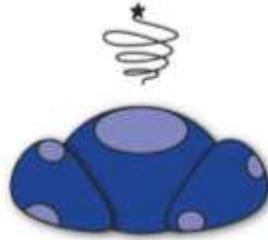




TOXI-LAB

BACTERIAL TOXICITY EDUCATIONAL KIT



STREAM



TABLE OF CONTENTS

Educator Version

About the Toxi-Lab™

Introduction	2
Purpose	2
Warranty	2
Concept: Toxicity	2-3
The Reaction	3

Preparation

Expectations	4
Contents of the Toxi-Lab™ Kit	4
Collecting the Samples	4
Preparation of the Samples	4
Student Station Preparation	5
Lab Safety Protocols	5

Procedure (Classroom Use)

6

Results and Analysis Guidelines

Recording the Results	7
Guidelines	7
Positive Control and Sample Results	8
Further Analysis (Questions and Answers for Students)	9

Student Lab Report

Introduction and Pre-Lab Notes

10

Preparation

Expectations	11
Collecting the Samples	11
Lab Station Preparation	11
Safety Protocols	11

Results and Analysis

Observing the Results	12
Recording the Results	13-15
Further Analysis	16



About the Toxi-Lab™ Red and Blue Chromogen

EDUCATOR
COPY



Introduction

EBPI's Toxi-Lab™ kit gives you the opportunity to determine environmental toxicity and the kit can be used for a variety of toxic substances such as heavy metals, as well as organic and inorganic pollutants. The test determines the relative toxicity of different samples at varying concentrations.

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 field itself is also undergoing rapid transformation with the advancement of related fields and technology including genomics, bioinformatics, biotechnology, and nearly all fields of the life sciences.

The Toxi-Lab™ is a test for the determination of environmental toxicity. It is sensitive to a wide spectrum of toxic substances such as heavy metals, and organic and inorganic pollutants, and may be used to detect the presence of toxicants in water and soil/sediment extracts. The test uses a non-pathogenic, harmless, mutated strain of *E. coli* specifically engineered to be highly sensitive to toxic substances. In microplate wells, the bacteria are exposed to different concentrations of a test chemical for a period of time, and the chemical's effect on the health and survival of the bacteria is measured. Since we can't directly visualize the health or viability of the bacteria in the wells of our microplate, we need to find an indirect method to measure any toxic effects the chemical might have.

WARRANTY

EBPI warrants that, at the time of shipment, the 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 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 – Toxi-Lab™ Red and Blue Chromogen

EDUCATOR
COPY



Expectations

The Toxi-Lab™ gives you the opportunity to determine environmental toxicity and the kit can be used to test a variety of toxic substances such as heavy metals, as well as organic and inorganic pollutants. The test determines the relative toxicity of different samples at varying concentrations. 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 Toxi-Lab™ is designed for students to work in groups of three to four with one microplate per group.

Contents of the Toxi-Lab™ Kit

Each kit contains sufficient components for 8x 96-well microplates. The bottles and vials in the Toxi-Lab™ are labelled with clear, bold letters.

<p>Each kit should contain the following:</p> <ul style="list-style-type: none"> ▪ Reaction Mixture 10 mL (8 vials) ▪ Toxi-Lab™ lyophilized bacteria 0.1 g (1 vial) ▪ Rehydration Solution 10 mL (1 vial) ▪ Standard Toxicant 2.5 mL (1 vial) ▪ Diluent 100 mL (1 bottle) ▪ Chromogen 10 mL (8 vials) ▪ 8x 96-well microplates ▪ 1x biohazard bag 	<p>Further materials required, but not included in the kit (contact EBPI if you require these items):</p> <ul style="list-style-type: none"> ▪ 20 units of 200uL micropipettes and tips ▪ up to 16 test tubes <p>Suggested Samples: bleach, drain cleaner, Windex™, alcohol, diluted laundry detergent, etc.</p>
---	--

Collecting the Samples

You have the option of either having your students acquire samples from pre-determined or assigned sources that you and/or your class discussed or you can acquire the samples. Two samples are required from each source. Ensure your students understand and follow safety measures when acquiring the samples. The samples you or the students acquire must be liquid or able to be made into a liquid or dissolved in a solvent such as water or dimethyl sulfoxide (DMSO). The class should have a total of 12 samples organized as follows:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 4	- Sample 7	- Sample 10	- Sample 13	- Sample 16
- Sample 2	- Sample 5	- Sample 8	- Sample 11	- Sample 14	- Sample 17
- Sample 3	- Sample 6	- Sample 9	- Sample 12	- Sample 15	- Sample 18

