STILLMEADOW
INCORPORATED

TITLE

PureCrop1
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test

TEST GUIDELINE

OCSPP 850.3020

AUTHOR

Cole Younger, PhD

STUDY COMPLETION DATE

14 September 2021

PERFORMING LABORATORY

STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

LABORATORY STUDY ID

24790-21

NO CLAIM OF CONFIDENTIALITY

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 § 10(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:_____
Name of Signer:_____
Sponsor: PureCrop1

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study and those required by United States Environmental Protection Agency FIFRA 40 CFR Part 160:

Section 160.31 (d) and 160.105 (a)(b)(e) Characterization and stability information was not provided to the testing facility.

Study Director:_____ Date: 14 Sep 21
Cole Younger, PhD
STILLMEADOW, Inc.

Sponsor:_____ Date:_____
Name of Signer:_____
Sponsor: PureCrop1

Submitter:_____ Date:_____
Name of Signer:_____
Submitter: PureCrop1

QUALITY ASSURANCE STATEMENT

Study Title: PureCrop1
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test

The study report and data have been audited in accordance with Good Laboratory Practice Standards and STILLMEADOW, Inc. Standard Operating Procedures (SOPs). The final report accurately reflects the study data. The Quality Assurance Unit has not been involved in the actual conduct of this study.

The Quality Assurance Unit performed a recent facility inspection on 22 Jul 21. All findings were reported to Management, and the report and responses are kept in the Quality Assurance files.

The findings from any study inspections and audits were reported to the Study Director and Management as follows:

Critical Phase Inspected	Date Inspected	Reported to Study Director	Reported to Management
Protocol Review	29 Jul 21	29 Jul 21	29 Jul 21
Dosing	04 Aug 21	04 Aug 21	04 Aug 21
Report/Data Audit	13 Sep 21	13 Sep 21	13 Sep 21

Richard L. Martin

Richard L. Martin, MS
Auditor, Quality Assurance
STILLMEADOW, Inc.

14 Sep 21
Date

TABLE OF CONTENTS

SUMMARY

In a 48-hour acute contact toxicity study, adult worker honey bees, *Apis mellifera,* were exposed to the test substance, PureCrop1, by direct topical application to their thorax at the nominal dose of 25 µg active ingredient (a.i.)/bee. The test bees were immobilized and randomly assigned to one of six groups. Deionized water with Polysorbate 80 was used as the vehicle for all groups. The test substance solution was individually administered to 100 bees as a single topical dose of 25 µg a.i./bee. A group of 100 bees was dosed with the vehicle only and served as vehicle controls. Another group served concurrently as the untreated controls. Three groups of 100 bees were dosed with the toxic standard, dimethoate, at 0.01 µg/bee, 0.1 µg/bee or 1.0 µg/bee and served as positive controls. The bees were observed for mortality and clinical signs of toxicity at 4, 24 and 48 hours post dose. Percent mortality at 48 hours in the untreated control, vehicle control, test substance, and positive control groups (0.01, 0.1 and 1.0 µg/bee of dimethoate) was 2.0%, 2.0%, 2.0%, 0.0%, 0.0% and 64.0%, respectively.

Since mortality in the test substance group did not exceed the mortality in the untreated and vehicle control groups and control mortality was less than 20%, the Median Lethal Dose (LD_{50}) for the test substance, PureCrop1, is considered to be greater than the nominal dose of 25 µg a.i./bee and was non-toxic when administered by contact to adult honey bees.

INTRODUCTION

The objective of this study was to assess the acute contact toxicity potential of the test substance, PureCrop1, when administered topically to adult worker honey bees in accordance with the Environmental Protection Agency Office of Chemical Safety and Pollution Prevention Guideline 850.3020. This study was conducted according to the approved protocol (included as report Appendix A) and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol that affected the quality or outcome of the study. The study was initiated on 02 Aug 21 and the laboratory portion of the study was conducted from 04 - 06 Aug 21. All raw data, the original protocol, the original final report, any amendment(s), and a retained test substance sample will be sent to Sponsor representative Raquel Hale at PureCrop1, 425 Kunzler Ranch Rd., Suite A, Ukiah, CA, 95482.

