MiSP Evolution / Bacterial Resistance Lab L1

Name _____ Date _____

LAB ACTIVITY: THE ANTIMICROBIAL EFFECT OF INCREASING CONCENTRATIONS OF TRICLOSAN

Introduction:

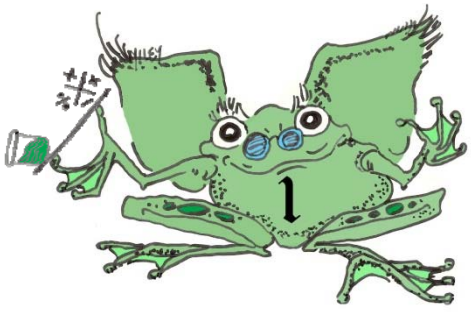
In this lab you will study the effects of the commonly used antimicrobial agent triclosan on *Bacillus cereus*, a type of soil bacteria. *Bacillus cereus* is not known to cause disease in any animal. Therefore, it can be used safely in the classroom. Triclosan is an antimicrobial agent that is effective 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.

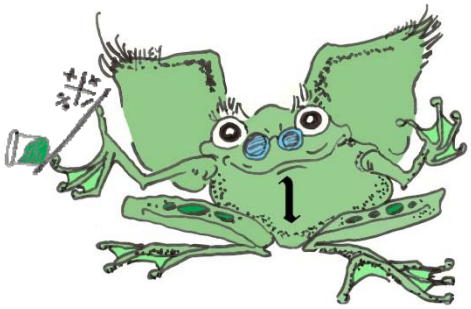
4. Give your plates to your teacher. Your teacher will incubate the plates at room temperature overnight.

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).



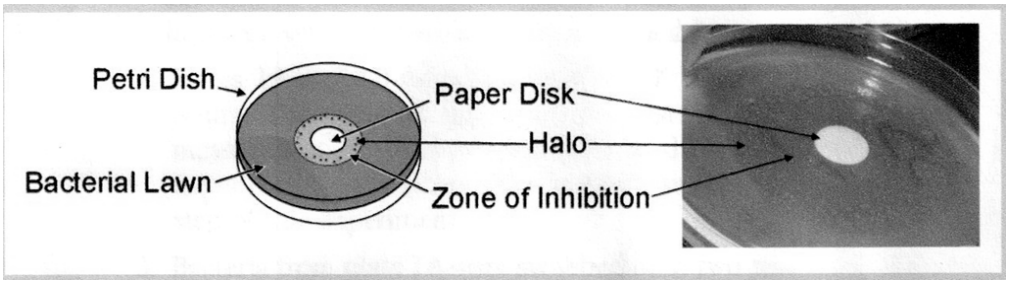


Figure 1. Diagram and picture of zone of inhibition and halo (from Serafini, A. and D. M. Matthews (2009). Microbial resistance to triclosan: A case study in natural selection. *American Biology Teacher* 71:536-540).

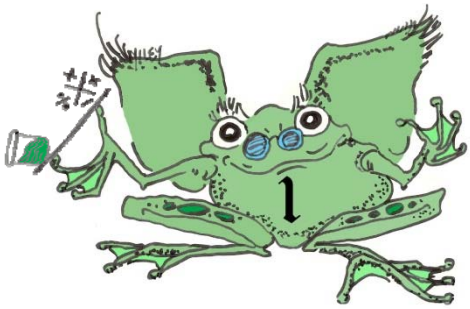
1. Turn your plates upside down and use a ruler to measure the diameter of the zone of inhibition in mm. Record this data in the chart below.

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?

_____

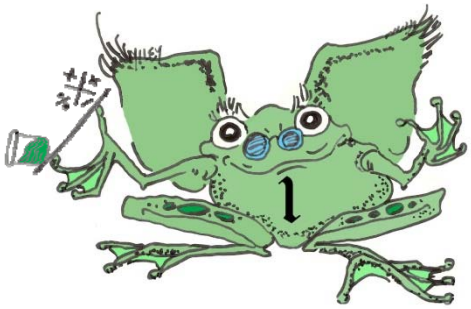
7. Scale the units on the *x*-axis appropriately.

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

_____

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

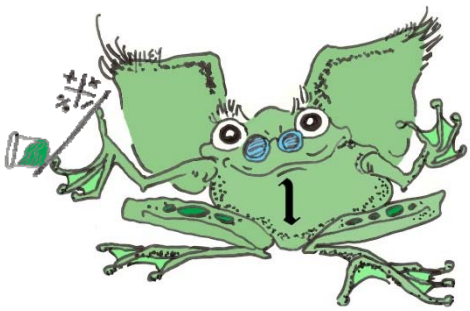
10. What is the range of the data to be graphed on the *y*-axis?

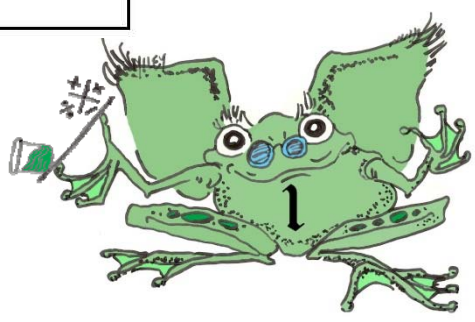


11. Scale the units on the *y*-axis appropriately.

12. Plot the points on the next page.

13. Connect the data points.





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?

   _____

   _____