Preparation of the Samples

Before the Experiment: Whether the students provide you the samples or you collect the samples, please note the following:

<p>If the sample is water-based, make sure that the samples are filter-sterilized to ensure no foreign bacteria contaminate your test, which may compromise the results. Aqueous samples may need to be filtered for sterility using a 0.22 µm filter.</p>	<p>If the sample is solid-based, the samples needs to be sterilized and in liquid form. It is recommended that 0.1 g of sample be dissolved in 0.5 mL of dimethyl sulfoxide (DMSO). This volume should be diluted with water to a total volume of 17.5 mL.</p>
---	---

Student Station Preparation

Below is the suggested distribution of supplies for the preparation of the lab stations for the day of the experiment:

STUDENT LAB STATIONS

FRONT OF THE CLASSROOM

Each group should have the following:

2x Bottles of Positive Control

- 1x 96-well Microplate
- 10 mL of diluent to each group
- 1x vial of blue of red chromogen to each group
- 1x vial of the Reaction Mixture with and without bacteria
- Test samples to each group

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

Storage:

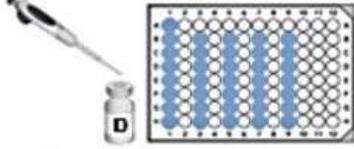
- All kit components can be stored at room temperature
- The main materials should be stored under refrigeration (2°C to 8°C)
- Protect from high temperatures and temperature changes
- If you plan to store the kit for a long period of time, keep the bacteria frozen
- Store the microplates in a dry, dark location at room temperature
- Note improper storage may result in instant colour change

Handling:

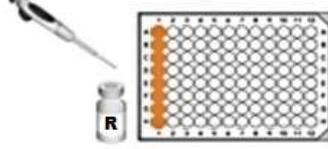
- Note that the bacterial strains used in the assay non-pathogenic laboratory strains, it is advised that good laboratory practice be used.
- Sterilization of the bacteria either in an autoclave or a bleach bath is recommended after use.



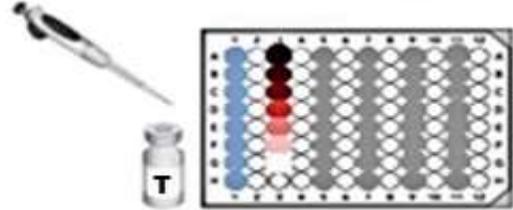
1. Add 100 uL diluent to column 1. Add 100 uL diluent to columns 3, 5, 7, & 9 from wells B to H.



2. Add 100 uL Reaction Mixture without bacteria to column 1.



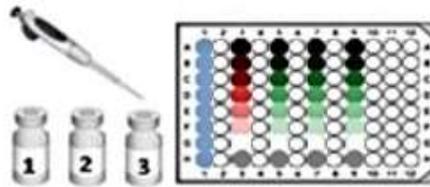
3. Add 200 uL standard toxicant to well A in column 3. Perform a serial dilution by transfer 100 uL from well A to well B, then from well B to well C, and so on. Take 100 uL from well G. Do **NOT** add anything to well H.



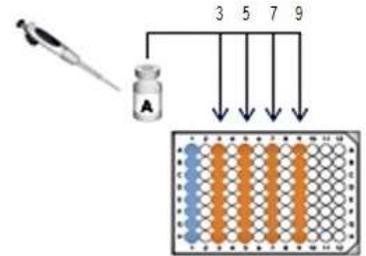
4. Rehydrate the bacteria in bottle B with the solution in bottle C. Mix well and leave for 15 minutes. Transfer 1 mL of bacterial suspension into Reaction Mixture.



5. Add 200 uL of sample 1 to well A in column 5. Perform a serial dilution similar to the previous step to well G. Do **NOT** add anything to well H. Perform the same steps for sample 2 in column 7 and sample 3 for column 9.



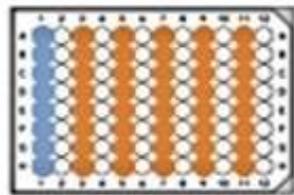
6. For columns 3, 5, 7, and 9, add 100 uL of bacterial suspension from reaction mixture. Do **NOT** mix.



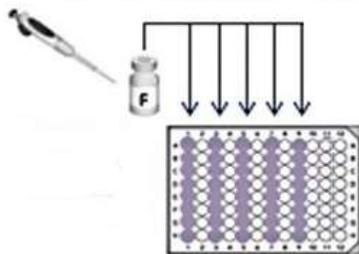
After Step 6, there are **two** different Chromogen steps to complete the lab experiment. Ensure that you follow the appropriate steps.