SPONSOR INFORMATION

Company Name:	PureCrop1
Address:	[redacted]

TEST SUBSTANCE

Label Identification:	PureCrop 1 Active Ingredients: Soybean Oil 10.0%; Corn Oil 5.0% Other Ingredients: 85.0%; Total 100.0% Net Contents: 16fl. Oz. (473ml)
Date and Quantity Received:	01 Jul 21; 527.5 g (GW)
Physical Description:	Light yellow liquid
Storage Conditions:	Room temperature
Purity & Composition:	Not provided to testing facility
Stability:	Not provided to testing facility

Data generated for characterization and stability, and the level of GLP compliance for that data, are the responsibility of the Sponsor. Records pertaining to identity, synthesis methods and location of documentation are the responsibility of the Sponsor.

CONTROL SUBSTANCES

Vehicle control:	Deionized water with Polysorbate 80 Polysorbate 80 (Mfr: Sigma-Aldrich; Lot: BCCF2192; Exp: Jan 2022) A copy of the manufacturer's Certificate of Analysis for the surfactant is included as report Appendix B.
Positive control (toxic standard):	Dimethoate (0.01, 0.1 and 1.0 µg/bee) CAS# 60-51-5 (Mfr: Chem Service, Lot: 8401400, Exp: 30 Apr 2022) A copy of the manufacturer's Certificate of Analysis for the positive control is included as report Appendix C.

TEST SYSTEM

Insect Species

Species / Strain / Source:	*Apis mellifera* / Italian honey bee / STILLMEADOW, Inc. bee colony, disease and pest-free with no previous pesticide exposure
Justification of Species:	The honey bee is the species required in the regulatory guidelines for this study.
Quantity:	600 bees. 20 bees per replicate; 5 replicates per treatment group; 6 groups.
Age at Dosing:	Young adult worker bees, similar in age
Identification:	Numbered cups with treatment identification
Acclimation and Health Status:	No acclimation was necessary. Normal appearance and behavior were factors used to select healthy bees from disease-free colonies for testing.

Insect Husbandry

Exterior housing:	Standard commercial honey beehive
Indoor Chambers:	16-ounce cardboard cup with screen lid
Environmental Controls Set to Maintain:	Incubator temperature at times of observation: 30 ± 5° C Incubator relative humidity at times of observation: 50 - 90% Lighting dark except when dosing or observations were made
Measured Incubator Temperature and Relative Humidity:	30°C / 48 - 60%
Handling:	Only as much handling as necessary to conform to the test procedures was allowed. The bees were shielded from excessive activity or other disturbance during holding and testing.
Food:	50:50 w/v sucrose: dechlorinated (DC) water solution; available *ad libitum* dispensed using saturated cotton balls replaced daily. (Sucrose: Mfr: Sigma Life Science, Lot: SLCG9891, Exp: Jun 2026)

No contaminants in the feed or water were expected to have been present that would have interfered with this study.

PROCEDURES

Preparation of Vehicle

The vehicle was prepared by mixing 50 mL of deionized water with 0.05 mL of Polysorbate 80.

Dose Calculation and Preparation of Dosing Solutions

With a target dose rate of 12.5 µg/mL, a dose amount of 2 µL per bee and a Sponsor provided active ingredient concentration of 15% (15 g a.i. / 100 mL), the amount of test substance required for 10 mL of solution was determined to be 0.833 mL. The dose calculations are as follows.

$$\textbf{Test Substance amount } (mL) = \frac{\text{Target dose * Unit Conversion}}{\text{Concentration of Active Ingredient}}$$

where: *Target Dose = 12.5 µg a.i./µL*
Dose volume = 2 µL per bee
Concentration of Active Ingredient = 15% = 15 g a.i. / 100 mL = 0.150 g a.i.
Unit Conversion = 1 g/1000 mg = 0.001 g/mg; µg/µL = mg/mL

$$\textbf{Test Substance amount } (mL) = \frac{12.5 \text{ µg a.i./µL} * 0.001 \text{ g/mg}}{0.150 \text{ g a.i.}} = 0.0833 \text{ mL per one mL of solution}$$

Approximately 0.833 mL of the test substance was placed in a 10 mL container and brought to volume with the vehicle to make a 12.5 µg a.i./µL solution. The positive control (dimethoate) solution at 0.5 mg/mL was prepared by mixing 0.005 g of dimethoate with 10 mL of the vehicle. This solution was then serially diluted to prepare 0.05 mg/mL and 0.005 mg/mL positive control dosing solutions. All solutions were dosed at 2 µL per bee.

