EBPI makes no other warranty, express or implied, with respect to the product. Notification of any breach of warranty must be made within 120 days of delivery. The sole and exclusive remedy of the customer for any liability of EBPI of any kind, including liability based upon warranty (express or implied, whether contained herein or elsewhere) is limited to the replacement of the product or the refund of the invoice price of the product.

Concept: Toxicity

About Toxicity

Toxicity is the degree to which a substance can damage an organism and also how much of the substance induces the damage. The importance & study of toxicology occurs for the following reasons:

- We look at the modern examination of changes in specific biochemical processes due to exposure to different substances
- We look at the consequences of what occurs when there is an accidental release of chemicals and raw materials into the environment
- We need to study, assess, and understand the potential health effects these substances might have on our environment, organisms, and ourselves

DDT and the Emergence of Environmental Awareness

While the study of chemicals, and more specifically of poisons, has been around for centuries, the impact of these chemicals on the environment is a more recent field of study. The history behind environmental toxicology and the environmental movement is intimately linked to the discovery and widespread use of insecticides. One in particular, dichlorodiphenyltrichloroethane (DDT), stands out from all the others. DDT was created in the 1800's, patented for use as an insecticide in 1940 by Paul Müller, a Swiss chemist, and quickly put to use during World War II as a method of killing insects. While many countries experienced much success with this new insecticide, the lack of initial testing on DDT would

later lead to significant problems. After the war, the widespread use of DDT and other pesticides and insecticides continued to grow in use in agriculture. However, the detrimental effects of these chemicals would be brought to the forefront by Biologist Rachel Carson in her book Silent Spring released in 1962. The book examined the environmental impact of DDT and other pesticides, and documented how these various pesticides were responsible for killing fish, reducing many bird populations including the Bald Eagle, and endangering human health.

Bioassays

We can evaluate toxicity by using a bioassay which involves a possible toxic chemical added to small petri dishes containing live organisms such as bacteria, cultured cells, or even invertebrate organisms such as Daphnia. After exposure to different concentrations of the chemical, we examine the cells or organisms to see if they have undergone a change. A scientist might look for effects such as death at a certain chemical concentration, or the inhibition or stimulation of cellular activities such as transport, movement, or cellular division. Bioassays are useful since you only require a small amount of your sample, bacteria respond much more quickly to toxicants, and the convenient testing protocol allows for the rapid testing of multiple chemical concentrations and samples simultaneously. The synthetic Toxi-Lab™ is a simulation of the bacterial based experiment. This experiment involves a simulation of a test that includes four unknown samples and three control samples: one positive sample (standard toxicant), one negative control, and one blank column.

The Reaction

The bacterial version of the Toxi-Lab™ takes advantage of E.coli's natural ability to produce an enzyme called β-galactosidase which is used by bacteria to metabolize the food source lactose. The E.coli bacteria was exposed to stressing conditions and need to first recover, before they will actively produce β-galactosidase. The bacteria was incubated in a cocktail containing essential factors required for the recovery of the bacteria, a specific inducer of β-galactosidase, and the test chemical. This bioassay is based on the knowledge that a biologically active toxicant or chemical can interfere with the bacteria's recovery process, diminishing β-galactosidase's action or preventing its production, effectively slowing down or even stopping the digestion of lactose.

Unfortunately, scientists can't "see" or measure this phenomenon. We provide a synthetic sugar (a chromogen called X-gal) to emulate the lactose. β-galactosidase recognizes this imposter molecule as "lactose" and cleaves (hydrolyzes) the X-gal, producing a blue colour in the well with the bacteria. The amount of blue chromogen released reveals the extent to which the chemical is harmful to this living cell. A dark blue colour indicates that a lot of β-galactosidase is being produced, and many molecules of X-gal are successfully being cleaved to the blue endpoint; hence, the chemical is not very toxic. If a light blue colour is produced in a well, it indicates that the test chemical is somehow inhibiting the production or function of β-galactosidase, and only a few molecules of X-gal are being cleaved to a blue endpoint. In extreme toxic conditions, a chemical could actually kill all the bacteria present, meaning there would be no β-galactosidase produced, no X-gal cleaved, and absolutely no blue colour produced (the well would appear clear).

Preparation
Synthetic Toxi-Lab™

EDUCATOR COPY

Expectations

The synthetic Toxi-Lab™ gives you the opportunity to determine environmental toxicity through a simulated experiment with unknown samples to demonstrate a simulation of chemical toxicity to bacteria. The synthetic Toxi-Lab™ kit is a realistic simulation of determining toxicity in unknown samples. Each student group will process four unknown samples and three control samples: one positive sample (standard toxicant), one negative control, and one blank column. Once your class completes the experiment and the colour change occurs, the results can be recorded to determine toxicity levels and each group will complete a lab report for evaluation. The synthetic Toxi-Lab™ is designed for students to work in groups of two to four with one microplate per group.

Contents of the Synthetic Toxi-Lab™ Kit

Each kit contains sufficient components for 12x 96-well microplates. The bottles and vials in the synthetic Toxi-Lab™ are labelled with clear, bold letters.

Each kit should contain the following:		Further materials required, but not included in the kit (contact EBPI if you require these items):
▪ 12x 96-well Microplates ▪ Sample 1 5 mL (1 unit) ▪ Sample 2 5 mL (1 unit) ▪ Sample 3 5 mL (1 unit) ▪ Sample 4 5 mL (1 unit) ▪ Diluent Buffer 100 mL (1 unit)	▪ Reaction Mixture without Bacteria 11 mL (1 unit) ▪ Reaction Mixture with Bacteria 10 mL (6 units) ▪ Standard Toxicant ($HgCl_2$) 5 mL (1 unit) ▪ Chromogen 5 mL (6 units)	• 20x units of 200 uL micropipettes & tips • 16x units of Test Tubes

Collecting the Samples and Preparation of the Samples

No samples or advance preparation is required.

Student Station Preparation

Below is the suggested distribution of the reagents needed for the experiment. The suggested distribution is assuming there will be 12 groups with 2 students per group.

STUDENT LAB STATIONS		FRONT OF THE CLASSROOM
Each group should have the following:		1 Unit of Reaction Mixture without Bacteria
- 1x 96-well Microplate - Test Tubes - Micropipettes	- ~ 8 mL of Diluent Buffer - ~ 5 mL of Reaction Mixture with Bacteria - ~ 2.5 mL of Chromogen	1 Unit of Standard Toxicant

Lab Safety Protocols – Review this with your class before beginning the experiment

Storage:
- All kit components can be stored at room temperature.
- The chromogen is light sensitive and is provided in a dark bottle.
- Store the 96-well microplates in a dry, dark location at room temperature to ensure optimum test performance (improper storage may result in an instantaneous colour change as opposed to a gradual colour change).

