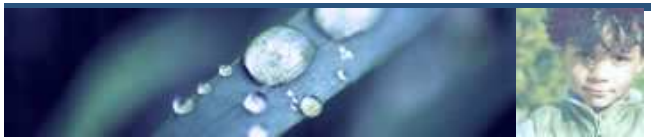

# Herbicide Bioassay Solution Preparation

**Brenda Johnson**

**02/11/2016**

# Solution Preparation

## Bioassay Definition

- **Appraisal of the biological activity of a substance (herbicide) by testing its effect on an organism (seed) and comparing the result to some agreed upon standard (control/check sample)**

## So how is it actually conducted?

- **Plant seeds in presence of herbicide OR spray seedlings that have grown and assess how each seedling looks compared to the control**

# Solution Preparation

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**Concentration of herbicide is determined during method validation**

**Depends on type of herbicide bioassay**

- **Spray method is typically at or close to field rate of application**
- **Substrate imbibition (rolled-towel) is typically more dilute than field rate**

# Calculating Concentration

**Herbicide concentration for testing trait purity of seeds is typically calculated in parts per million (ppm)**

- **1ppm is equal to 0.0001%**
- **Just like a percent, ppm is unitless**
- **Examples:**

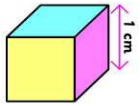
  **1 minute in 20,000 years**

  **1¢ in $10,000**

  **1 inch in 16 miles**

# Understanding ppm

**1ppm is also equal to 1mg/L, important when calculating herbicide concentrations**

- **ppm=mg/L**
- **g=mL=cm³ (water)** 


$$\frac{1\text{mg}}{1\text{L}} \quad \text{Some math} \quad = \quad \frac{\text{g}}{\text{mL}}$$

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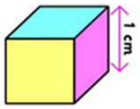
- **g=mL=cm³ (water)** 


$$\frac{1\cancel{mg}}{1L} \times \frac{1g}{1000\cancel{mg}} = \frac{g}{mL}$$

# Understanding ppm

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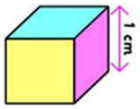
- **g=mL=cm$^3$ (water)** 


$$\frac{1\text{mg}}{1\text{L}} \times \frac{1\text{g}}{1000\text{mg}} \times \frac{1\text{L}}{1000\text{mL}} = \frac{\text{g}}{\text{mL}}$$

# Understanding ppm

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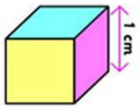
- **g=mL=cm$^3$ (water)** 


$$\frac{1\text{mg}}{1\text{L}} \times \frac{1\text{g}}{1000\text{mg}} \times \frac{1\text{L}}{1000\text{mL}} = \frac{1\text{g}}{1{,}000{,}000\text{mL}}$$

- **That's 1ppm!**

# Calculating Concentration (ppm)

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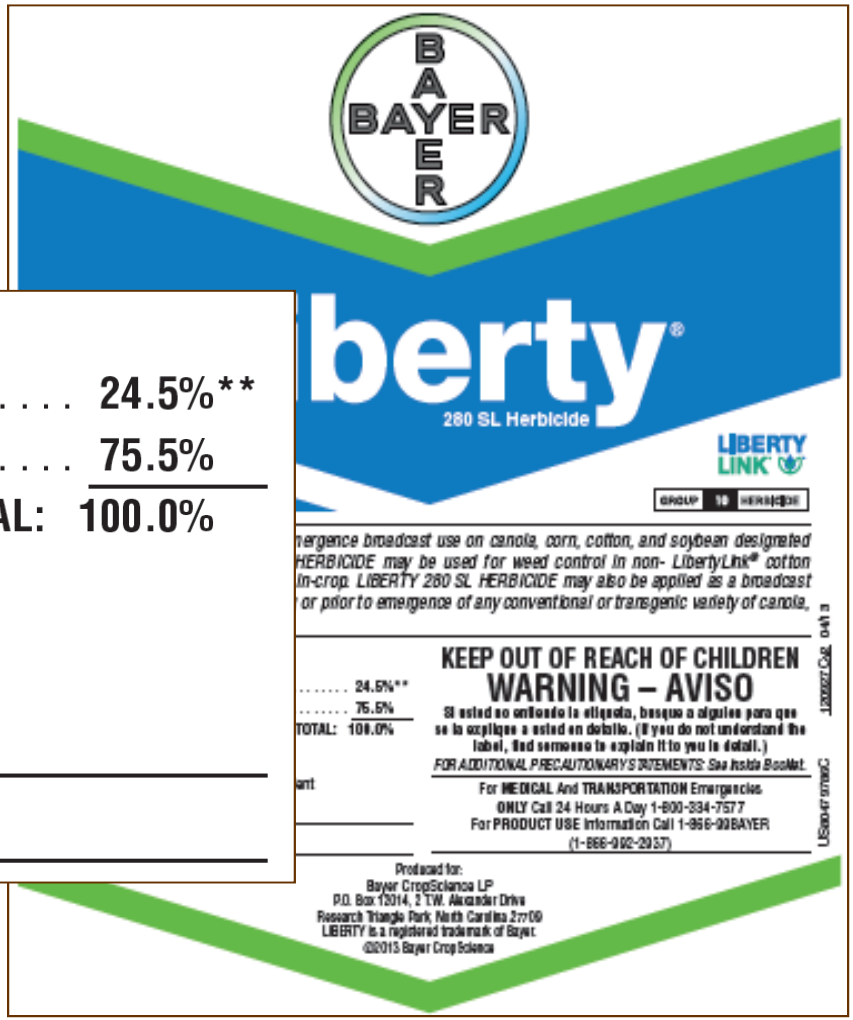
## Gather appropriate information from product label