Blue Chromogen

7. Leave the microplate overnight. Make sure the lid is on properly.



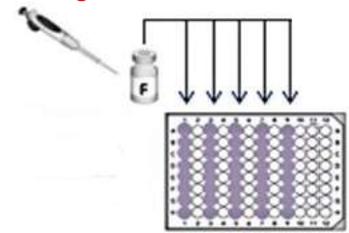
8. Add 100 uL of chromogen to each well that was used (well A to well H). Do **NOT** mix.



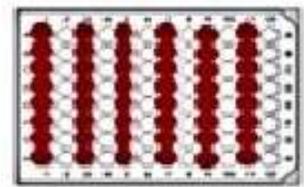
9. Leave the microplate for 30 minutes or until the blue colour appears. Record your results.

Red Chromogen

7. Add 50 uL of chromogen to each well that was used (well A to well H). Do **NOT** mix.



8. Leave the microplate overnight. Make sure the lid is on properly.



9. The next day, you can record your results.

Do not forget to clean up once your experiment is done. Place all used material in the autoclave bag for sterilization and disposal. Remember that proper disposal is essential.



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

Recording the Results: Once the student groups complete their experiment, they can record the results for their microplates using the Tables provided. They will then complete the % Toxicity, calculate the mean average and standard deviation, as well as graph their results. Once this is complete, students should complete their analysis. 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.

Blue Chromogen

Please note the following regarding the procedure:

Once students return to the class the day after the experiment, the blue chromogen will be added immediately and the blue colour will be visible within 30 minutes.

Red Chromogen

Once the red chromogen is added, the results will develop overnight and will be available to record the results next day.

Guidelines

Determine if the Blank column (Column 1) performed as expected. Once you add the **blue** or **red** chromogen added, the wells should be colourless, indicating no unwanted bacterial contamination. If there is any colour present in these wells, the results would be invalid.

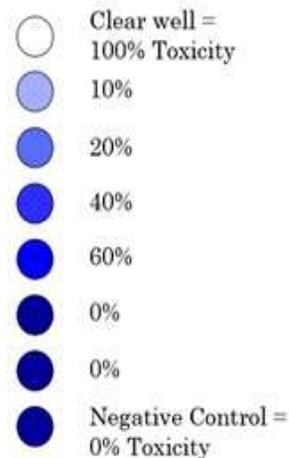
Determine if the positive control worked. If no colour appears in the lower wells of Column 2 (containing the lowest concentrations of the standard toxicant), then the Toxi-Lab™ bacteria were not functioning properly and the results are invalid.

For the the negative control, observe Well H of each of the samples tested. These wells contain bacteria and **blue** or **red** chromogen with no potentially toxic compounds added. This should result in the deepest **blue** or **red** colour.

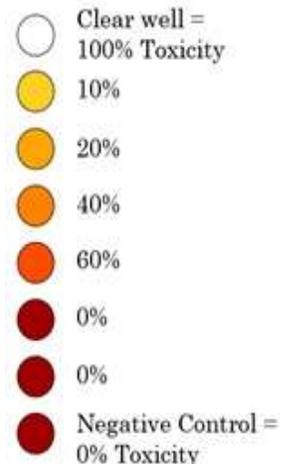
Students will perform a visual analysis on the intensity of the blue or red colour in each well. The visual diagrams on the right will assist you with the estimates. There will be student copies to help with their analysis. Have students complete the % Toxicity Table using the assigned % toxicity values.

- The colours should appear in different intensities depending on the concentration of the sample. The last well Column (Row H) will have the most intense **blue** or **red** colour because it does not contain any toxicant. You will treat the rows as 0% toxicity since no bacteria died due to toxins.
- Look at the other wells that contain **blue** or **red** colour. Rate each one in comparison to Well H. Use the colour scheme on the right to determine the levels of toxicity. Use your judgement, approximate values are acceptable.

