

Date: July 2022

Sponsor:Bee Friendly Pest Control, LLC.

Study:Honey Bee Direct 22 Test Method: 311

## REPORT TITLE

Evaluation of Bee friendly Yard and Garden Concentrate when applied as a Direct Spray Application Against Honey Bees *Apis mellifera* 

## **STUDY**

Honey Bee Direct 22

TRIAL

**APISME** 

## **SPONSOR CODE**

N/A

## EXPERIMENTAL START DATE

June 14, 2022

## **EXPERIMENTAL COMPLETION DATE**

June 16, 2022

#### REPORT DATE

July 21, 2022

# **TEST FACILITY/AUTHORS**

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## **SPONSOR**

Bee Friendly Pest Control, LLC.



Date: July 2022

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## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA  $\S10(d)(1)(A)$ , (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter:	Date:
Typed Name of Signer:	
Typed Name of Company: Bee Friendly Pest Control, LLC.	

Date: July 2022

Sponsor:Bee Friendly Pest Control, LLC.

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# **COMPLIANCE STATEMENT**

This study was <u>NOT</u> conducted in compliance with Good Laboratory Practice Standards as described by EPA (40 CFR Parts 160 and 792) and was never intended for that purpose.

	Em /e		
Test Facility Management: 07/21/22			Date: _
	Eric J. Snell	Snell Scientifics, LLC.	
Study Director:		of st	
Date:			
	Todd Smith	Snell Scientifics, LLC.	
Sponsor:			
			Date: _
Submitter:			
			Date: _



Date: July 2022 Sponsor:Bee Friendly Pest Control, LLC. Study:Honey Bee Direct 22

Test Method: 311

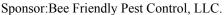
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# **TABLE OF CONTENTS**

Statement of No Data Confidentiality Claims	2
Compliance Statement	3
Table of Contents	4
Study Objective(s)	5
Test Substance Information	5
Test System Information	5
Materials and Methods	5
Results	8
Statisical Analysis	8
Tables	8
Appendix A: Photographs	9
Appendix B: Raw Data	11

Date: July 2022



Study:Honey Bee Direct 22 Test Method: 311



## **STUDY OBJECTIVE(S):**

To determine the mortality associated with the Bee Friendly Yard and Garden concentrate when applied as a direct spray application against honey bees *Apis mellifera*.

## TEST SUBSTANCE INFORMATION:

#	Test Substance	Dilution Rate	Active Ingredients	EPA Reg. No. and/or Est. No.	Lot and/or Batch#	Snell Receipt Code
1	Controls - Untreated	N/A	N/A	N/A	N/A	N/A
2	Bee Friendly Yard & Garden	1:64	N/A	N/A	N/A	052022-1-A-BEE

## **TEST SYSTEM INFORMATION:**

Trial	Test System	Strain	Stage	Source	Test Environmental Conditions
APISME	Honey Bee (Apis mellifera)	Field	Workers	Field Collected	Incubator at 77°F/80%RH with a 12:12 L:D photoperiod

#### **MATERIALS AND METHODS:**

#### 311.1 Materials:

Test Arena Information:

- 311.1.1 Treatment Arenas: 2.25" CPVC mesh cartridge with Tulle mesh. The Test Arenas were used to contain the test systems during the test substance applications.
- Post-Treatment Arenas: 20oz SOLO cup. The Post-Treatment arenas were used to contain the test systems in a clean environment after exposure to the test substance(s).
- 311 1 3 Food/Moisture: 50:50 sucrose: water-soaked cotton ball

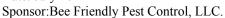
## *Test Equipment:*

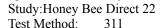
- Volumetric Measuring Equipment: Graduated cylinders and/or beakers were used as needed in preparing and/or measuring the flow rates of the test substance(s).
- 311.1.5 Digital Balance(s): Balances were used as needed in preparing and/or weighing the test substance canisters before and after applications.
- 311.1.6 CO<sub>2</sub> and Regulator: A standard CO<sub>2</sub> cylinder with regulator was used to anesthetize the test systems and sort them into the test arenas (prior to exposure to the test substances). The test

Page 6 of 12

This work was conducted under sound scientific principles but does not comply with GLP standards under 40 CFR 160









systems were allowed to adequately recover from anesthetizing before being exposed to the test substance(s), and they were not anesthetized at any point following exposure to the test substance(s). Any additional transfers required after exposure to the test substances was conducted using methods that did not involve anesthetizing.

- 311.1.7 Intermediate Sorting/Transfer Containers: Additional sorting and transfer containers were -used to aid in moving the test systems from the primary rearing/collection containers and into the treatment and/or post-treatment arenas.
- 311.1.8 Metronome/Timing Equipment: A metronome and/or other timing equipment were used as needed to assist in the timing when conducting the applications and/or when collecting the observations.

