

# THE EFFECT OF COMMON HOUSEHOLD PAINTS ON *DROSOPHILA MELANOGASTER*

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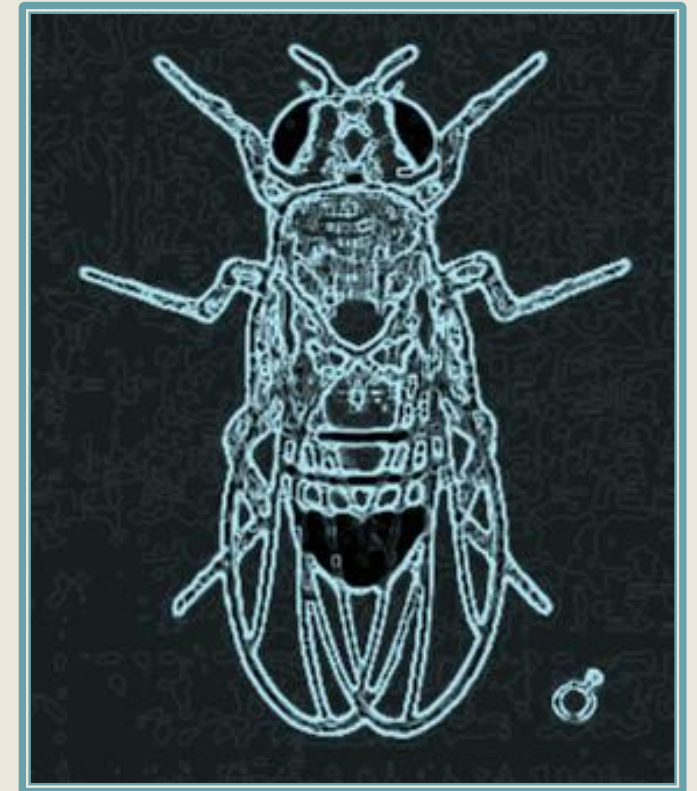
RISE ONLINE STEM RESEARCH INSTITUTE

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# Purpose

- To determine the effect of common household paints, and the volatile organic compounds (VOCs) they contain, on *Drosophila melanogaster* (the common fruit fly)
- These effects are measured with 3 metrics:
  - *Climbing ability*
  - *Reproduction*
  - *Burrowing Behavior*



# Hypothesis

1. If the fruit flies are exposed to common household paints containing VOCs, then they will suffer damage to the central nervous system, respiratory system, and ability to reproduce.
2. If the flies are exposed to VOCs over a longer period of time, then they will suffer more damages. With each successive generation exposed to paint fumes, the effects will worsen.

# Background Information: VOCs

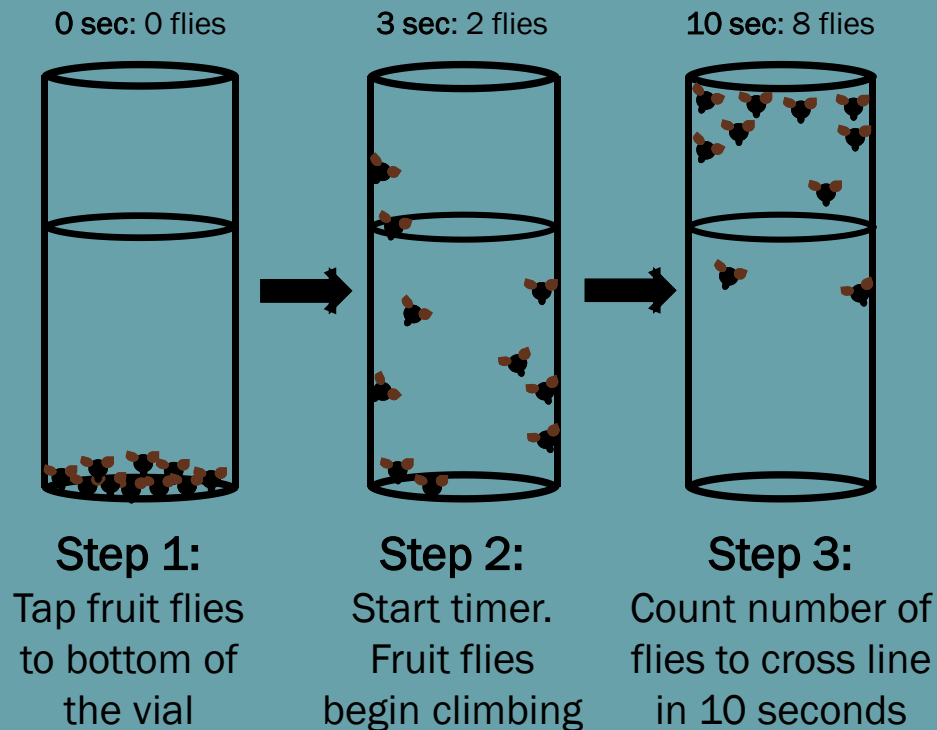
- **Volatile organic compounds (VOCs)** are carbon-containing compounds emitted as gases by certain solids and liquids.
- Most people are exposed to VOCs indoors, as outgassing from paints, furniture, glues, etc. (Chin et al., 2014).
- VOCs have been linked to numerous health problems.
  - **Disruption to the central nervous system (CNS):** such as headaches and seizures (Dick, 2006). Many VOCs are lipophilic, which makes it easy for them to be absorbed into the bloodstream and accumulate in the brain (National Research Council, 2009).
  - **Development of asthma and other respiratory conditions:** In a study done in Sweden, it was found that propylene glycol and glycol ethers (PGEs) were associated with a “1.5-fold greater likelihood of asthma” (Choi et al., 2010)