Blue Chromogen Guideline Results



Red Chromogen Guideline Results



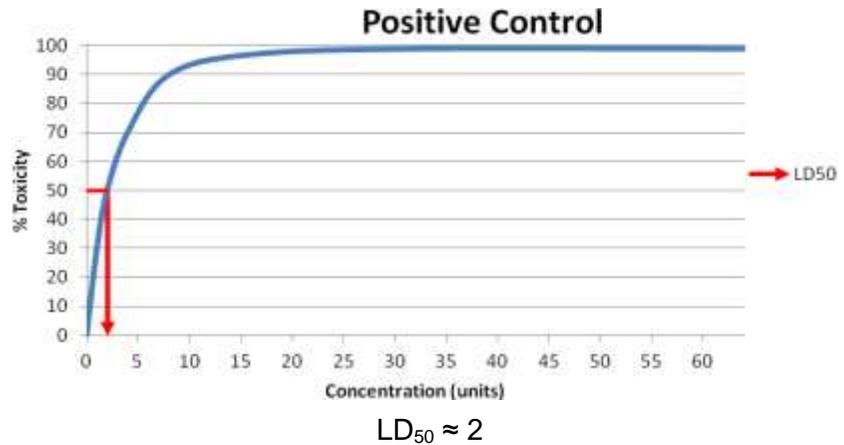
The mean and standard deviation provide the information to properly analyze the results and determine the dose-response. Students will calculate the mean toxicity % and standard deviation at each concentration for the positive control and each sample and record the results. Once the calculations are done, students will create dose response curves for each sample and the positive control with concentration units on the x-axis and % toxicity on the y-axis. 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 LD₅₀ of the different samples and classify them from most toxic to least toxic in the table.

Positive Control and Sample Results

The following information contains positive control and chemical sample results once students complete the Toxi-Kit™ 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.

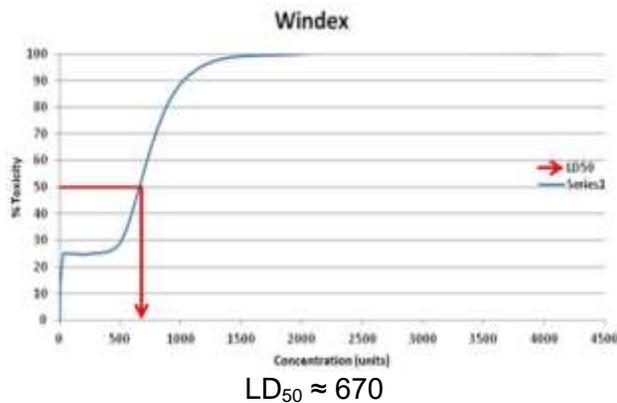
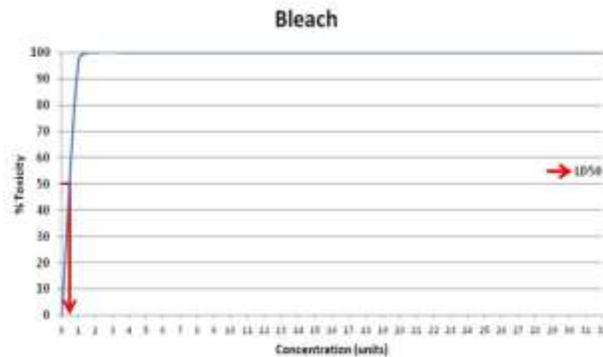
Part A: Positive Control

For the positive control, 70% ethanol is used. The results for this should be approximately the same as the figure given below:



Part B: Samples

Given below are results for some common household substances. Please note that these results serve as a guide and may be used as a reference to compare results between different student groups along with the positive control.



Further Analysis: You can pick further analysis questions based on your preference and the project stream's overall objectives.

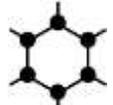
Questions and Answers

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.



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.

<p>Pre-Lab Notes</p>	
-----------------------------	--

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 – Toxi-Lab™ Red and Blue Chromogen



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

Expectations

The Toxi-Lab™ gives you the opportunity to determine environmental toxicity and the kit can be used for a variety of toxic substances such as heavy metals, as well as organic and inorganic pollutants. The test determines the relative toxicity of different samples at varying concentrations. Once your group completes the experiment and the colour change occurs, record the results to determine toxicity levels and each group will complete a lab report for evaluation.

Collecting the Samples

Your educator will discuss whether your group will need to acquire samples from a pre-determined or assigned sites or if your educator will provide the samples. If you will be doing field work, ensure you read and understand the safety measures. The samples you acquire must be a liquid or able to be made into a liquid or dissolved in a solvent such as water. The class samples will be organized by the following:

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
- Sample 1	- Sample 4	- Sample 7	- Sample 10	- Sample 13	- Sample 16
- Sample 2	- Sample 5	- Sample 8	- Sample 11	- Sample 14	- Sample 17
- Sample 3	- Sample 6	- Sample 9	- Sample 12	- Sample 15	- Sample 18

If you are completing the site sample study: Once you acquired your three samples, submit your samples to the Educator.

