A Simple Qualitative Test For Surfactants In Glyphosate-Based Herbicides

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Introduction

Surfactant - amphiphilic substance that reduces the surface tension of a liquid

- Harmful to organisms since they disrupt cell membranes
- **Triton X-100** is the control known detergent in this experiment

Amphiphilic - possessing a hydrophilic (lipophobic) part and a hydrophobic (lipophilic) part

When surfactants are immersed in water:

- Hydrophilic heads associate to form a sphere where water cannot enter, as seen in Figure 1.
- This 3D aggregate is called a **micelle**.

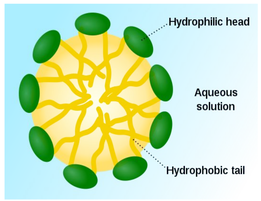

Figure 1: Diagram of a micelle

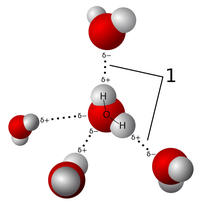

Figure 2: Diagram of Hydrogen Bonds

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Water

- Polar substance
- Hydrogen part of one water molecule attracted to the oxygen part of another molecule forms **hydrogen bonds** like in Figure 2
- **Cohesive properties of water create a high surface tension**
- When a surfactant is introduced to a sample of pure water it breaks the H-bonds between water molecules, **breaking the surface tension**

Glyphosate-Based Herbicides (Round-up® and Rodeo®)

- In 2016, **286 million pounds** of glyphosate (active ingredient in GBHs) were used on crops in the United States
- Most GBHs contain surfactants to allow the glyphosate to penetrate the plant's cuticles
- Potential **ecological threat**

This experiment used a simple loss of surface tension as a test for the presence of surfactant in the glyphosate-based herbicides(GBHs) **Round-up® and Rodeo®.**

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Acknowledgements

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Abstract

In 2016, 286 million pounds of glyphosate were used on crops in the United States. Glyphosate is the active ingredient in glyphosate-based herbicides (GBHs), such as Round-up®and Rodeo®. Only 2% of the commercial final product applied to crops is glyphosate, with the other 98% composed of "undisclosed "or "other" ingredients. To allow the glyphosate to get past the cuticle of plants, many GBHs contain surfactants formulated in these undisclosed ingredients. A surfactant can be defined as a substance which reduces the surface tension of a liquid. Surfactants disrupt cell membranes and are known to be toxic to aquatic organisms. Considering the broad use of GBHs, this presents a potential ecological threat. This investigation devised a simple method to determine whether there was surfactant present in the GBHs Round-up and Rodeo. The presence of surfactant in herbicides and controls was tested using a qualitative loss of surface tension. For each test, six milliliters of pure reverse osmosis water were placed on a standard microscope slide cleaned with isopropyl alcohol. This was tested to be the maximum amount of water that could be held on a slide when 5µL of pure water as a negative control was added to the slide. If the surface tension broke after the addition of 5µL of a test herbicide, it was recorded to contain a surfactant. Triton X-100 was used as a positive control and pure reverse osmosis water was the negative control. The Triton X-100 was diluted to concentrations of 100, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625% and the Round-up and Rodeo were diluted to concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625%. It was found that the surface tension did not break at any levels of concentration tested of Rodeo while the surface tension broke for high levels of concentration (100, 50, 25%) of Round-up. All trials of the Triton X-100 broke the surface tension at a concentration of 0.625% and above, and some trials of the Triton X-100 broke the surface tension at concentrations of 0.3125 and 0.15625%. All trials of the negative control did not break the surface tension. These results suggest that there is little to no surfactant in the herbicide Rodeo but there is some surfactant in the herbicide Round-up. We hope this method can be used to quickly determine whether any of the many GBHs contain surfactants. These results also suggest that the high amounts of GBHs being applied to crops may present an ecological threat to water supplies.

Results/Discussion

Our results suggest that **Rodeo®** has **little to no surfactant** (all trials did not break surface tension) and the **Round-up®** has **some** surfactant (surface tension broke until 50% of Round-up®, compared with 0.625% of Triton).