# Background Information: Fruit Flies

- *Drosophila melanogaster*, the common fruit fly, is an excellent model organism because of **easy breeding and short life span**.
- **Fruit flies and the CNS:**
  - Fruit flies can be used to **investigate depression of the CNS**, which can cause everything from deteriorating motor function to behavioral changes.
  - A **climbing assay test** can be used to determine how VOCs effect the CNS (Dangond, n.d.).
- **Fruit flies and the respiratory system:**
  - Fruit flies can be used to **assess respiratory damage**, such as **asthma-like symptoms**
  - Fruit flies have a tracheal system with many similarities to the human lung. Fruit fly larvae have behavioral characteristics that cause them to come near the surface when deprived of oxygen. (Roeder, Isermann & Kabesch, 2009).
  - Measuring larval **burrowing behavior** can reveal respiratory damage.



# Methods: Climbing Assay

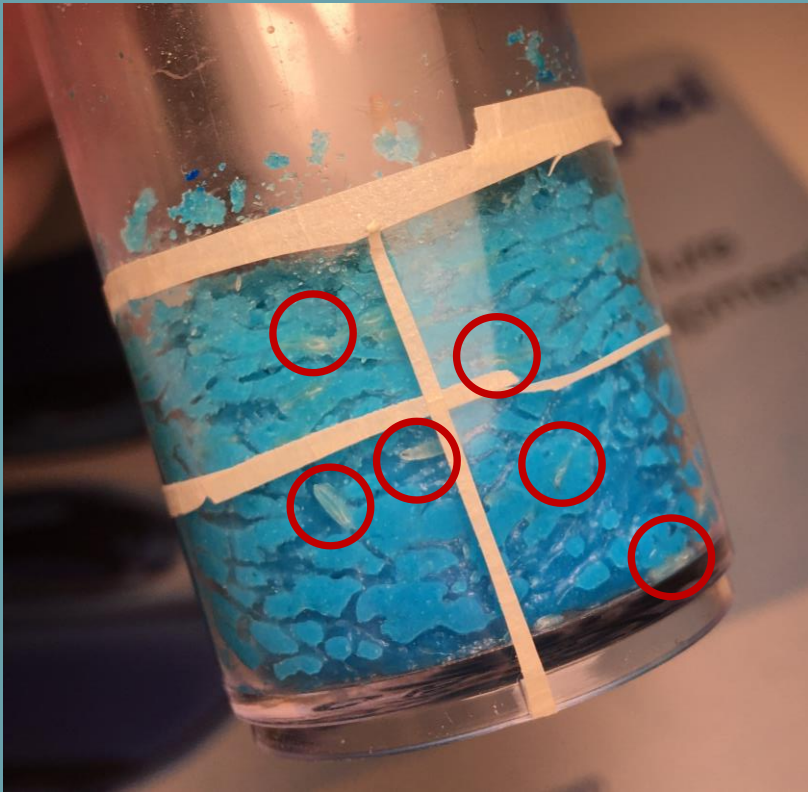


- Place 10 fruit flies into an empty vial marked with a line 6 cm above the bottom of the vial.
- Tap down vial and count how many flies cross the 6 cm line in 10 seconds.
- Do five trials per vial.