# Application Equipment:

311.1.9 Application Equipment: Stihl SR 200 Backpack Mister.

### 312.2Methods:

## Test Design:

- 311.2.1 The evaluations of this study followed the photographs in the Appendix A: Photograph section of the report.
- Each Treatment and/or Post-Treatment Arena was labeled with a test substance code and a replicate number. The arenas were positioned on a clean tray and grouped together per test substance type. The tray(s) with the Treatment and Post-Treatment Arenas were also labeled using the study name, trial name, and the study initiation date (as a duplicate means of ensuring accurate data collection).
- 311.2.3 The test systems were sorted into the Treatment Arenas using the appropriate methods based on the species type.
- 311.2.4 All test systems were confirmed to be of "good vigor" (alive) prior to exposure to the test substance(s).
  - 311.2.4.1 Only live test systems were selected for use in the study.
  - After all test systems were transferred into the test arenas, they were confirmed to be alive and exhibiting normal behavior before continuing with the study.
- 311.2.5 The number of replicates conducted per test substance and the number of test systems evaluated per replicate and trial were as follows:

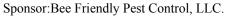
# Reps per Substance	# Systems per Rep	# Systems per Substance	# Test Substances	Total # Systems
4	10	40	2	80

*Test Substance Preparation & Applications:* 

311.2.6 The test substance was diluted with water and adequately shaken prior to applications.







Study:Honey Bee Direct 22 Test Method: 311



311.2.7 The applications were conducted using an approximate 2 gal/10,000 sq. ft. application rate with a Stihl SR 200 backpack sprayer:

Sprayer settings

Nozzle setting = 2

Spray speed setting = low

Flow rate = 1 gal/6.5 min (3.785 mL/390 sec = 9.7 mL/sec)

Spray distance/measurements

Spray distance = 6 ft

Spray swath =  $\sim$ 24 inches

Application details

2 gal (7,571mL)/10,000 sq. ft. (1,440,000 sq. in.) = 0.005mL/sq. in.

40 linear inches (960 sq. in.) sprayed in 0.5 second to apply approximately 4.8mL

#### Observation Methods:

- The number of "Alive", "Knockdown (KD)", and "Dead" test systems per arena were 311.2.8 recorded prior to applications (Pre-trt), and at 30 mins, 1 hr, 2 hrs, 4 hrs, 24 hrs, and 48 hrs after the applications.
- 311.2.9 The observations were collected by raising the test arenas and gently blowing air on the test systems to provoke movement, lightly prodding the test systems, or the test arenas were shaken/agitated to provoke test system movement.
- The test systems were transferred from the Treatment Arenas into the clean Post-Treatment 311.2.10 Arenas 1-hour after the applications.
- 311.2.11 Definitions of "Alive", "Knockdown (KD)", and "Dead":
  - Alive Test System exhibited normal forward motion and/or the ability to fly. 311.2.11.1
  - Knockdown (KD) Test System exhibited some movement but could not crawl 311.2.11.2 and/or flv.
  - 311.2.11.3 Dead - Test System exhibited no movement when stimulated.

## Environmental Conditions:

- The test systems were tested under the following conditions. 311.2.12
- 311.2.13 Average environmental conditions recorded during the study:

311.2.13.1	Applications:	Temperature: 85°F	Humidity: <u>70%</u>
311.2.13.2	Laboratory:	Temperature: 71°F	Humidity: <u>54%</u>
311.2.13.3	Incubator:	Temperature: 77°F	Humidity: 80%



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Study:Honey Bee Direct 22 Test Method: 311

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## **RESULTS:**

The results of this study are shown in Table 1, which illustrates the percent mortality and Abbott's corrected percent mortality for the honey bees after applications.

Percent Mortality = Number Dead/Total Population X 100 Abbott's Percent Mortality = (1-(1-Treatment Mortality) / (1-Control Mortality))

The honey bees that were treated with Bee Friendly Yard and Garden formulation (1:64 dilution) recorded 3% mortality at 24 and 48 hours after the applications. The untreated population recorded 0% mortality during the study.

## **STATISTICAL ANALYSIS:**

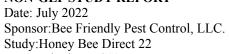
Due to the minimal mortality associated with the Bee Friendly Yard and Garden treated population, no statistical analysis was conducted on the results.

## **TABLES:**

#### Table 1.

Р	ercent M	ortality					
Test Substance:	Pre-trt	30 min	1 hr	2 hr	4 hr	24 hr	48 hr
Control - Untreated	0%	0%	0%	0%	0%	0%	0%
Bee Friendly Yard and Garden (1:64 dilution rate)	0%	0%	0%	0%	0%	3%	3%
Abbott's I	Percent N	Mortality (	(1925.)				
Bee Friendly Yard and Garden (1:64 dilution rate)	0%	0%	0%	0%	0%	3%	3%





Test Method: 311



# **APPENDIX A: PHOTOGRAPHS**

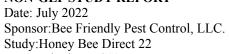
#### Photograph 1. **Test Substance**



Photograph 2. Mesh Cartridge with Honey Bees







Test Method: 311



Application with Stihl SR 200 Sprayer Photograph 3.



Photograph 4. Honey Bees in Post-Treatment Arena



Date: July 2022 Sponsor:Bee Friendly Pest Control, LLC.

Study:Honey Bee Direct 22

Test Method: 311

# **APPENDIX B: RAW DATA**

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