Test Substance and Control Administration

A limit test of 25 µg a.i./bee was conducted with the test substance administered in the vehicle. On day 0, the bees in the holding chambers were immobilized using CO_2. A single dose of the test substance, the vehicle or the positive control was applied to each bee's thorax via a microapplicator. All bees were dosed topically on the dorsal side of their thorax with 2 µL of the appropriate solution. A group of 100 bees (five replicates of 20 bees each) was dosed with the vehicle only and served as vehicle controls. Another group of 100 bees (five replicates of 20 bees each) served concurrently as the untreated controls. Three dose levels (0.01, 0.1, 1.0 µg/bee) of the toxic standard, dimethoate, were administered to five replicates of 20 bees each and served as positive controls.

PROCEDURES (cont.)

Observations

All bees were observed at approximately 4, 24 and 48 hours after dosing for mortality and clinical signs of toxicity, particularly signs of intoxication (ataxia, lethargy, hypersensitivity, etc.). Dead bees were not removed until the end of the study and bees that were still alive were frozen and disposed of. Relative humidity and temperature were recorded at each observation time prior to mortality observations.

Test Validity

For this test to be considered valid, no more than 20% of the bees in either the untreated or the vehicle control could be dead at the end of the test.

Statistical Analysis

A one-way parametric analysis of variance (ANOVA), with Dunnett's Multiple Comparisons Post Test if significance was indicated ($p<0.05$), was performed on mortality using GraphPad InStat, version 3.10 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com.

Evaluation of Results

Results were evaluated by comparing mortality between the treated and untreated control groups. Results were evaluated using the following formula:

$$\textbf{Percent Mortality}\ (\%) = \left(100 \times \frac{\text{Total number of dead honey bees in group}}{\text{Total number of bees in group}}\right)$$

RESULTS AND DISCUSSION

Protocol Deviation

Relative humidity was two degrees below the protocol-specified range on day 2. This protocol deviation did not affect the integrity or outcome of the study.

Mortality Observations and Percent Mortality

Mortality observations and percent mortality are presented in Table 2. Percent mortality at 48 hours in the untreated, vehicle control, test substance, and positive control groups (0.01, 0.1 and 1.0 µg/bee) was 2.0%, 2.0%, 2.0%, 0.0%, 0.0% and 64.0%, respectively (Table 1).

Since mortality in the test substance group (2.0%) did not exceed the mortality in the untreated and vehicle controls (2.0%) and control mortality was less than 20%, the LD_{50} for the test substance, PureCrop1, was considered to be greater than the nominal dose of 25 µg a.i/bee.

Table 1 - Cumulative Mean and Percent Mortality Summary

Group [b]	Mean Dead[a] / % Mortality		
	4 Hours	24 Hours	48 Hours
Untreated	0.0 a / 0.0	0.0 a / 0.0	0.4 a / 2.0
Vehicle Control	0.0 a / 0.0	0.0 a / 0.0	0.4 a / 2.0
PureCrop1	0.2 a / 1.0	0.2 a / 1.0	0.4 a / 2.0
Dimethoate 0.01	0.0 a / 0.0	0.0 a / 0.0	0.0 a / 0.0
Dimethoate 0.1	0.0 a / 0.0	0.0 a / 0.0	0.0 a / 0.0
Dimethoate 1.0	2.0 b / 10.0	12.4 b / 62.0	12.8 b / 64.0
p value	*< 0.0001*	*< 0.0001*	*< 0.0001*

[a] Different letters within the same column indicate significance compared to untreated at $p<0.05$.
[b] Each group began with 100 honey bees on day 0.

CONCLUSION

In a 48-hour acute contact toxicity study, adult worker honey bees, *Apis mellifera,* were exposed to the test substance, PureCrop1, by direct topical application to their thorax at the nominal dose of 25 µg a.i./bee. The test bees were immobilized and randomly assigned to one of six groups. Deionized water with Polysorbate 80 was used as the vehicle for all groups. The test substance solution was individually administered to 100 bees as a single topical dose of 25 µg a.i./bee. A group of 100 bees was dosed with the vehicle only and served as vehicle controls. Another group served concurrently as the untreated controls. Three groups of 100 bees were dosed with the toxic standard, dimethoate, at 0.01 µg/bee, 0.1 µg/bee or 1.0 µg/bee and served as positive controls. The bees were observed for mortality and clinical signs of toxicity at 4, 24 and 48 hours post dose. Percent mortality at 48 hours in the untreated control, vehicle control, test substance, and positive control groups (0.01, 0.1 and 1.0 µg/bee of dimethoate) was 2.0%, 2.0%, 2.0%, 0.0%, 0.0% and 64.0%, respectively.