*If fewer fruit flies cross the 6cm line, this can indicate a problem with the CNS*

- *Motor Function Deterioration*
- *Behavioral change*

# Methods: Reproduction

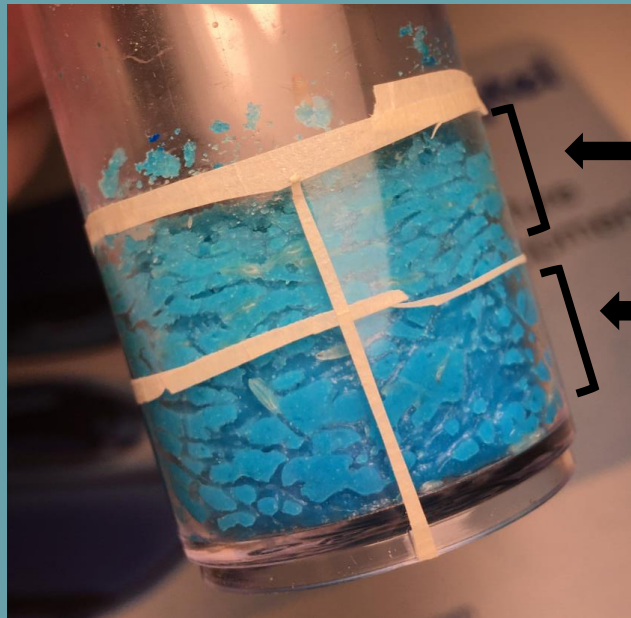


- Count total number of visible larvae found in the medium between generations

*Compare number of larvae between different paint groups.*

*Significantly less larvae in one group can indicate reproductive damage or that the eggs are not developing.*

# Methods: Burrowing Behavior



- Mark a line on the vial that divides the medium into upper and lower halves.
- Count the number of visible larvae in the upper vs. lower half of the medium.

*Fruit fly larvae come close to surface when oxygen deprived:*

*Less larvae in the lower half is an indication of possible asthmatic symptoms or other respiratory disorders.*



# Methods: Paints Tested – VOC Content

## Rust-Oleum Spray

Propane  
n-Butyl Acetate  
Acetone  
Propylene Glycol Monobutyl  
Ether  
Titanium Dioxide  
n-Butane  
Solvent Naphtha  
Light Aromatic  
Xylenes (o-, m-, p- isomers)  
Barium Sulfate  
Ethylbenzene

535 g/L

## Rust-Oleum Topcoat

Limestone  
Hydro-treated Light Distillate  
Titanium Dioxide  
Mineral Spirits.

410 g/L

## Behr Marquee Low VOC

Titanium dioxide  
Nepheline Syenite  
Anhydrous aluminum silicate  
Amorphous silica  
Aluminum hydroxide  
Coalescing aid  
Ethylene Glycol

71 g/L

# Methods: Prepare Vials

## Painting the Vials

Separate 20 vials into groups of 5, labeled according to paint type.

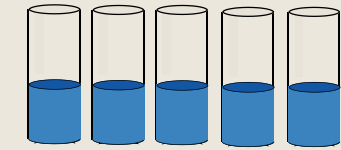
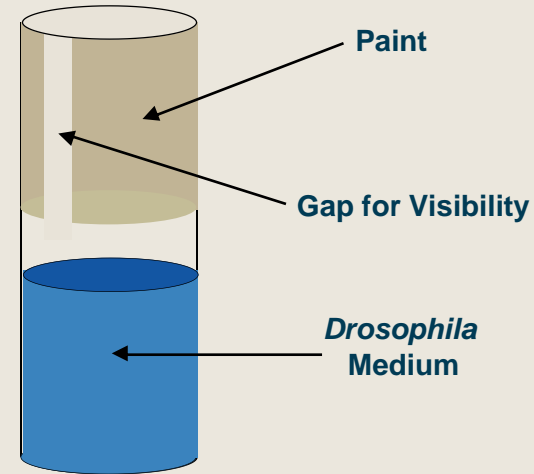
Paint the top 4 cm of each non-control vial.

Give each vial three coats of paint.

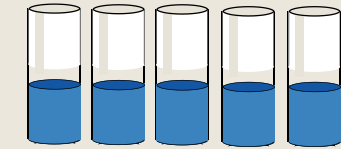
## Add Medium

Add 15 mL *Drosophila* medium and 15 mL water to each vial.

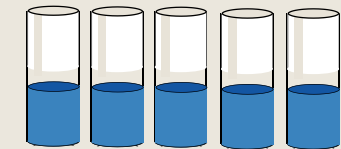
Add 3 grains of yeast to each vial.