Handling:
- The simulated reaction mixture with bacteria contains ammonium hydroxide. Appropriate safety precautions should be taken (eye protection and gloves).
- Keep container tightly sealed in a dry and well-ventilated place. This reagent is a single-use reagent.
- Once opened and used for this experiment, this reagent should be disposed of according to appropriate legal requirements.

Procedure – Synthetic Toxi-Lab™

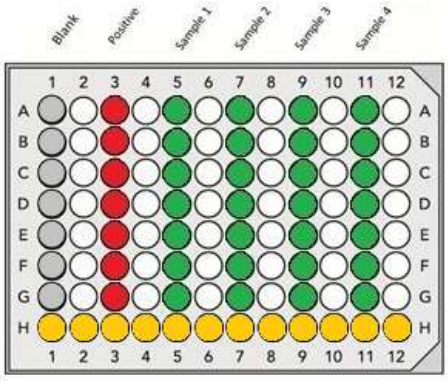
Please note the following before beginning the experiment:

Column 1 is the blank control

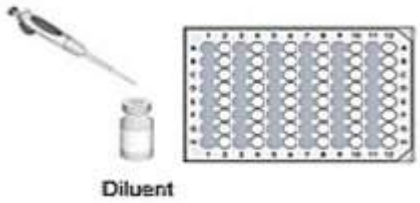
Column 3 is the positive control

Row of Well H is the negative control

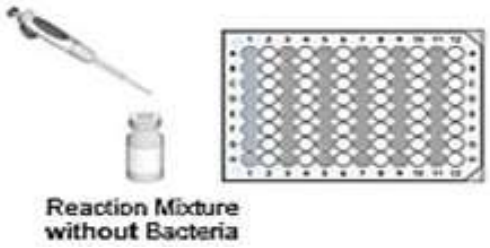
Columns 5, 7, 9, & 11 are Samples 1, 2, 3, & 4 respectively



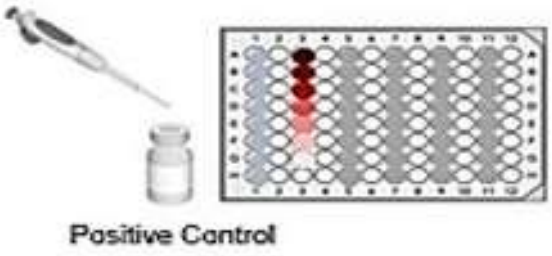
1. Add 100 uL of diluent to each well in column 1. Add 100 uL of diluent to wells B to H in columns 3, 5, 7, 9, & 11. Do NOT add diluent to well A in any of those columns as the positive control & samples will be tested at full strength.



2. Add 50 uL of **Reaction Mixture without Bacteria** to each well in column 1.

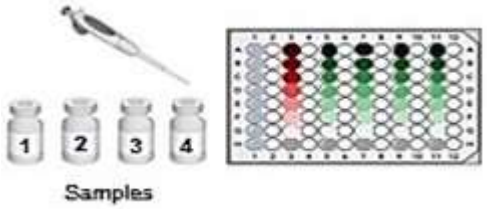


3. In column 3, add 200 uL of the positive control to well A. Perform a **serial dilution** by transferring 100 uL from well A to well B, then transfer 100 uL from well B to well C, and so on until you reach well G. Remove 100 uL from well G. Do NOT add anything to well H.

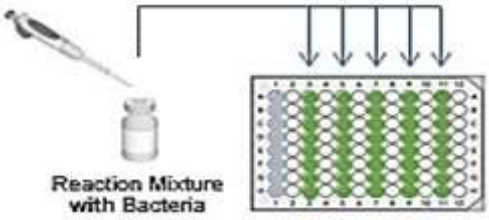


4. Repeat Step 3 using Samples 1 to 4.
- in column 5, add 200 uL of sample 1 to well A.
- in column 7, add 200 uL of sample 2 to well A.
- in column 9, add 200 uL of sample 3 to well A.
- in column 11, add 200 uL of sample 4 to well A.

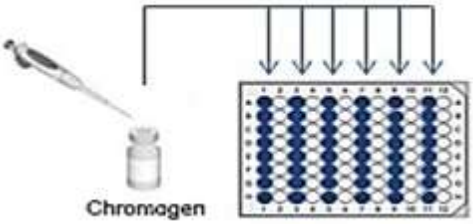
Perform serial dilutions from well A to well G.
Remove 100 uL to well G. Do **NOT** add anything to well H.



5. For columns 3, 5, 7, 9, & 11, add 50 uL of Reaction Mixture with Bacteria to wells A to H. Do **NOT** mix.



6. Add 40 uL of chromogen to every well used in all columns.



7. Clean up. All materials can be disposed of through regular solid waste disposal.

To Educators: Please review the information as a visual guideline of the experiment results, recording the results significance, and the answer sheet for the questions.

Recording the Results

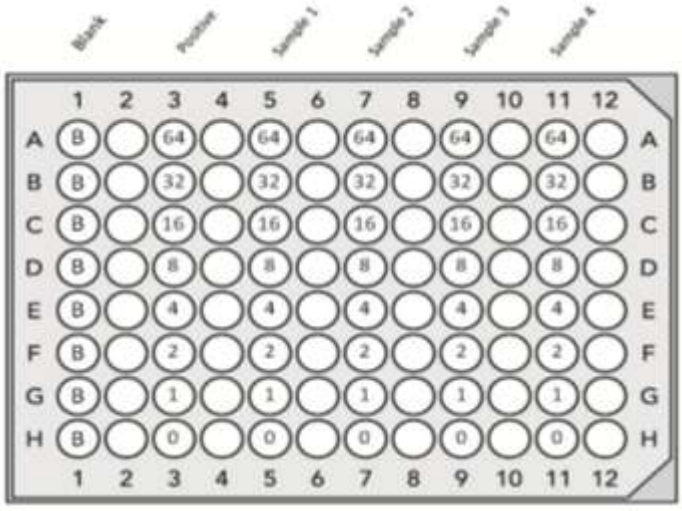
Once the experiment is done, students will be able to record the results for their plate using the Tables provided. This includes completing the % Toxicity, calculating the mean average and standard deviation, as well as graphing the results of each sample. Once this is complete, students should complete their analysis.