Since mortality in the test substance group did not exceed the mortality in the untreated and vehicle control groups and control mortality was less than 20%, the LD_{50} for the test substance, PureCrop1, is considered to be greater than the nominal dose of 25 µg a.i./bee and was non-toxic when administered by contact to adult honey bees.

SIGNATURE

Cole Younger, PhD
Study Director
Entomologist, STILLMEADOW, Inc.

14Sep21
Date

STUDY PERSONNEL

Technical Staff
Stephen Balestrier, BS
Hugo Martinez
Yudith Amaya, AAS
Mariana Cortez, AAS

Technical Writer
Monica Dunn, BS

Table 2 - Mortality Observations and Percent Mortality
Honey Bee, *Apis mellifera,* Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

UNTREATED

Time Post Dose	Cumulative Number Dead							% Mort.	Observations
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5	Mean[a]	Sum[b]		
4 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
48 Hours	0	1	1	0	0	0.4	2	**2.0%**	Rest NOA
Total % Mortality	**0.0%**	**5.0%**	**5.0%**	**0.0%**	**0.0%**				

VEHICLE CONTROL

Time Post Dose	Cumulative Number Dead							% Mort.	Observations
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5	Mean[a]	Sum[b]		
4 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
48 Hours	1	1	0	0	0	0.4	2	**2.0%**	Rest NOA
Total % Mortality	**5.0%**	**5.0%**	**0.0%**	**0.0%**	**0.0%**				

PureCrop 1

Time Post Dose	Cumulative Number Dead							% Mort.	Observations
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5	Mean[a]	Sum[b]		
4 Hours	0	1	0	0	0	0.2	1	**1.0%**	Cup 3: 1 moribund Rest NOA
24 Hours	0	1	0	0	0	0.2	1	**1.0%**	Cup 3: 1 moribund; Rest NOA
48 Hours	0	1	0	0	1	0.4	2	**2.0%**	Cup 3: 1 moribund; Rest NOA
Total % Mortality	**0.0%**	**5.0%**	**0.0%**	**0.0%**	**5.0%**				

Note: 20 bees were added to each cup on day 0 (100 bees per group).
Percent Mortality = (number of dead bees / total number of bees) * 100
% Mort., Cumulative % Mort. by day. Total % Mortality, final percent mortality per cup by 48 hours.
[a] Mean number of dead bees in all five cups. [b] Sum of the number of dead bees in all five cups.
NOA, no observable abnormalities

Table 2 - Mortality Observations and Percent Mortality (cont.)
Honey Bee, *Apis mellifera,* Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

Dimethoate 0.01 µg/bee

Time Post Dose	Cumulative Number Dead							% Mort.	Observations
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5	Mean[a]	Sum[b]		
4 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
Total % Mortality	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**				

Dimethoate 0.1 µg/bee

Time Post Dose	Cumulative Number Dead							% Mort.	Observations
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5	Mean[a]	Sum[b]		
4 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	Cup 5: 1 moribund; Rest NOA
Total % Mortality	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**				

Dimethoate 1.0 µg/bee

Time Post Dose	Cumulative Number Dead							% Mort.	Observations
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5	Mean[a]	Sum[b]		
4 Hours	3	3	1	1	2	2.0	10	**10.0%**	Cup 2: 3 moribund; Cup 5: 4 moribund; Rest NOA
24 Hours	13	11	16	12	10	12.4	62	**62.0%**	Cup 5: 1 moribund; Rest NOA
48 Hours	13	11	16	12	12	12.8	64	**64.0%**	Rest NOA
Total % Mortality	**65.0%**	**55.0%**	**80.0%**	**60.0%**	**60.0%**				

Note: 20 bees were added to each cup on day 0 (100 bees per group).
Percent Mortality = (number of dead bees / total number of bees) * 100
% Mort., Cumulative % Mort. by day. Total % Mortality, final percent mortality per cup by 48 hours.
[a] Mean number of dead bees in all five cups. [b] Sum of the number of dead bees in all five cups.
NOA, no observable abnormalities

Appendix A - Protocol
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

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PROTOCOL FOR STUDY 24790-21

Study Title: Honey Bee, *Apis mellifera,* Acute Contact Toxicity Limit Test (OCSPP 850.3020)