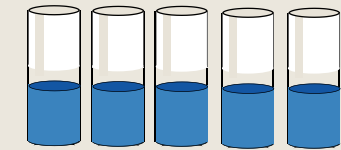
Control



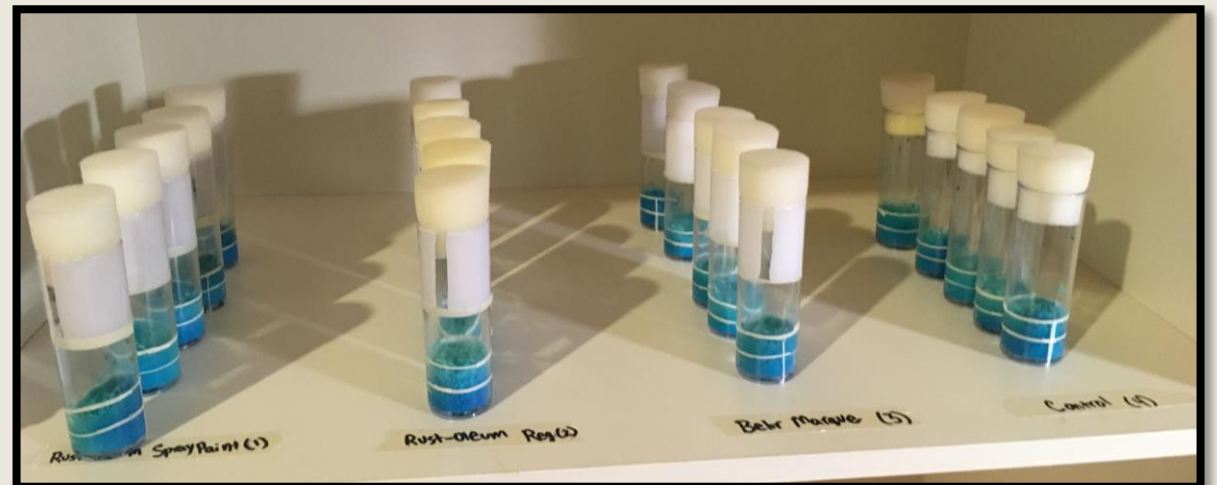
Rust-Oleum Spray



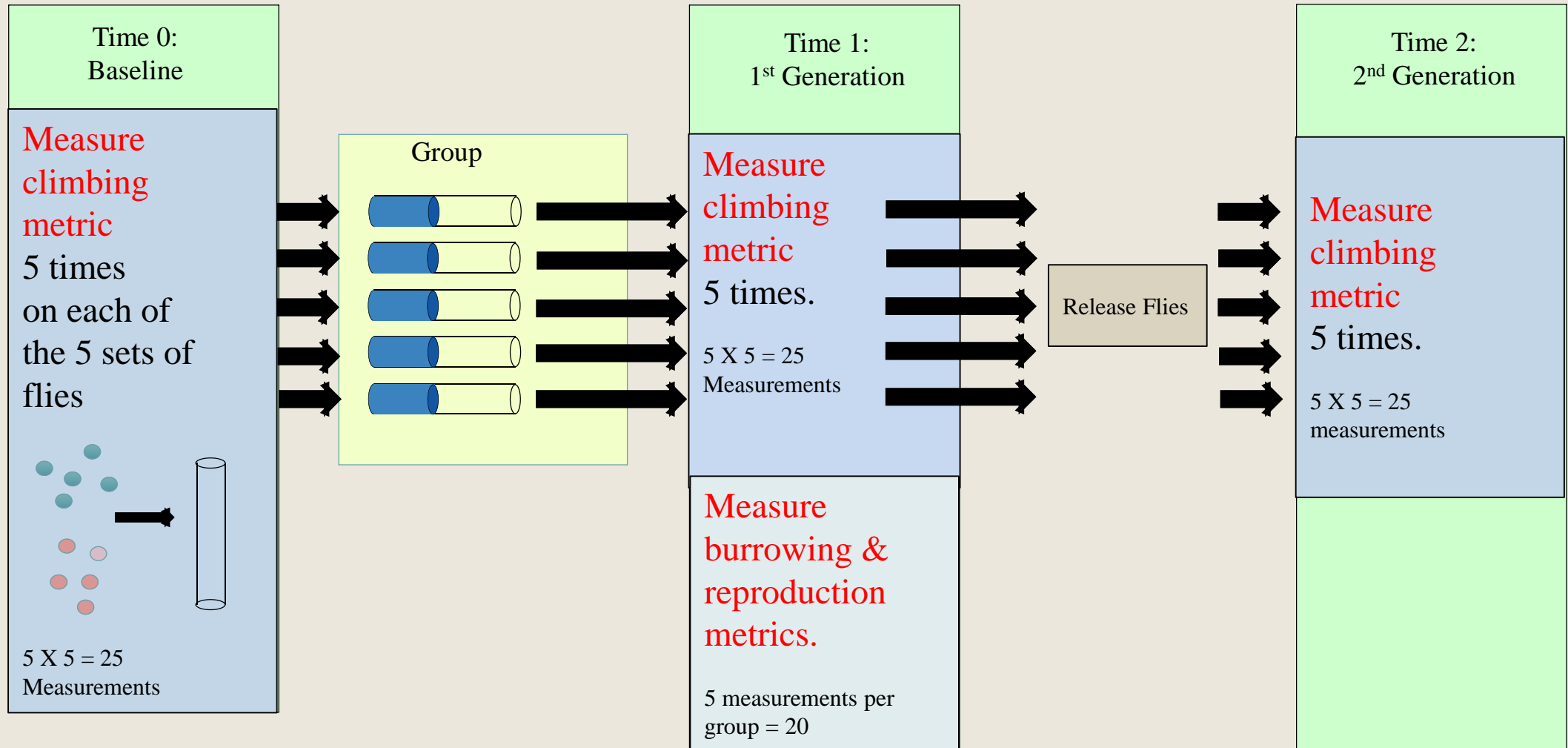
Rust-Oleum Topcoat



Behr Marquee



# Methods: Taking Measurements



Repeat for each group: Rust-Oleum Spray, Rust-Oleum Topcoat, Behr Marquee and Control