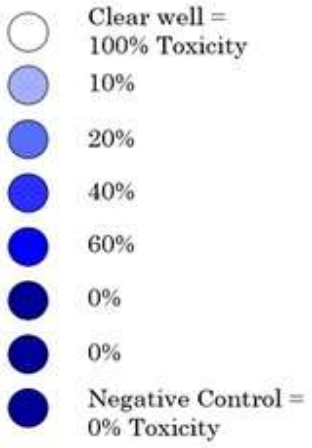
Please Note: Once the chromogen is added, the results will develop instantaneously and will be stable for approximately 15 minutes, so it is important that the results are recorded once the chromogen is added.

During this time, you can also assess and evaluate their results to note accuracy and any possible errors. Students should be encouraged to record their errors in their lab reports.

Guidelines

A. Immediately after the chromogen is added, students should place the microplate on top of a white piece of paper to better observe the colours. Final concentrations are indicated in the figure below where B refers to blank, each number refers to the concentration of that well relative to the undiluted sample (row A), and 0 indicates absence of sample and absence of standard.

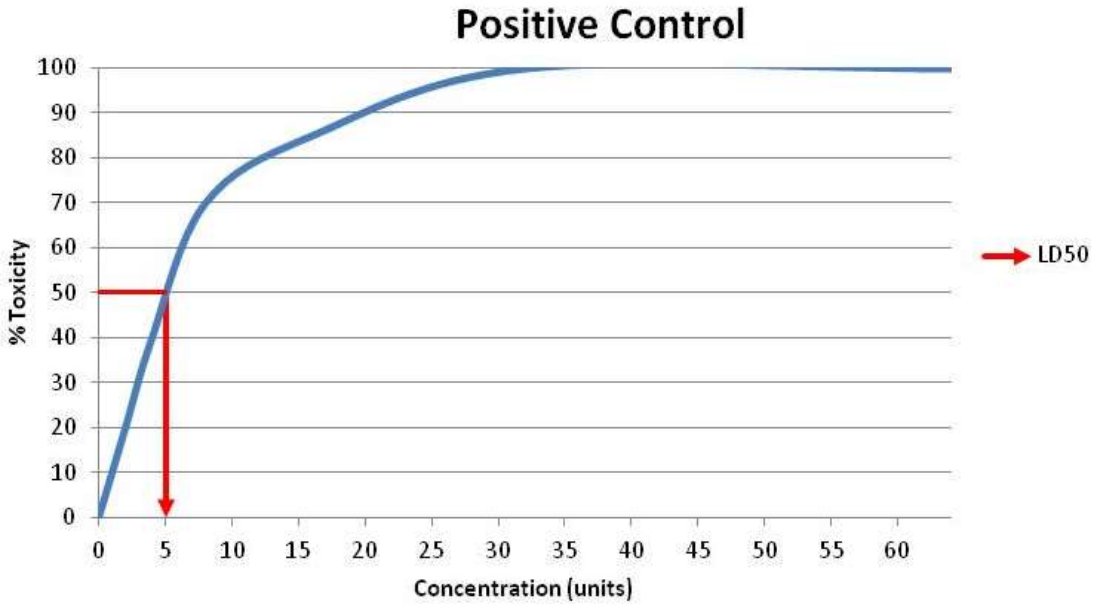




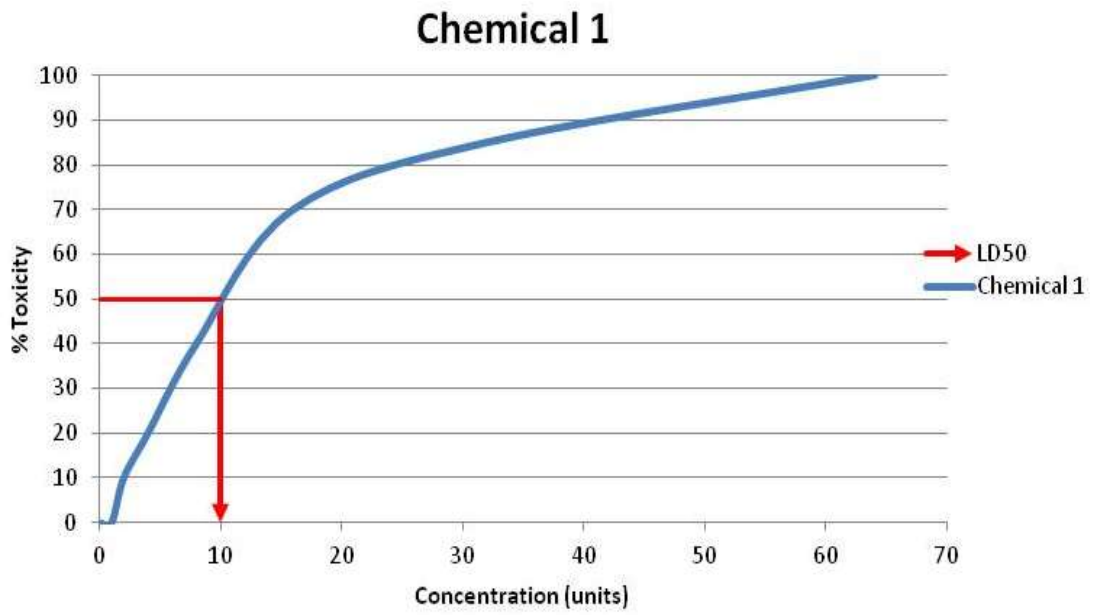
B. Students will perform a visual analysis on the intensity of the blue colour on each well and assign the toxicity values. The last well in each column (Well H) should have the highest intensity blue colour as these wells did not contain any sample or toxicant. These wells should be treated as 0% toxicity. All other wells should be compared to these 0% toxicity wells. Have students use their judgement.

Positive Control and Sample Results: The following information contains sample results about the simulated samples once they undergo the synthetic Toxi-Lab™ experiment. Presented below are approximate dose-response graphs for the positive control, as well as samples that would represent the results you are looking for. Students will generate one graph for each sample with the dose response curves agreeing somewhat closely with the results presented below. It is expected that there will be some leeway since the subjective assignment of toxicity can result in slightly different shapes and LD50 values.