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

- 2x bottles of Positive Control

GROUP LAB STATION

- 1x 96-well Microplate
- 10 mL of diluent to each group
- 1x vial of blue of red chromogen to each group
- 1x vial of the Reaction Mixture with and without bacteria
- Test samples to each group

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 main materials should be stored under refrigeration (2°C to 8°C)
- Protect from high temperatures and temperature changes
- If you plan to store the kit for a long period of time, keep the bacteria frozen
- Store the microplates in a dry, dark location at room temperature
- Note improper storage may result in instant colour change

Handling:

- Note that the bacterial strains used in the assay non-pathogenic laboratory strains, it is advised that good laboratory practice be used.
- Sterilization of the bacteria either in an autoclave or a bleach bath is recommended after use.



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 your group completes the experiment, be sure to look for the following (be sure you look at the appropriate chromogen experiment):

Blue Chromogen

Please note the following regarding the procedure:

Once students return to the class the day after the experiment, the blue chromogen will be added immediately and the blue colour will be visible within 30 minutes.

Red Chromogen

Once the red chromogen is added, the results will develop overnight and will be available to record the results next day.

Criteria for a Valid Test:

Determine if the Blank column (Column 1) performed as expected. Once you add the **blue** or **red** chromogen added, the wells should be colourless, indicating no unwanted bacterial contamination. If there is any colour present in these wells, the results would be invalid.

Determine if the positive control worked. If no colour appears in the lower wells of Column 2 (containing the lowest concentrations of the standard toxicant), then the Toxi-Lab™ bacteria were not functioning properly and the results are invalid.

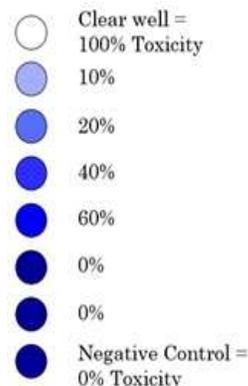
For the the negative control, observe Well H of each of the samples tested. These wells contain bacteria and **blue** or **red** chromogen with no potentially toxic compounds added. This should result in the deepest **blue** or **red** colour.

Visual Guidelines:

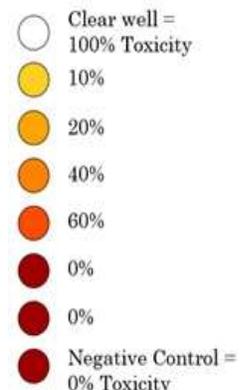
The visual diagrams on the right will assist with your estimates.

- The colours should appear in different intensities depending on the concentration of the sample. The last well Column (Row H) will have the most intense **blue** or **red** colour because it does not contain any toxicant. You will treat these rows as 0% toxicity since no bacteria died due to toxins.
- Look at the other wells that contain **blue** or **red** colour. Rate each one in comparison to Well H. Use the colour scheme on the right to determine the levels of toxicity. Use your judgement, approximate values are acceptable.

Blue Chromogen Guideline Results



Red Chromogen Guideline Results



Recording the Results

1) Table of % Toxicity Values: Record the % toxicity values section using the charts provided.

Concentration (Units)	Positive Control			Sample _____			Sample _____			Sample _____		
	% Toxicity	Avg.	Stand. Dev.	% Toxicity	Avg.	Stand. Dev.	% Toxicity	Avg.	Stand. Dev.	% Toxicity	Avg.	Stand. Dev.
64												
32												
16												
8												
4												
2												
1												
0												