# Experimental Error

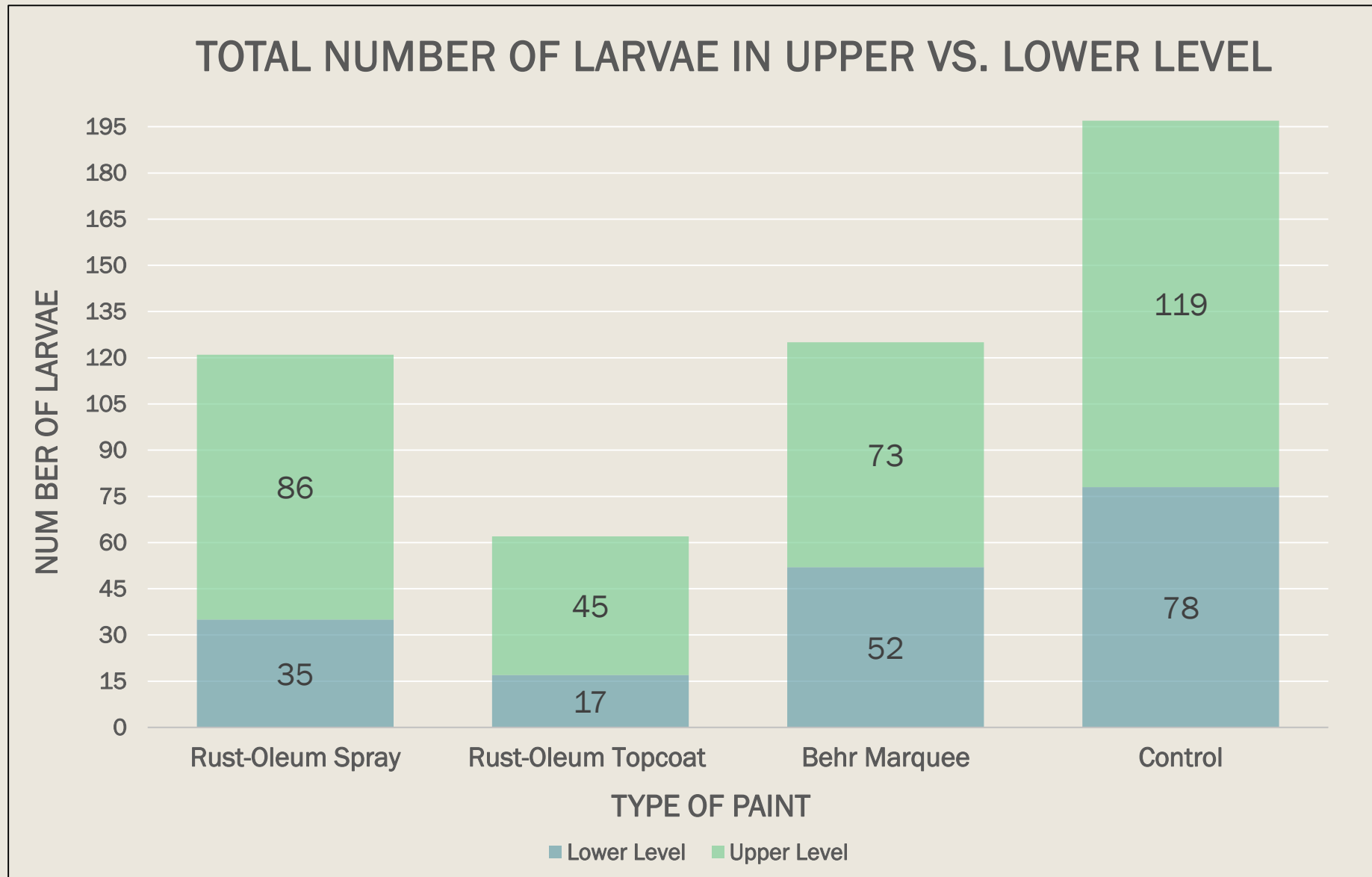
1. If the flies didn't fully wake up after being sedated, errors could have occurred during the climbing assay
  - *Prevented by giving flies 1.5 hours to wake up*
2. Errors related to not having a large enough sample size
  - *Prevented by doing repeated trials and having multiple groups*

