MiSP Evolution / Bacterial Resistance Lab L3

Name _____ Date _____

LAB ACTIVITY: THE ANTIMICROBIAL EFFECT OF INCREASING CONCENTRATIONS OF TRICLOSAN

Introduction:

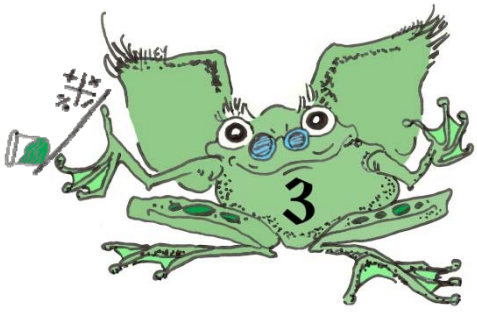
In this lab you will study the effects of the commonly used antimicrobial agent triclosan on *Bacillus cereus*, a type of soil bacteria. *Bacillus cereus* is not known to cause disease in any animal. Therefore, it can be used safely in the classroom. Triclosan is an antimicrobial agent that is effective against bacteria, fungi, and viruses. It was introduced as a surgical scrub in 1972 to limit the spread of infections in health care settings. Triclosan is bactericidal (it kills bacteria) at concentrations at and above 0.3%. Triclosan is now marketed to the general consumer as a component in many personal care products such as toothpaste, deodorant soaps, hand lotions and soaps, mouthwashes, and underarm deodorants. Triclosan is typically present in these products at 0.1% concentration. This lower concentration is bacteriostatic, meaning that it slows the growth of bacteria but does not kill all of the bacteria. This is in contrast to higher concentrations (0.3%) that are bactericidal, meaning that the concentration kills all of the nonresistant bacteria.

This lab will allow you to test the ability of triclosan to inhibit the growth of *Bacillus cereus*. You will expose the bacteria to different concentrations of triclosan and measure the diameter of the zone of inhibition created by each concentration. You will determine whether a given concentration of triclosan always results in the same amount of inhibition and whether a higher concentration of the antimicrobial substance always results in greater inhibition.

Day 1

Work in groups of three to four. Each group will need:

- 5 culture plates containing nutrient agar
- an agar plate containing individual colonies of *Bacillus cereus*
- sterile swabs
- sterile forceps
- a marker
- sterile gloves
- sterile filter disks



- 5 tubes, each containing one of the following: 50% ethanol, 0.1% triclosan, 0.3% triclosan, 0.5% triclosan, and 0.7% triclosan

1. Mark each agar plate with the date, your initials, and one of the following labels:
   - 0% triclosan = 50% ethanol = negative control
   - 0.7% triclosan
   - 0.5% triclosan
   - 0.3% triclosan
   - 0.1% triclosan

2. Use a sterile swab to transfer a single colony of *Bacillus cereus* to each plate, and spread the bacteria evenly on the plate using the sterile swab.

3. Place a filter soaked in the appropriate concentration of triclosan in the center of each labeled plate of bacteria. To soak the disk, first turn the tube containing the triclosan or ethanol upside down and then open the cap. A small drop of liquid will remain in the cap. Use sterile forceps to pick up a sterile disk, dip the disk in the drop of liquid in the cap, and then place it in the center of the agar plate.

4. Give your plates to your teacher. Your teacher will incubate the plates at room temperature overnight.

Day 2

You will need:

- bacterial plates from day 1
- sterile gloves
- a metric ruler

1. Measure the diameter of the zone of inhibition on your plates. Bacteria should grow and make a whitish film over the entire surface of your plates except in areas where growth has been inhibited by triclosan. Notice that in a circular area close to the disk containing triclosan, the agar is totally transparent. Farther away from the disk, the zone of inhibition may look hazy. Some small colonies of bacteria are able to grow in this hazy area, which is known as a halo (see figure 1).