# Calculating Concentration (ppm)

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## Percent Glufosinate-ammonium…24.5%

- By weight?  By volume?
- What is the “active ingredient”?

**ACTIVE INGREDIENT:**

Glufosinate-ammonium* . . . . . 24.5%**

**OTHER INGREDIENTS:** . . . . . 75.5%

**TOTAL:** 100.0%

*CAS Number 77182-82-2

**Equivalent to 2.34 pounds of active ingredient per U.S. gallon.

**EPA Reg. No. 264-829**

Not enough information!

# Calculating Concentration (ppm)

**2.34 pounds of ai per gallon

- Convert from lb/gal to ppm (or g/L to ppm, any mass/volume)

**ACTIVE INGREDIENT:**
Glufosinate-ammonium* . . . . . 24.5%**
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# Calculating Concentration (ppm)

**2.34 pounds of ai per gallon

- Convert from lb/gal to ppm (or g/L to ppm, any mass/volume)

$$\frac{2.34\text{lb}}{1\text{gal}} \times \frac{454\text{g}}{1\text{lb}} \times \frac{1000\text{mg}}{1\text{g}} \times \frac{1\text{gal}}{3.785\text{L}} = 280676\frac{\text{mg}}{\text{L}}$$

- Since there are only 3 digits in the given number we are converting (2.34), round to 281000mg/L or 281000ppm

# Calculating Concentration (ppm)

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## Creating Working Solution From Stock

- **C1 x V1 = C2 x V2**

**where**

**C1=concentration (or dilution) of stock**

**V1=volume of stock to pipet**

**C2=concentration of working solution**

**V2=final total volume** (volume of source extract and diluent)

# Calculating Concentration (ppm)

**Example: Create a 4 Liters of 1000ppm working solution from the 281000ppm stock.**

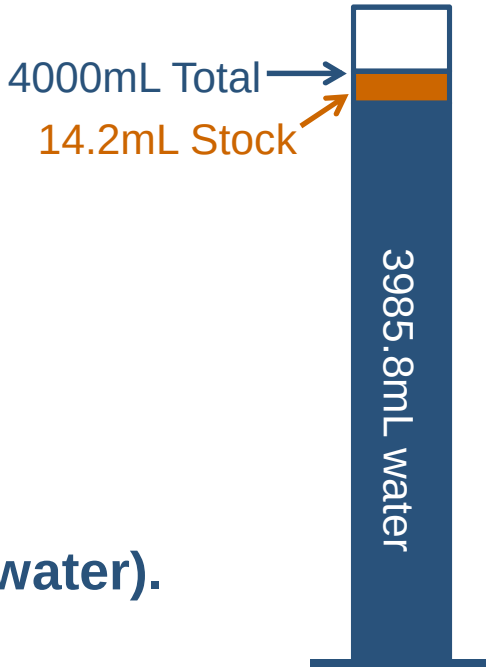
**C1 x V1 = C2 x V2**

**281000ppm x V1 = 1000ppm x 4L**

$$V1 = \frac{1000ppm \times 4L}{281000ppm}$$

**$V_1$ = 0.0142L = 14.2mL**

**Add 14.2mL of Liberty to 3985.8mL of diluent (water).**



# Another Example

**“Okay, okay, okay… everyone just calm down and we’ll try this thing one more time.”**

***The Far Side*** **by Gary Larson**

# Calculating Concentration (ppm)

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**Calculate the final concentration of a working solution of Roundup WeatherMax produced by adding 2.0mL of stock solution to 1L.**

ACTIVE INGREDIENT:

*Glyphosate, N-(phosphonomethyl)glycine,
in the form of its potassium salt ..... 48.8%

OTHER INGREDIENTS: ..... 51.2%

100.0%

*Contains 660 grams per liter or 5.5 pounds per U.S. gallon of the active ingredient glyphosate, in the form of its potassium salt. Equivalent to 540 grams per liter or 4.5 pounds per U.S. gallon of the acid, glyphosate.