# Variables

- **Independent Variable:** the type of paint the fruit flies were exposed to
- **Dependent Variable:** the effect of those VOCs on the fruit flies
- **Controlled Variables:** amount of food/yeast per vial, length of time exposed to fumes, amount of paint, surface area paint covers, # of male/female flies
- **Control/Comparison Group:** the vials in which the flies were NOT exposed to paint fumes



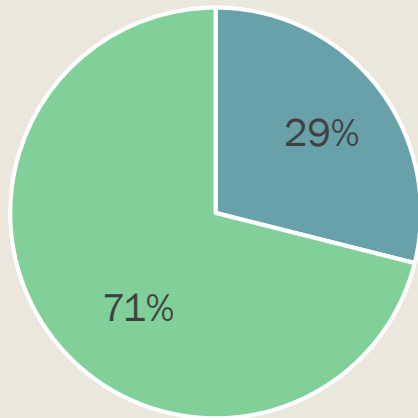
# Results: Reproduction



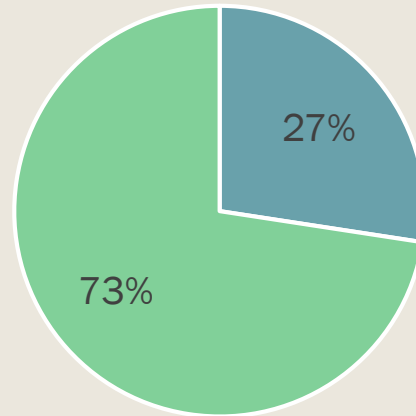
# Results: Burrowing Behavior

■ Lower Level  
■ Upper Level

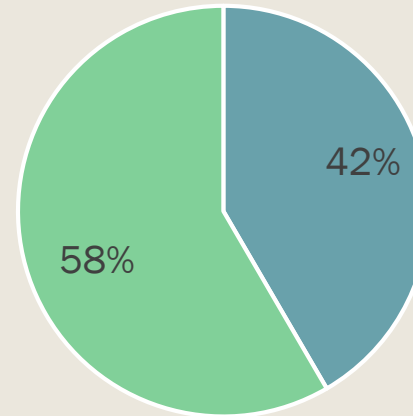
Rust-Oleum Spray



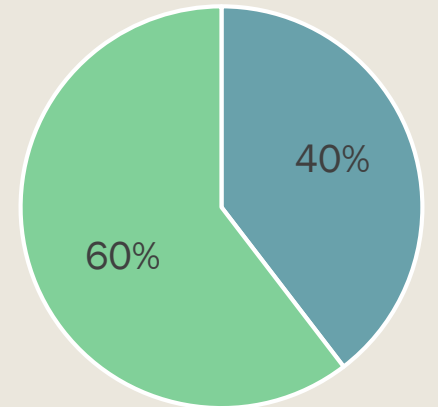
Rust-Oleum Topcoat



Behr Marquee



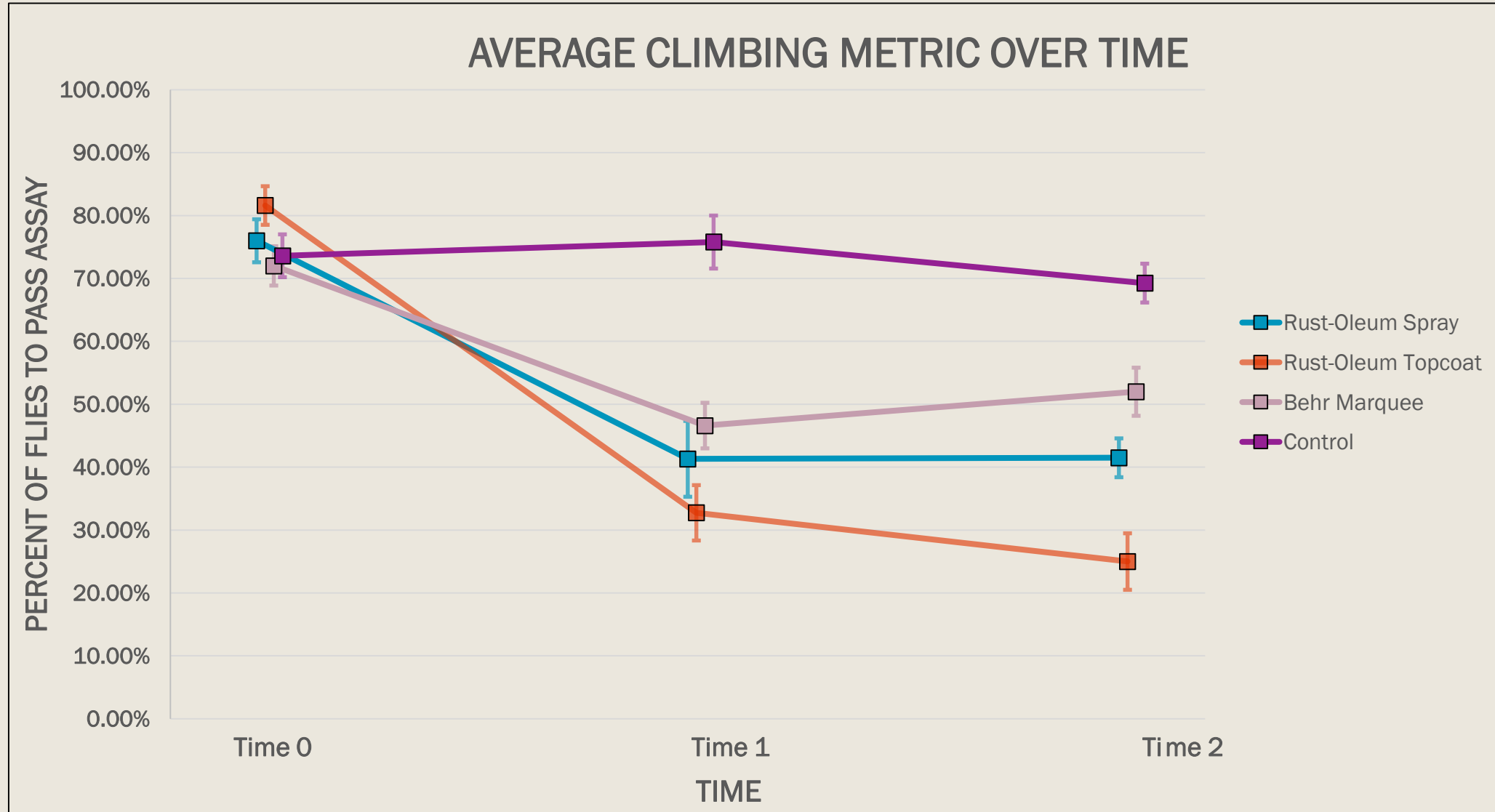
Control



# Results: Climbing Ability Damage

	Rust-Oleum Spray	Rust-Oleum Topcoat	Behr Marquee	Control
<b>Baseline (Time 0)</b>	<b>76.00%</b> (72.584% - 79.416%)	<b>81.60%</b> (78.514% - 84.686%)	<b>72.00%</b> (68.881% - 75.119%)	<b>73.60%</b> (70.261%- 76.939%)
<b>First Generation (Time 1)</b>	<b>41.31%</b> (35.291% - 47.329%)	<b>32.74%</b> (28.331% - 37.149%)	<b>46.61%</b> (42.977% - 50.243%)	<b>75.79%</b> (71.571%- 80.009%)
<b>Second Generation (Time 2)</b>	<b>41.49%</b> (38.404% - 44.576%)	<b>25.00%</b> (20.498% - 29.502%)	<b>52.00%</b> (48.181% - 55.819%)	<b>69.26%</b> (66.173% - 72.347%)

# Results: Climbing Ability Damage



# Statistical Analysis of Climbing Metric: Repeated Measure ANOVA and p-value

An **Analysis of Variance** is a statistical test to measure differences in group means when there are two or more groups.

A repeated measures analysis of variance is a type of ANOVA used when the experiment has repeated measures.

Because this experiment measures climbing ability 5 times for each vial at 3 different times, this is a repeated measures experiment and it is better to analyze differences in group means using a repeated measures ANOVA.



# Statistical Analysis for Climbing Metric

Data from times A and B  
for a given paint



## ANOVA for Time Comparison Table

Test difference between means at times A and B for a given paint, taking the repeated measures into account.



## P-Value

One of the outputs of the ANOVA is the p-value.

Represents the probability that you find a difference at least as extreme when there actually is no difference.

When this value is small, there is strong evidence that there really is a difference.

## Bonferroni Correction for Multiple Tests

Usually, you choose to accept a  $5/100 = 1/20$  chance that you get a result “by chance.” With multiple tests, the significance should be corrected.