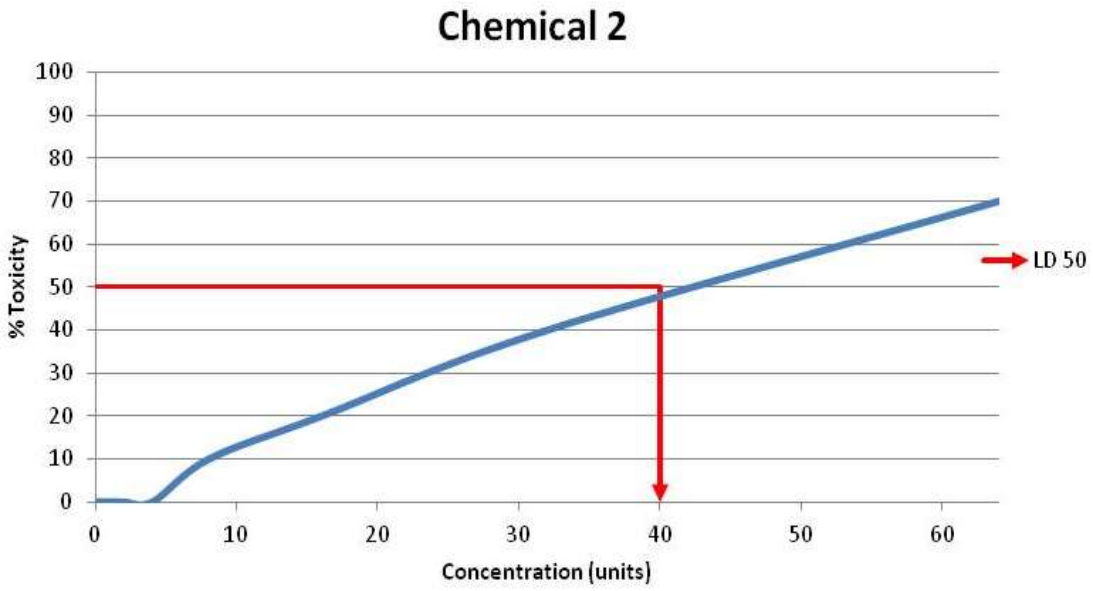
Graph 1:
Dose Response for Positive Control



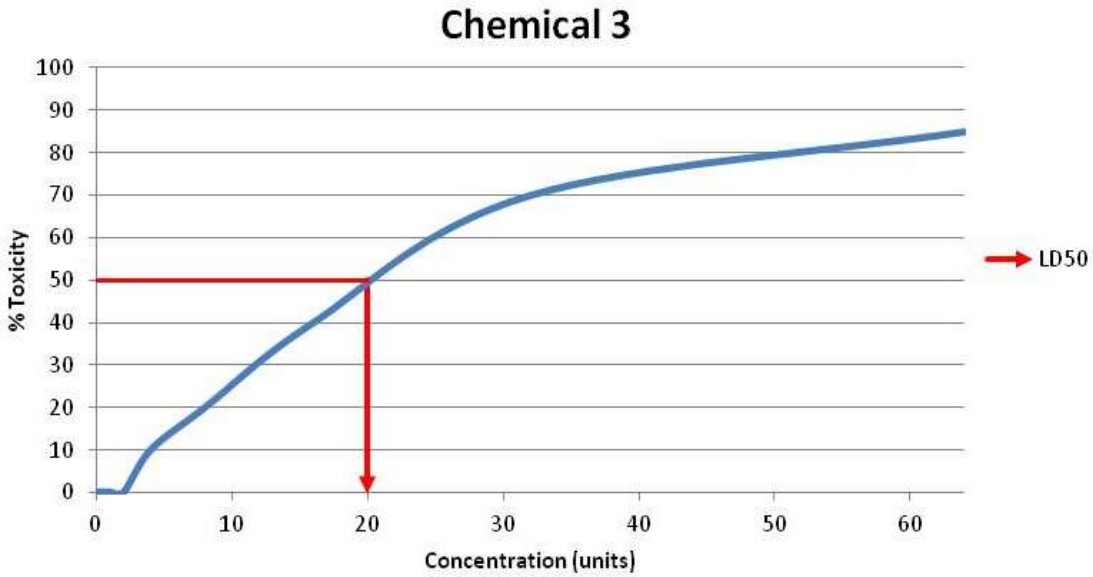
Graph 2:
Dose Response for Sample 1



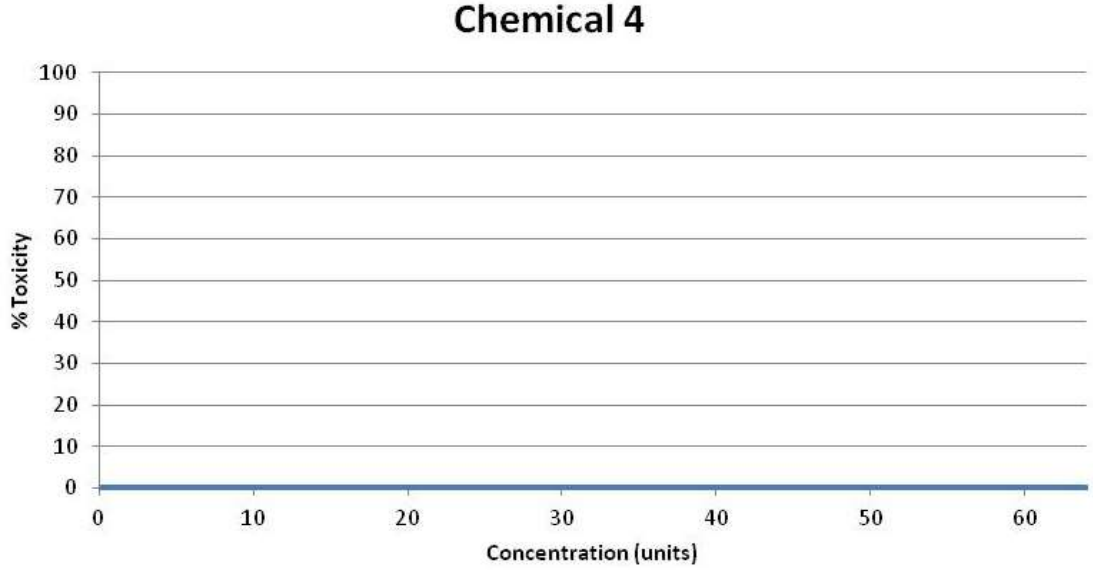
Graph 3:
Dose Response for
Sample 2



Graph 4:
Dose Response for
Sample 3



Graph 5:
Dose Response for
Sample 4



Interpreting the Results:

1. Using the information from '*Recording the Results Guidelines*,' the student groups would complete the % Toxicity Table using the assigned % toxicity values.

2. Students will then complete the mean average and the standard deviation to provide all the information needed to properly analyze the results and determine the dose-response.

3. Students will create a dose response curve for each sample and the positive control with concentration units on the x-axis and % toxicity on the y-axis. Students should plot the average response calculated from all group estimates. As well, above and below each point, place markers to show the range of the standard deviation. Students should draw a smooth curve through the data on each graph and try to determine at which concentration 50% survival occurred for each sample. This is the LD50. Students should then compare the LD50s of the different samples and classify them from most toxic to least toxic in the Table.