This product is protected by U.S. Patent No's. 5,668,085, RE 37,866 and 6,365,551. Other Patents Pending. No license granted under any non-U.S. patent(s).

# Calculating Concentration (ppm)

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**$C_1$= ? (Need to calculate this from the label)**

**$V_1$=2.0mL**

**$C_2$= ? (This is the answer)**

**$V_2$=1L + 2mL = 1002mL**

ACTIVE INGREDIENT:
*Glyphosate, N-(phosphonomethyl)glycine,
in the form of its potassium salt ..... 48.8%
OTHER INGREDIENTS: ..... 51.2%
100.0%

*Contains 660 grams per liter or 5.5 pounds per U.S. gallon of the active ingredient glyphosate, in the form of its potassium salt. Equivalent to 540 grams per liter or 4.5 pounds per U.S. gallon of the acid, glyphosate.

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# Calculating Concentration (ppm)

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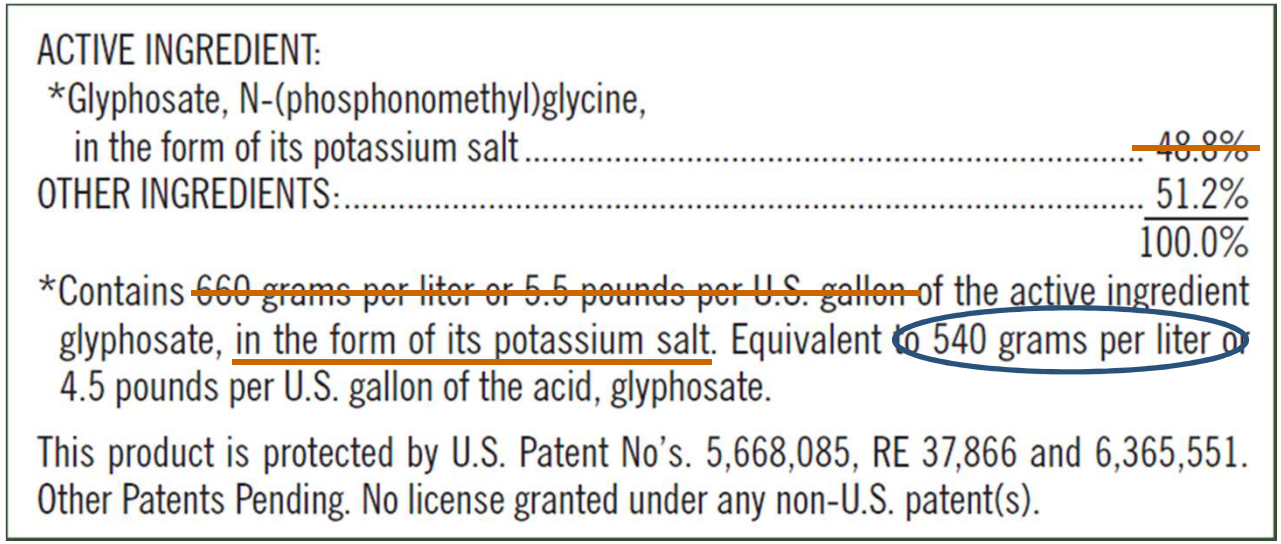
**C1 = ? ppm**

***540 grams of ai per Liter**

- **Convert from g/L to mg/L (aka ppm)**



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# Calculating Concentration (ppm)

**C1 = ? ppm**

***540 grams of ai per Liter**

- **Convert from g/L to mg/L (aka ppm)**

$$\frac{540\,g}{1\,L} \times \frac{1000\,mg}{1\,g} = 540{,}000\,\frac{mg}{L} = 540{,}000\,ppm$$

- **C1=540,000ppm**

**Now use C1 x V1 = C2 x V2**

# Calculating Concentration (ppm)

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$C_1$ = 540,000

$V_1$ = 2.0mL

$C_2$ = ? (This is the answer)

$V_2$ = 1L + 2.0mL = 1002mL

$C_1 \times V_1 = C_2 \times V_2$

540,000ppm x 2.0mL = $C_2$ x 1002mL

$$C_2 = \frac{540{,}000\text{ppm} \times 2.0\text{mL}}{1002\text{mL}} = 1077.8443\ldots\text{ppm}$$

$C_2$= 1080ppm when rounded

# Solution Preparation

## Important notes

- **Proper measuring of reagents is important, i.e. selecting the most appropriate measurement device (pipet, graduated cylinder)**
- **Adequate mixing is essential to ensure even distribution of herbicide throughout the solution**

# Solution Preparation

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## Questions?