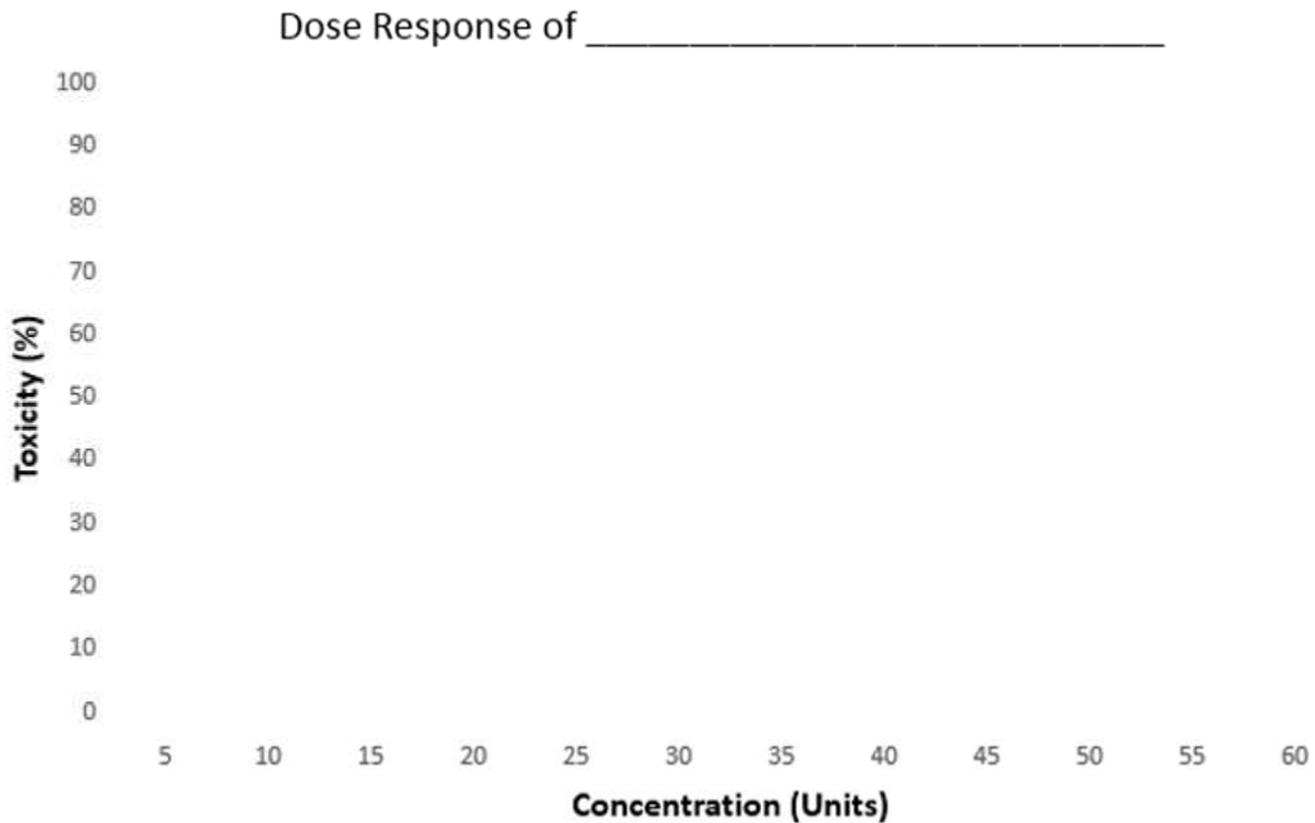
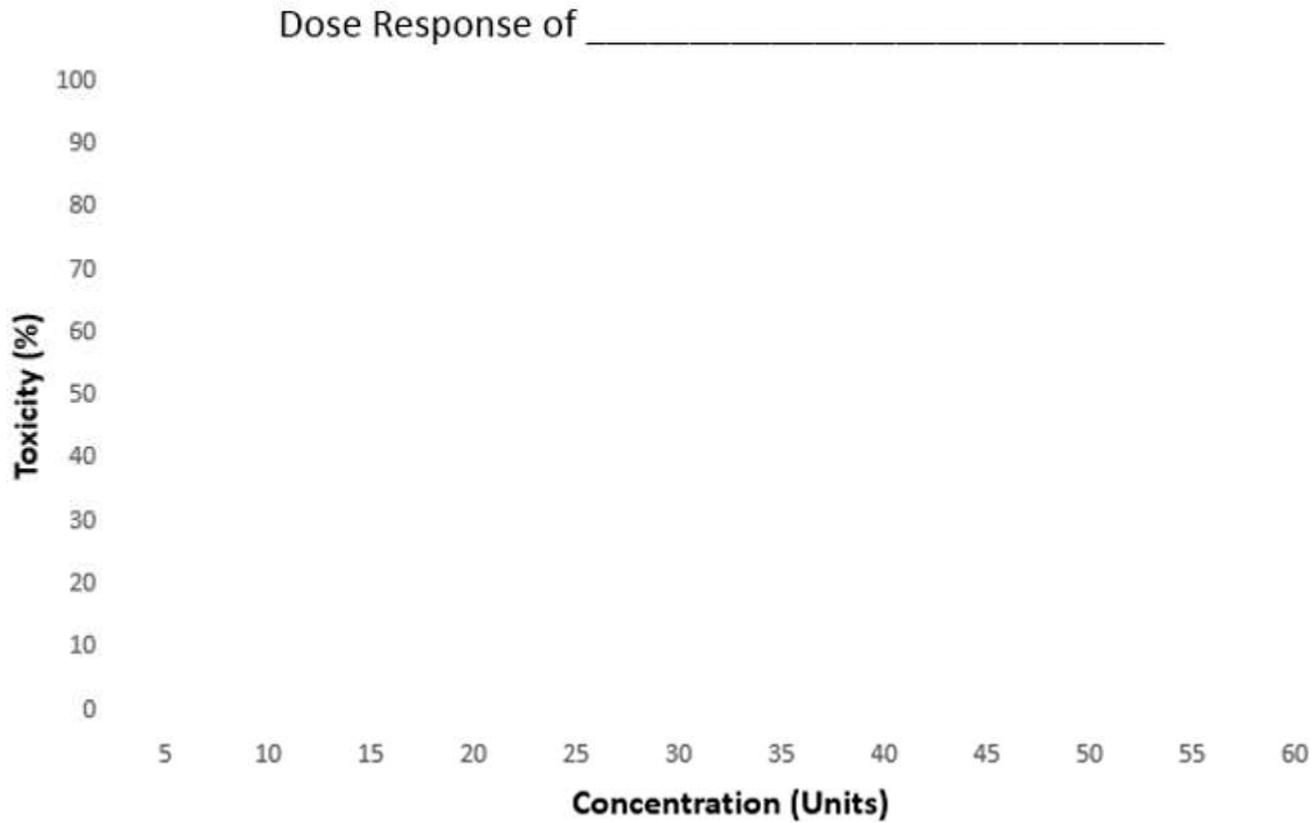
Table: LD₅₀ and relative toxicity ranking for positive control and each sample.