Further Analysis:

You can pick further analysis questions based on your preference and what you want your students to learn.

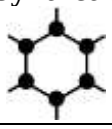
Questions and Answers

1. Name three factors which affect the toxicity of a compound.	Factors that affect the toxicity of a compound are the dose, the route of exposure, duration of exposure, individual susceptibility, and the properties of the compound.
2. Explain the concept of dose response. Make sure to mention the NOEL and LD50 in your answer.	Dose-response is simply a measure of the adverse health effects (or in the case of bacteria in this test, death) as a function of the amount of the compound of interest. Typically, as the dose increases, the measured response increases. There may be no response at low doses, however, this will eventually rise at the NOEL (No Observed Effect Level). As the dose is increased, it will reach the LD50, the point at which only 50% of the organisms exposed survive the dose. This will increase to 100% toxicity at a high enough dose, as anything will be toxic at high enough concentrations.
3. Have students reply to the main question: Which sample had the lowest concentration to reach LD50? How does this relate to the % toxicity? Explain the significance of this using the concepts learned in class (i.e. dose, duration, LD50, etc.).	This answer will vary and depend on what samples were collected. Ensure that students have the concepts (dose, duration, LD50, etc.) included in their answers.

Student Lab Report

Group #	Group Student Names	Role

Checkmark the appropriate Stream below:

Water 	Chemical 	Soil 	Synthetic 

Note: At the beginning of class, your teacher will have the lab stations ready. Remember to check your lab station, ensure you have the appropriate supplies, and read over the safety protocols.

Pre-Lab Notes	

Instructions: Read over the preparation and the procedure for the specific biotechnology kit that you will be working on. Complete the lab and record the results.

Preparation – Synthetic Toxi-Lab™

To Students: Please follow the instructions and information below to help prepare for the lab experiment.

Expectations

The synthetic Toxi-Lab™ kit is a realistic simulation of determining toxicity in unknown samples. Your group will process four unknown samples and three control samples: one positive sample (standard toxicant), one negative control, and one blank column. Once your class completes the experiment and the colour change occurs, the results can be recorded to determine toxicity levels and your group will complete a lab report for evaluation.

Lab Station Preparation

At the beginning of the lab, you should have the following available in the front of the classroom and lab station:

FRONT OF THE CLASSROOM

- 1 Unit of Reaction Mixture without Bacteria
- 1 Unit of Standard Toxicant

GROUP LAB STATION

- 1x 96-well Microplate
- Test Tubes
- Micropipettes
- ~ 8 mL of Diluent Buffer
- ~ 5 mL of Reaction Mixture with Bacteria
- ~ 2.5 mL of Chromogen

Before starting an experiment it is important to ensure all of the materials, supplies and equipment needed are available and ready. Be sure to speak to your educator if there is anything missing.

Ensure that you review the Lab Safety Protocols before beginning the experiment

Storage:

- All kit components can be stored at room temperature.
- The chromogen is light sensitive and is provided in a dark bottle.
- Store the 96-well microplates in a dry, dark location at room temperature to ensure optimum test performance (improper storage may result in an instantaneous colour change as opposed to a gradual colour change).

Handling:

- The simulated reaction mixture with bacteria contains ammonium hydroxide. Appropriate safety precautions should be taken (eye protection and gloves).
- Keep container tightly sealed in a dry and well-ventilated place. This reagent is a single-use reagent.
- Once opened and used for this experiment, this reagent should be disposed of according to appropriate legal requirements.

Results & Analysis
Synthetic Toxi-Lab™

Instructions: Once you obtained the results, complete the following steps below. Read over the guidelines before recording your results.

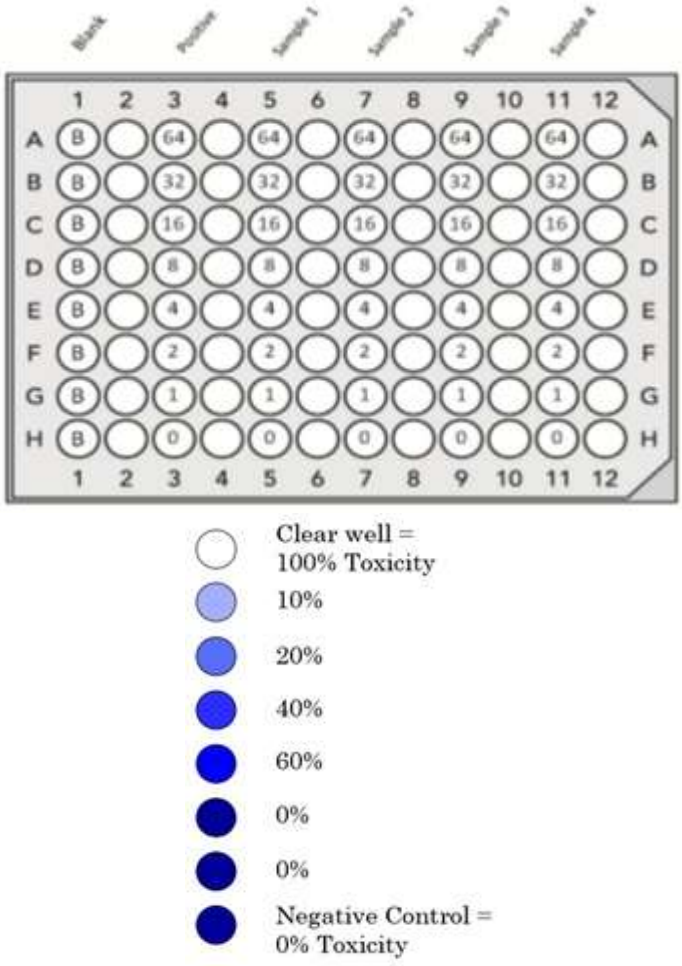
If you have any errors from the experiment, please describe them here and the reasons why this occurred:

Observing the Results

Once the chromogen is added, the results will develop instantaneously and will be stable for approximately 15 minutes, so it is important that you record the results once the chromogen is added. Record the results for your plate using the Table provided. This includes completing the % Toxicity, calculating the mean average and standard deviation, as well as graphing the results of each sample. Once this is complete, your group can complete the analysis for evaluation.

Visual Guidelines

A. Immediately after the chromogen is added, place the microplate on top of a white piece of paper to better observe the colours. Final concentrations are indicated in the figure below where B refers to blank, each number refers to the concentration of that well relative to the undiluted sample (row A), and 0 indicates absence of sample and the absence of standard.