Test Substance: PureCrop1

Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

Approved: _____ 02 AUG 21
Cole Younger, PhD
Study Director
STILLMEADOW, Inc.
Date

Approved: _____ 29JUL21
Management
STILLMEADOW, Inc.
Date

Reviewed: _____ 29 Jul 21
Kristina Rodrigue, RQAP-GLP
Director, Quality Assurance Unit
STILLMEADOW, Inc.
Date

Sponsor: PureCrop1
[redacted]
707-272-8053
shale@purecrop1.com

Approved: _____ 8/2/2021
Shelby Hale
Vice President
Date

12852 Park One Drive ■ Sugar Land, Texas 77478 ■ 281 240-8828 ■ Fax 281 240-8448
www.stillmeadow.com

Appendix A - Protocol (cont.)
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

PROTOCOL FOR STUDY 24790-21

A. GENERAL

1. Study Title: Honey Bee, *Apis mellifera,* Acute Contact Toxicity Limit Test

2. Purpose: To assess the acute contact toxicity potential of the test substance when administered topically to adult worker honey bees.

3. Methods Guidelines: This study will be conducted according to OCSPP 850.3020, Honey Bee Acute Contact Toxicity Test.

4. Regulatory Compliance: This study will be conducted in compliance with Good Laboratory Practice Standards: EPA FIFRA: 40 CFR Part 160.

The dose mixtures will not be verified according to the requirements specified in 40 CFR 160.113(a) as the active ingredients in the test substance are unable to be analyzed separate from the other components of the test substance by normal analytical methods.

In the event of a regulatory inspection, Regulatory Inspectors will be provided with all study documentation requested. The Sponsor will be notified of the inspection of their study.

All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).

5. Quality Assurance: The Quality Assurance Unit (QAU) will review the protocol. The study information will be entered into the Master Schedule. In-progress inspection(s) will be performed to ensure the integrity of the study. Any deviations from SOPs, the Protocol or Good Laboratory Practice Standards will be immediately reported to the Study Director and Management. The report and raw data will be audited, and a statement prepared and signed which will specify the dates that the inspections were made and findings reported to Management and the Study Director.

6. Test Substance: PureCrop1. Test substance identification should include the name, batch number and purity. The Sponsor should also provide information regarding safety, stability, storage conditions and disposal. The Sponsor assumes responsibility for the test and reference substances' purity, stability, identity, synthesis methods and location of documentation.

7. Positive Control Substance: Dimethoate (CAS# 60-51-5).

8. Proposed Schedule: Proposed Experimental Start Date: 09 Aug 21
Proposed Experimental End Date: 13 Aug 21

Study duration: at least 48 hours and may be extended to a maximum of 96 hours after dosing.

Appendix A - Protocol (cont.)
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

A. GENERAL (cont.)

9. Study Director:	Cole Younger, PhD
10. Experimental Summary:	Test bees will be immobilized and randomly assigned to dose groups or controls. The test substance will be administered separately with the appropriate vehicle as a single topical dose of 25 µg active ingredient (a.i.)/bee. The bees will be observed for mortality and clinical signs of toxicity at –4, 24 and 48 hours after dosing. Observations may be extended to 96 hours after dosing. A negative control group will remain untreated and will be conducted concurrently. A vehicle control group dosed with the vehicle only will be conducted concurrently. A toxic standard with three dose levels will also be tested.
11. Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director and recorded in writing.
12. Sponsor Audits:	The Sponsor may send an authorized representative to inspect the test system and/or data on the STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Insects:

a. Species/Source:	Italian honey bee, *Apis mellifera* STILLMEADOW, Inc. bee colony or other suitable supplier, disease and pest-free with no previous pesticide exposure.
b. Justification of Species:	The honey bee is the species required in the regulatory guidelines for this study.
c. Quantity:	600 bees. 20 bees per replicate; 5 replicates per treatment group; 6 groups.
d. Age at Dosing:	Young adult worker bees, similar in age
e. Identification:	Replicates will be labeled according to treatment.
f. Acclimation and Health Status:	No acclimation is necessary. Normal appearance and behavior will be factors used to select healthy bees from disease-free colonies for testing.