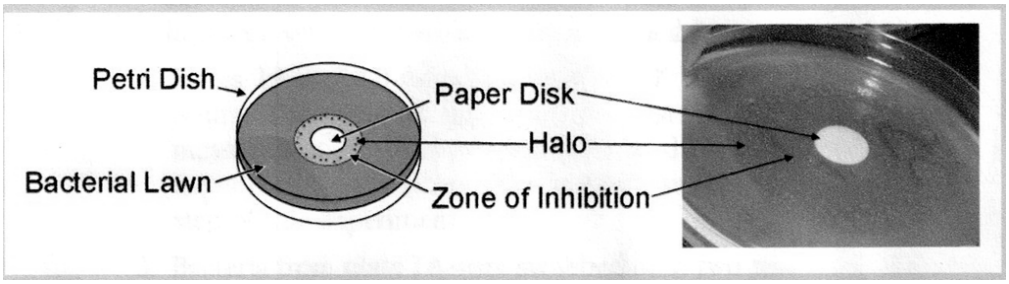


Figure 1. Diagram and picture of zone of inhibition and halo (from Serafini, A. and D. M. Matthews (2009). Microbial resistance to triclosan: A case study in natural selection. *American Biology Teacher* 71:536-540).

1. Turn your plates upside down and use a ruler to measure the diameter of the zone of inhibition in mm. Record this data in the chart below.

2. Your teacher will collect data from the entire class. Put this information in the worksheet. Average the data.

Table 1. Zone of inhibition for different concentrations of triclosan

Concentration of Triclosan	**Diameter of the Zone of Inhibition (mm)**
0% (control)	
0.1%	
0.3%	
0.5%	
0.7%	

Table 2. Diameter of the zone of inhibition for different concentrations of triclosan: Group and class average data

Concentration of Triclosan	Diameter of the Zone of Inhibition (mm)					
	Group 1	Group 2	Group 3	Group 4	Group 5	Average
0% (control)						
0.1%						
0.3%						
0.5%						
0.7%						

3. Use the average data to graph the relationship between the concentration of triclosan and the diameter of the zone of inhibition.

4. What is the independent/manipulated variable in this experiment?

_____

5. Label the *x*-axis for the independent variable.

6. What is the range of the data to be graphed on the *x*-axis?

_____

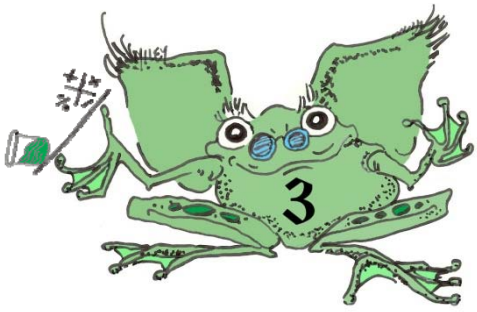
7. Scale the units on the *x*-axis appropriately.

8. What is the dependent/responding variable in this experiment?

_____

9. Label the *y*-axis for the dependent variable.

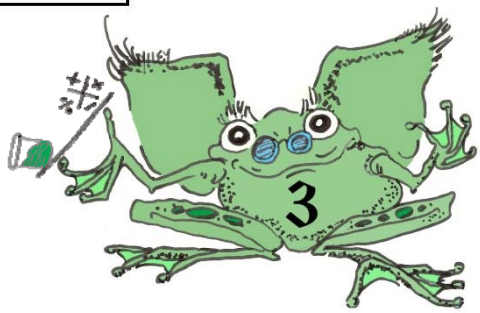
10. What is the range of the data to be graphed on the *y*-axis?



11. Scale the units on the *y*-axis appropriately.

12. Plot the points on the next page.

13. Connect the data points.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Discussion Questions:

1. What happens to the diameter of the zone of inhibition as the concentration of triclosan increases?

   _____

2. Look at the lines that connect each pair of points. Are all of the lines equally steep? _____

3. If the lines are not of equal steepness,

   a. between which two points is the line the steepest? _____

   b. between which two points is the line the least steep? _____

4. Is the line horizontal between any two points? _____

5. If the line between any two points is horizontal, what does this mean?

   _____

   _____

6. The unit rate of change in the zone of inhibition between different concentrations of triclosan can be found by dividing the change in the diameter of the zone of inhibition (mm) by the change in concentration of triclosan (%).

   $$\text{Unit rate of change} = \frac{\Delta y}{\Delta x} = \frac{(y_2 - y_1)}{(x_2 - x_1)} = \frac{\Delta \text{ diameter of zone of inhibition (mm)}}{\Delta \text{ \% triclosan}}$$

   Use the table on the next page to find the unit rate of change (slope) of the line between the following intervals:

   0.1% triclosan and 0.3% triclosan
   0.3% triclosan and 0.5% triclosan
   0.5% triclosan and 0.7% triclosan

Ordered Pairs (x_1, y_1) (x_2, y_2)	Δ diameter of zone of inhibition (mm) Δy	Δ % triclosan Δx	**Unit Rate of Change (slope)** $\Delta y / \Delta x$
0% - 0.3% triclosan			
0.3% - 0.5% triclosan			
0.5% - 0.7% triclosan			

7. In which range of concentrations is the effectiveness (ability to inhibit bacterial growth) of triclosan increasing the most?