B. Perform a visual analysis on the intensity of the blue colour on each well and assign the toxicity values. The last well in each column (Well H) should have the highest intensity blue colour as these wells did not contain any sample or toxicant. These wells should be treated as 0% toxicity. All other wells should be compared to these 0% toxicity wells. Complete the % Toxicity table on the next page.

Recording and Interpreting the Results:

1. Complete the % Toxicity Table using the assigned % toxicity values.

Concentration (Units)	Positive Control	Sample 1	Sample 2	Sample 3	Sample 4
64					
32					
16					
8					
4					
2					
1					
0					

2. The mean and the standard deviation provide all the information needed to properly analyze the results and determine the dose-response. Calculate the average toxicity % and standard deviation at each concentration for the positive control and each sample and record the results.

Formula to calculate the mean:

$$\bar{X} = \frac{\sum_{i=1}^{i=n} X_i}{n}$$

Mean ($\bar{X}$) is the sum of all observations that the other student groups made ($\sum_{i=1}^{i=n} X_i$) …

… Divided by the number of student groups (n)

Formula to calculate the standard deviation:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n}}$$

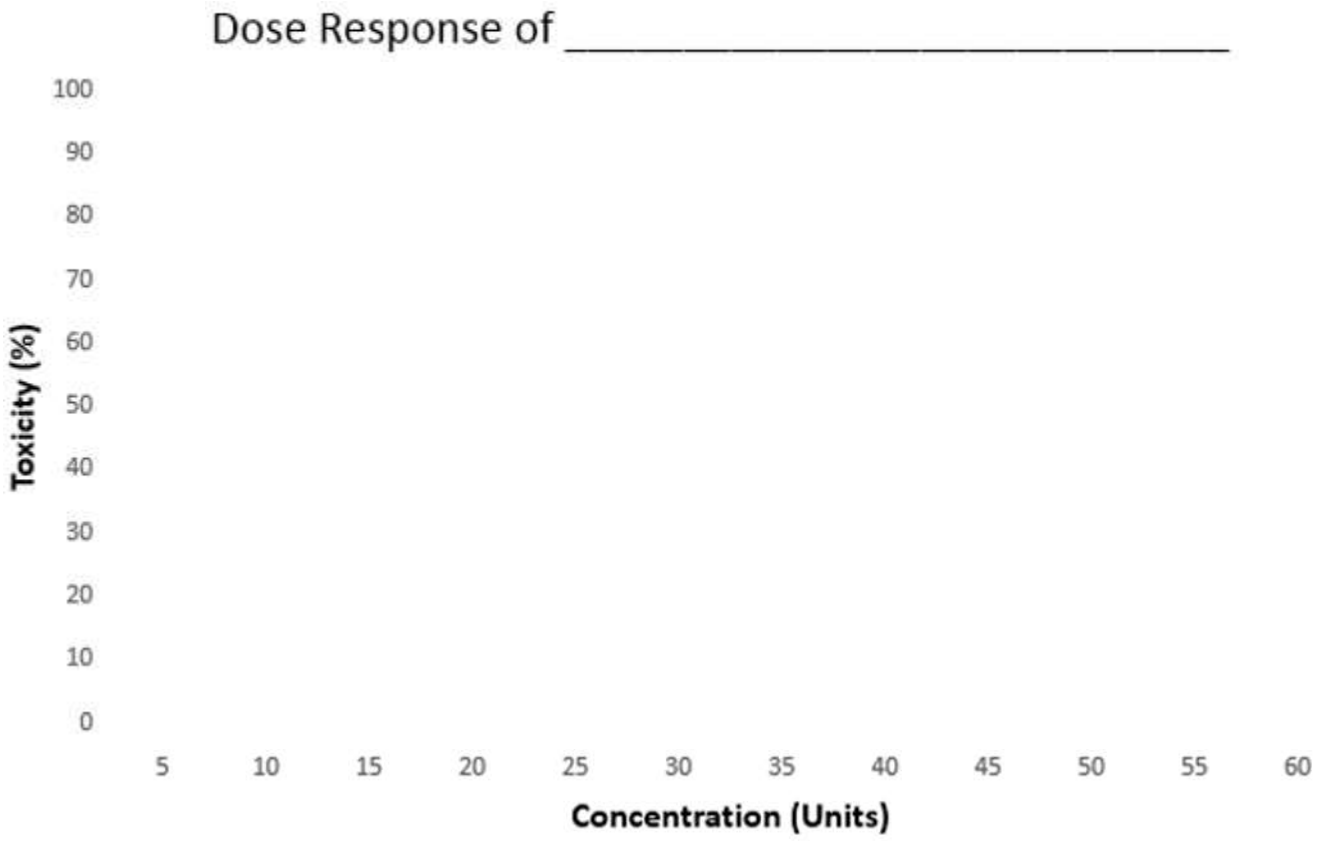
- **σ is the standard deviation**
- **$(x_i - \bar{x})^2$ = difference between each observation and the mean of all the observations, which is then squared**
- **$\sum_{i=1}^{n} i$ = sum of the squared difference between the mean and each observation**
- **'n' = number of observations (i.e. number of student groups)**

Concentration (Units)	Positive Control		Sample 1		Sample 2		Sample 3		Sample 4	
	Avg	Std Dev	Avg	Std Dev	Avg	Std Dev	Avg	Std Dev	Avg	Std Dev
64										
32										
16										
8										
4										
2										
1										
0										