Negative Control (pure reverse osmosis water) - all three trials did **not break** the surface tension as predicted.
Positive Control (Triton X-100) - should have broken the surface tension of the water

- For low concentrations of **0.3125% and 0.15625%** of the Triton X-100, resulted in **no breakage** of surface tension.
- Potential sources of error:
  - Less water added to microscope slides than absolute maximum amount possible to prevent surface tension from breaking due to small gusts of wind
  - Foaming of the Triton X-100 and dust that may have been on the microscope plates even after cleaning

In future studies:

- Mix the solutions by pipetting up and down
- Using these new methods, re-testing 0.3125% and 0.15625% concentrations and testing more concentrations between 50% and 25% of the Round-up® would be useful in further clarifying the amount of surfactant in the herbicides.

Table 1: *Test Results for Whether Surface Tension Was Broken or Not for Different Concentrations of Rodeo®, Round-up®, and Triton X-100 and the Control (Pure Reverse Osmosis Water)*

	Rodeo®			Round-up®			Triton X-100			Pure Reverse Osmosis Water		
Percent Concentration	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
100	X	X	X	O	O	O	O	O	O	X	X	X
50	X	X	X	O	O	O	NT	NT	NT			
25	X	X	X	O	X	X	NT	NT	NT			
12.5	X	X	X	X	X	X	NT	NT	NT			
10	NT	NT	NT	NT	NT	NT	O	O	O			
6.25	X	X	X	X	X	X	NT	NT	NT			
5	NT	NT	NT	NT	NT	NT	O	O	O			
3.125	X	X	X	X	X	X	NT	NT	NT			
2.5	NT	NT	NT	NT	NT	NT	O	O	O			
1.5625	X	X	X	X	X	X	NT	NT	NT			
1.25	NT	NT	NT	NT	NT	NT	O	O	O			
0.625	NT	NT	NT	NT	NT	NT	O	O	O			
0.3125	NT	NT	NT	NT	NT	NT	X	O	O			
0.15625	NT	NT	NT	NT	NT	NT	X	O	X			

Key:
NT = Not Tested
O = Surface Tension Broken
X = Surface Tension Not Broken
T1 = Trial 1
T2 = Trial 2
T3 = Trial 3

Methods

Concentrations to Test:
Different liquid concentrations were tested to get a better sense of the amount of surfactant in the Round-up® and Rodeo®
Pure Reverse Osmosis Water
Triton X-100 % (100, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625)
Round-up® % (100, 50, 25, 12.5, 6.25, 3.125, 1.5625)
Rodeo® % (100, 50, 25, 12.5, 6.25, 3.125, 1.5625)

Cleaning the Microscope Slides:

- Isopropyl alcohol sprayed on non-frosted side and dried using a paper towel
- Slides were placed on a table frosted side down

Preparing the Microscope Slides:

- To find the best amount of pure reverse osmosis water to put on each microscope slide, I used trial and error
- **6mL** was the optimal for maximal "beading" on slides
- Pipette used to place water on microscope slides, 1mL at a time with 3 slides for each concentration

Making the Different Concentrations:
Round-up®/Rodeo®

- 10µL of Round-up®/Rodeo® inserted in a micro centrifuge tube
- 5µL moved to a new micro centrifuge tube (tube 2)
- 5µL of pure RO water added to tube 2 and mixed by placing on a vortex mixer for 1 or 2 seconds
- This process was repeated until total of 7 concentrations

Triton X-100

- At first tried using the same process as the Round-up® and Rodeo® but found the Triton X-100 did not mix well and foamed
  - We lowered the concentration and also placed the micro centrifuge tube on the mixer for a very short period of time
- 10µL of Triton X-100 in the micro centrifuge tube(tube A)
- 1µL moved from tube A to a new micro centrifuge tube(tube B)
- 9µL of pure RO water added to tube B and mixed for less than a second on the vortex mixer
- The same process as the Round-up® and Rodeo®, with slight change of shorter time on mixer
- Process repeated until total of 8 concentrations of Triton X-100

Testing the Different Concentrations:

- Triton X-100 - **positive control**, pure RO water - **negative control.**
- 5µL of the liquid concentration being tested slowly inserted into the 6mL water on the slides, right above the center of the slide, using a 20µL pipette
- Surface Tension breakage recorded on a piece of paper and by taking a video of the process.