Corrected significance level =  $0.05/30 = 0.001677$



## Testing Significance

If  $p\text{-value} < 0.001677$

Then the difference is statistically significant

Data from paint groups A and B  
at a given time



## ANOVA for Paint Comparison Table

Test difference between means for paint groups A and B at given time, taking the repeated measures into account.



## P-Value

One of the outputs of the ANOVA is the p-value.

Represents the probability that you find a difference at least as extreme when there actually is no difference.

When this value is small, there is strong evidence that there really is a difference.



## Testing Significance

If  $p\text{-value} < 0.001677$

Then the difference is statistically significant



Repeat 12 times  
12 pairs of time



Repeat 18 times  
18 pairs of paint groups

# Results: Climbing Ability Damage

P-value for paint comparison ANOVAs

		Rust-Oleum Spray	Rust-Oleum Topcoat	Behr Marquee	Control
<b>Time 0</b>					
	<b>Rust-Oleum Spray</b>		0.158424	0.387993	0.584652
	<b>Rust-Oleum Topcoat</b>			0.036039	0.058205
	<b>Behr Marquee</b>				0.725552
	<b>Control</b>				
<b>Time 1</b>					
	<b>Rust-Oleum Spray</b>		0.364475	0.524910	0.004264
	<b>Rust-Oleum Topcoat</b>			0.035944	<b>0.000179</b>
	<b>Behr Marquee</b>				<b>0.000515</b>
	<b>Control</b>				
<b>Time 2</b>					
	<b>Rust-Oleum Spray</b>		<b>0.000021</b>	<b>0.001409</b>	<b>0.000000</b>
	<b>Rust-Oleum Topcoat</b>			<b>0.000001</b>	<b>0.000000</b>
	<b>Behr Marquee</b>				<b>0.000006</b>
	<b>Control</b>				

Statistically significant differences are in red.

The significance level is 0.001667

This is 0.05 significance level corrected by the Bonferroni correction:  $0.05/30 = 0.001667$ .

# Results: Climbing Ability Damage

P-value for time comparison ANOVAs

		Time 0	Time 1	Time 2
<b>Rust-Oleum Spray</b>				
	Time 0		0.015970	<b>0.000877</b>
	Time 1			0.980309
	Time 2			
<b>Rust-Oleum Topcoat</b>				
	Time 0		<b>0.000566</b>	<b>0.000051</b>
	Time 1			0.064974
	Time 2			
<b>Behr Marquee</b>				
	Time 0		0.008091	0.003685
	Time 1			0.135204
	Time 2			
<b>Control</b>				
	Time 0		0.728769	0.230066
	Time 1			0.227407
	Time 2			

Statistically significant differences are in red.

The significance level is 0.001667

This is 0.05 significance level corrected by the Bonferroni correction:  $0.05/30 = 0.001667$ .

# Hypothesis 1:

If the flies were exposed to paints containing VOCs, then they would suffer damages

**Result: Supported**

1. All 3 paints led to decreased climbing abilities relative to the control
  - *Neurological damage*
2. All 3 paints led to reproductive damage, with less total larvae being seen in all the paint trials
  - *Reproductive damage*
3. For burrowing behavior, both Rust-Oleum paints did more damage
  - *Respiratory damage*

# Hypothesis 2:

If multiple generations of fruit flies are exposed to paints, then over time and with successive generations, the negative effects will increase.

**Result: Partially Supported**

1. Only the Rust-Oleum Topcoat saw climbing abilities decrease between two generations

*Measured solely through climbing assay*

- *indicates neurological damage over time*



# Methods: Paints Tested – VOC Content

## Rust-Oleum Spray

Propane  
n-Butyl Acetate  
Acetone  
Propylene Glycol Monobutyl Ether  
Titanium Dioxide  
n-Butane  
Solvent Naphtha  
Light Aromatic  
Xylenes (o-, m-, p- isomers)  
Barium Sulfate  
Ethylbenzene

535 g/L

## Rust-Oleum Topcoat

Limestone  
Hydro-treated Light Distillate  
Titanium Dioxide  
Mineral Spirits.

410 g/L

## Behr Marquee Low VOC

Titanium dioxide  
Nepheline Syenite  
Anhydrous aluminum silicate  
Amorphous silica  
Aluminum hydroxide  
Coalescing aid  
Ethylene Glycol

71 g/L

# Impact

- Understanding how the chemicals that we expose ourselves to on a daily basis effect our health is vital.
- Only if consumers are made aware of the dangers of VOCs, like those found in paints, will they be able to choose to avoid them.
- Researching and understanding how VOCs effect the health of fruit flies is an important first step to preventing conditions like asthma or damage to the central nervous system.
- Further experimentation on the topic could focus on isolating specific VOCs, as well as further testing how other common household items effect our health.