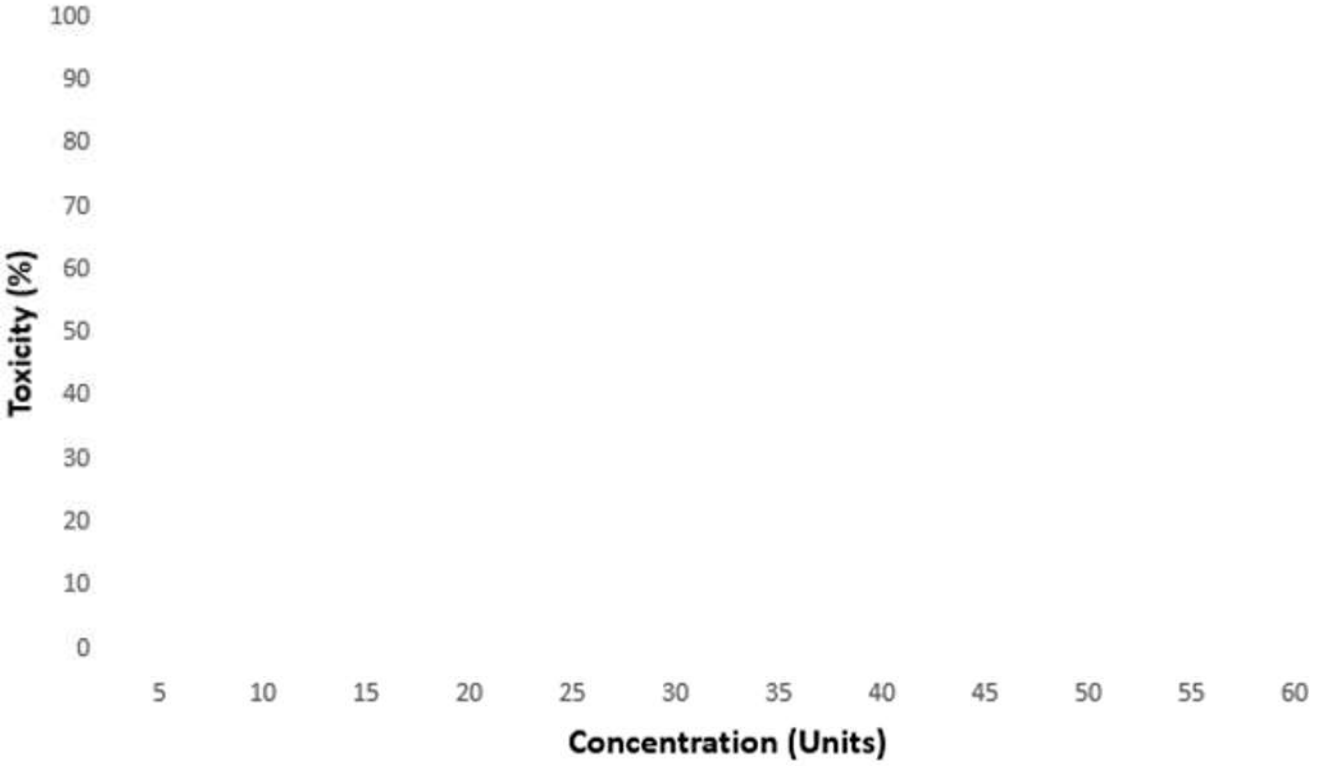
3. Create a dose response curve for each sample and the positive control with concentration units on the x-axis and % toxicity on the y-axis. Plot the average response calculated from all group estimates. As well, above and below each point, place markers to show the range of the standard deviation. Draw a smooth curve through the data on each graph and try to determine at which concentration 50% survival occurred for each sample. This is the LD50. Compare the LD50s of the different samples and classify them from most toxic to least toxic in the Table.

Table: LD_{50} and relative toxicity ranking for positive control and each sample.

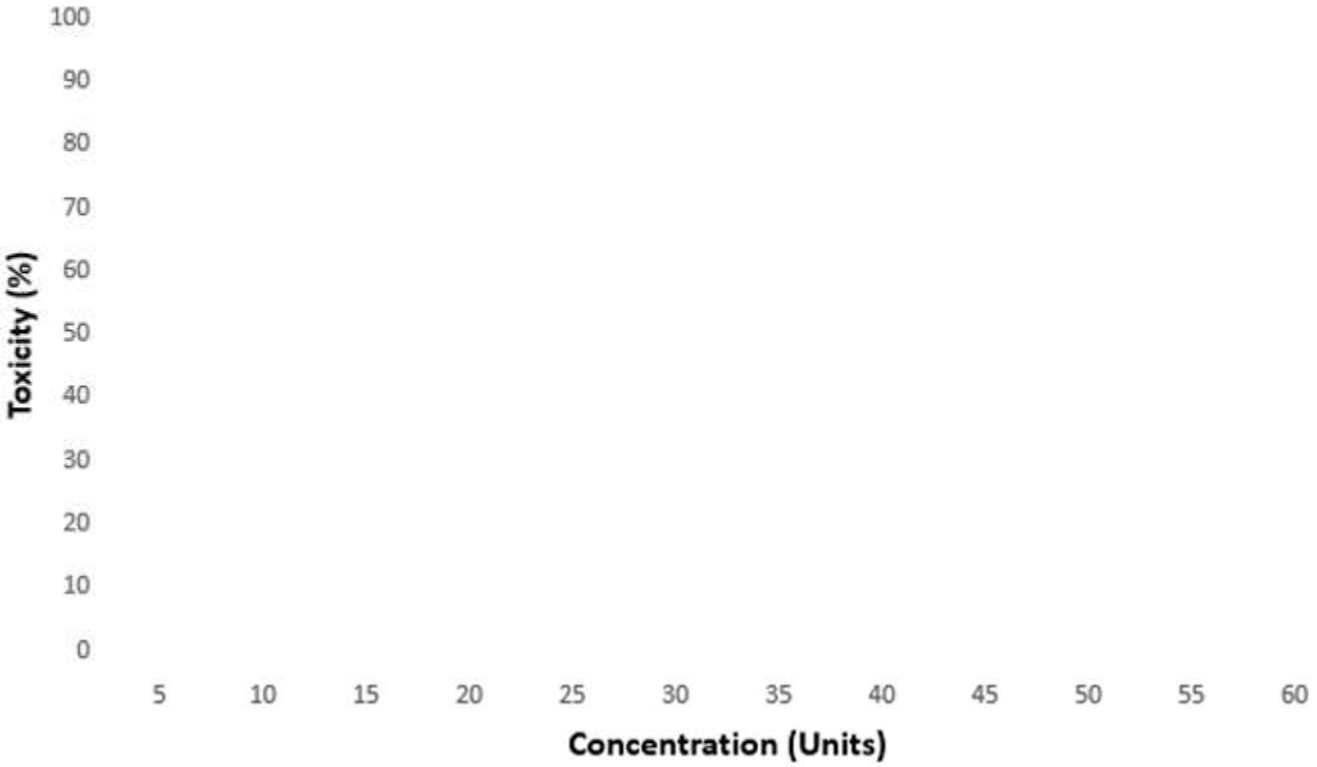
Sample	LD_{50}	Toxicity Rank
Positive Control		
Sample 1		
Sample 2		
Sample 3		
Sample 4		