2) Mean and Standard Deviation: The mean and standard deviation provide all the information needed to properly analyze the results and determine the dose-response. Calculate the mean toxicity % and standard deviation for each concentration and each sample and record your values next to your samples. See below for the formulas.

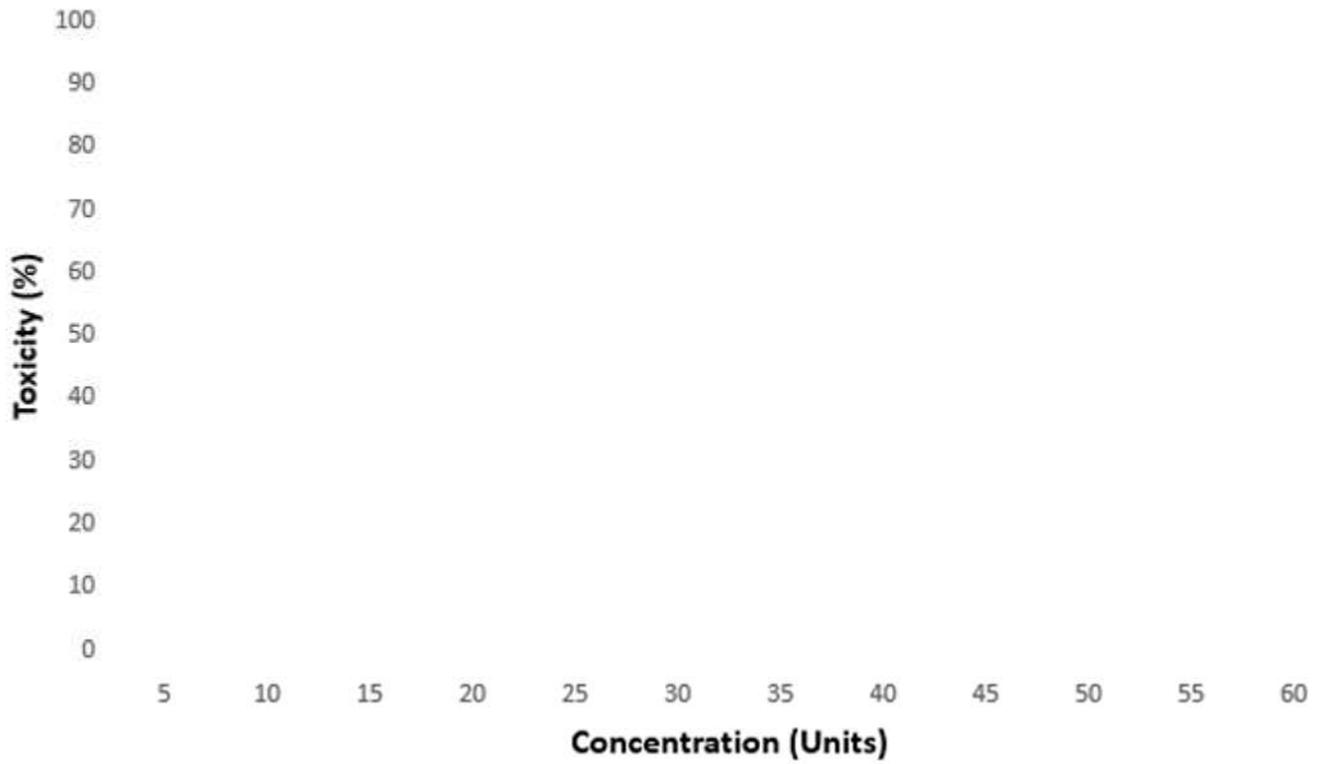
<p>Formula to calculate the mean:</p> $\bar{X} = \frac{\sum_{i=1}^{i=n} X_i}{n}$ <p>Mean (\bar{X}) is the sum of all observations that the other student groups made ($\sum_{i=1}^{i=n} X_i$) ... / ... Divided by the number of student groups (n)</p>	<p>Formula to calculate the standard deviation:</p> $\sigma = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n}}$ <p>σ - Standard Deviation $(x_i - \bar{x})^2$ - difference between each observation and mean of all 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)</p>
---	---