   _____

8. Does your answer to #7 agree with your answer to #3? _____

If not, reevaluate your answers to both questions and make changes as appropriate.

9. If the 0% triclosan control produced a zone of inhibition, provide an explanation for this result.

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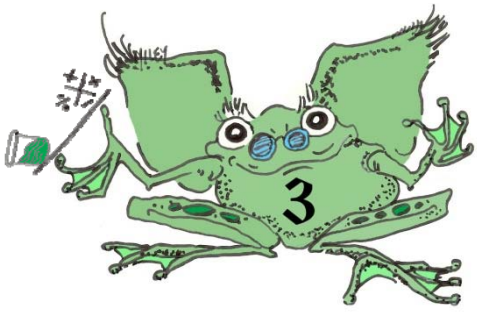
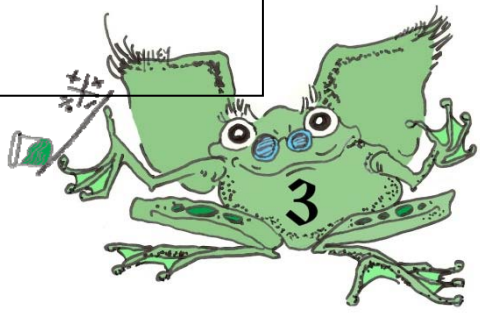


10. Consider the line on your graph between 0.1% triclosan and 0.3% triclosan. Use the slope you calculated in #6 the equation for a line, and one of the ordered pairs on your graph to find the *y*-intercept. The equation for a line is

    $y = mx + b$
    where m is the unit rate of change (slope) and
    b is the y-intercept

Y-Intercept
m = Ordered pair (x, y) = (___ , ___) $y = mx + b$ Solve for b:

11. Write an equation for the line between 0.1% triclosan and 0.3% triclosan, using the slope that you calculated in #6 and the *y*-intercept calculated in #10.



Logically your *y*-intercept should be = 0 (0% antimicrobial agent should yield a zone of inhibition of 0 mm). If the zone of inhibition on your control plate was not 0, your *y*-intercept will not be equal to 0. This means that 50% ethanol has an antimicrobial effect. Because all of the triclosan concentrations were dissolved in 50% ethanol, it is reasonable to assume that the inhibition due to ethanol is the same for all of the plates. You could go back to your data table and subtract the measurement of the zone of inhibition for your control plate from the zone of inhibition for all of the other plates. However, you can also make the adjustment directly on your graph.

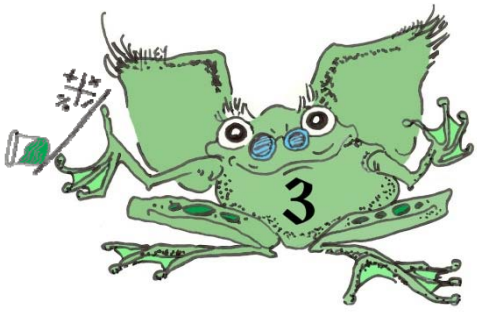
12. Explain how you could manipulate the line on the graph and your linear equation so they reflected only the inhibition due to triclosan. Draw the new line on your graph using a different color of pencil.

    _____

    _____

    _____

13. Look at the class data table. Do all of the zones of inhibition for a given concentration of triclosan have the same diameter? _____



14. If the diameters of the zones of inhibition for a given concentration of triclosan are not all the same, how would you explain this observation?

    a. Some of the explanations should be related to experimental variability. List three possible sources of experimental variability.

        i. _____

        ii. _____

        iii. _____

    b. Another explanation is related to variability among the bacteria that grew on the plate. In what way are the bacteria different? How did this happen?

        _____

        _____

        _____

15. The zone of inhibition on any given plate is usually not equally transparent across its entire diameter. The edge of the zone should be hazy. This is called a halo. If you look closely, there are usually small colonies of bacteria in this zone. The bacteria in these colonies are resistant to triclosan.
    Look at the edge of the zone of inhibition on all of the plates treated with the same concentration of triclosan. (Use a dissecting microscope to look at the plates if one is available.) Do all of the plates with the same concentration of triclosan have the same number of resistant colonies? _____ Explain your observation.

    _____

    _____

    _____