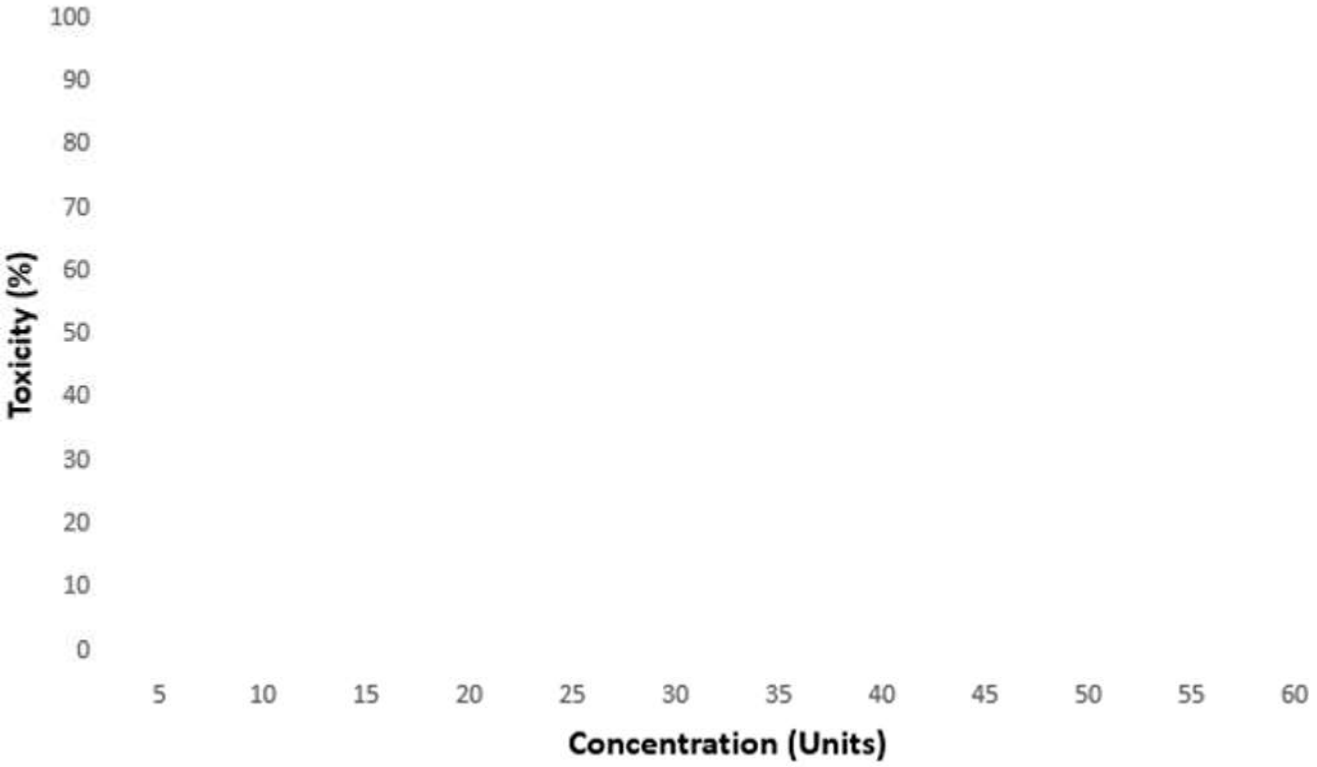
Dose Response of _____



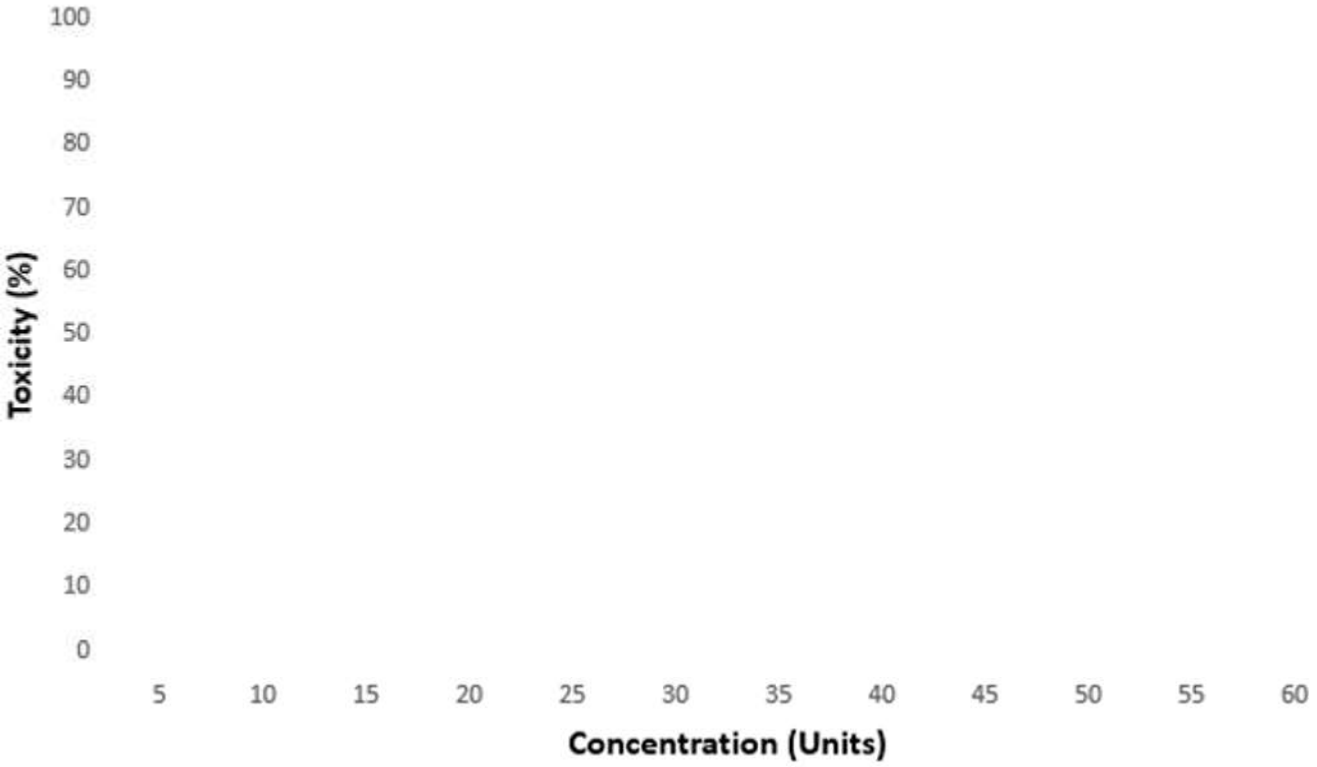
Dose Response of _____



Dose Response of _____



Dose Response of _____



Further Analysis – Please complete the following questions below.

Name three factors which affect the toxicity of a compound.

Explain the concept of dose response. Make sure to mention the NOEL and LD50 in your answer.

Which sample had the lowest concentration to reach LD50? How does this relate to the % toxicity? Explain the significance of this using your results and the concepts.