Sample	LD ₅₀	Toxicity Rank
Positive Control		
Sample 1		
Sample 2		
Sample 3		

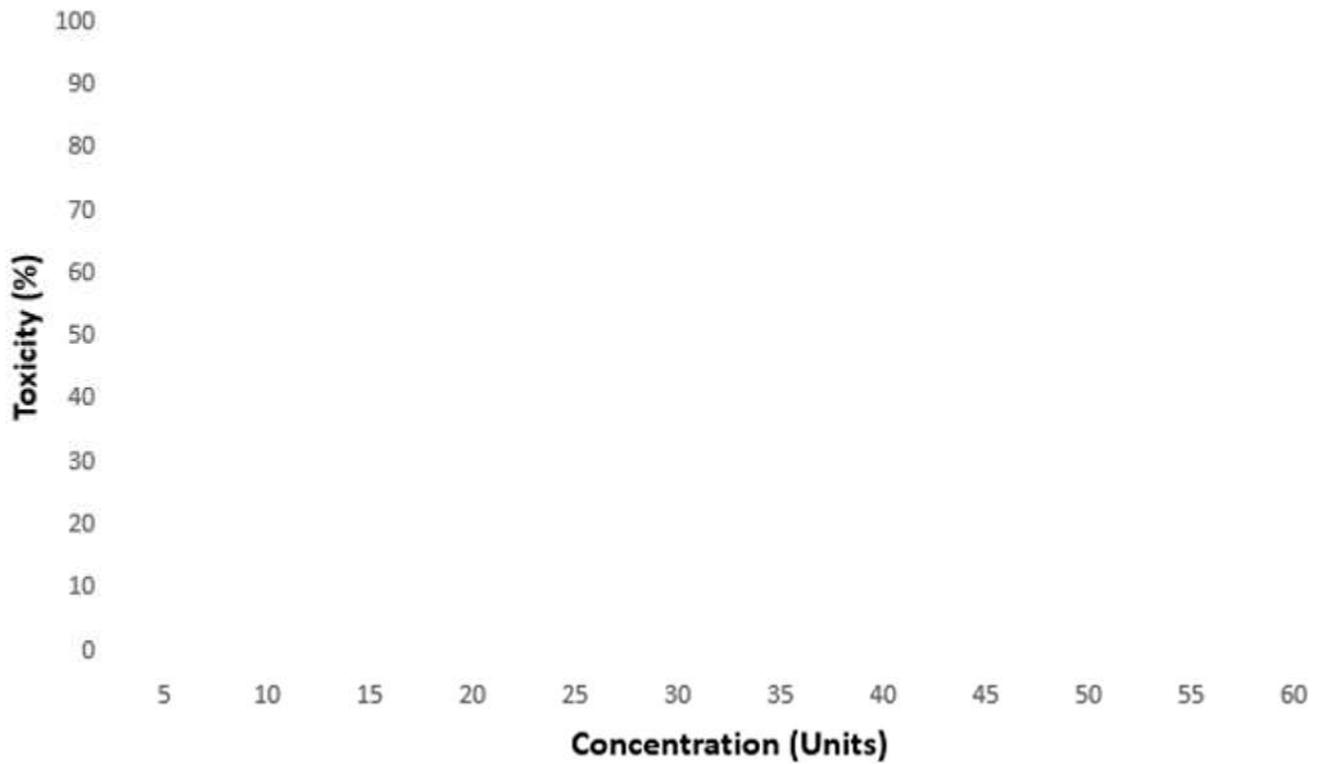
- 3) Creating a Dose Response Curve: Create a dose response curve for each sample and the positive control with concentration units on the x-axis and % toxicity on the y-axis. As well, above and below each point, place markers to show the range of the standard deviation. Students should draw a smooth curve through the data on each graph and try to determine at which concentration 50% survival occurred for each sample. This is the LD₅₀.



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.