Appendix A - Protocol (cont.)
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

B. EXPERIMENTAL DESIGN (cont.)

2. Insect Husbandry:

a. Exterior housing:	Standard commercial honey beehive
b. Indoor Chambers:	Disposable cardboard containers with a screened lid
c. Food:	50:50 w/v sucrose:dechlorinated (DC) water solution; available *ad libitum*
d. Environment:	Incubator temperature at times of observation of 30°C ±5° Incubator relative humidity at times of observation of 50-90% Honey bees will be kept in dark except when dosing or making observations.
e. Handling:	Only as much as is necessary to conform to test procedures; shielded from excessive activity or other disturbance during holding and testing.
f. Contaminants:	There are no known contaminants in the feed or water available to laboratory insects that would be expected to interfere with this study.

3. Dose Level: A limit test of 25 µg a.i./bee will be conducted with the test substance being administered with the appropriate vehicle as a single dose level to five replicates of 20 bees. Per Sponsor information, the test substance has a combined active ingredients concentration of 15%.

4. Vehicle Selection: Per Sponsor information, deionized (DI) water will be used as the vehicle. A surfactant will be used with the water and the surfactant will also be added to the vehicle control group. The positive control will be diluted with the same vehicle chosen for the test substance.

5. Test Substance and Control Administration:

a. Reason for Route of Administration:	For honey bee toxicity testing, direct contact dosing is an acceptable route of administration.
b. Randomization:	Honey bees will be taken randomly by manual selection from the collection container and placed in one of six groups.

Appendix A - Protocol (cont.)
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

B. EXPERIMENTAL DESIGN (cont.)

5. Test Substance and Control Administration: (cont.)

c. Test and Control Substance Administration: On Day 0, the bees in the holding chambers will be immobilized using CO_2. A single dose of the test substance, the vehicle control or the positive control will be applied to the dorsal side of each bee's thorax via a microapplicator. Dosing will be administered as follows:

Group I - Untreated Control
Group II - Vehicle Control
Group III - Test Substance 25 µg a.i./bee
Group IV - Dimethoate 0.01 µg/bee
Group V - Dimethoate 0.1 µg/bee
Group VI - Dimethoate 1.0 µg/bee

6. Controls: A negative control and a vehicle control will be conducted concurrently. Five replicates of 20 bees will remain untreated to serve as a negative control. Five replicates of 20 bees will receive only the vehicle to serve as a vehicle control. If DI water is chosen as the vehicle, then a surfactant will be used with the water and the surfactant will also be added to the vehicle control group. The volume administered will be equal to the volume administered to test bees.

7. Toxic Standard: A toxic standard (positive control) will be conducted concurrently. The toxic standard will use the same vehicle as the test substance. Three dose levels (0.01, 0.1, 1.0 µg/bee) of the toxic standard, dimethoate, will be administered to five replicates of 20 bees (100 bees for each dose level). Toxic standard groups will receive the dimethoate in the same manner as test and controls.

8. Observations: All bees will be observed at ~4, 24 and 48 hours after dosing for mortality and clinical signs of toxicity, particularly signs of intoxication (ataxia, lethargy, hypersensitivity, etc. If the mortality rate for the test substance group increases by more than 10% (i.e. from 2% to 13% or from 0% to 11%), then observations will be extended to 72 and 96 hours, provided that control mortality does not exceed 20%. Any dead bees will not be removed until the end of the study and bees that are still alive will be frozen and disposed of. Temperature and relative humidity will be recorded first at each observation time prior to doing mortality observation.

9. Test Validity: For the test to be considered valid, no more than 20% of the bees in either the negative or the vehicle control can be dead at the end of the test.

Appendix A - Protocol (cont.)
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

B. EXPERIMENTAL DESIGN (cont.)

10. Evaluation of Results: The effects of treatment will be determined by:

$$\textbf{Percent Mortality}\ (\%) = 100 \times \frac{\text{Total number of dead honey bees in group}}{\text{Total number of bees in group}}$$

or other appropriate formula will be employed.

Statistical analysis comparing treatment groups will be performed using the appropriate statistical methods when possible.

11. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. The test substance container(s) will be weighed when received at this facility, and a record of all test substance use will be maintained. Test substance and test substance dosing solutions will be stored in the original containers or equivalent, or in glass containers with polyethylene screw-type caps.

12. Disposal of Unused Test Substance: Unused test substance will be disposed of at the Sponsor's expense after the termination of the study.

13. Safety Precautions: General safety precautions required by laboratory SOPs will be followed. The Sponsor will supply basic toxicity data on the test substance to be used. However, since the toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. The Sponsor or Sponsor's Representative shall be notified of any exposures requiring a physician's examination or care.

Appendix A - Protocol (cont.)
Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Test Substance: PureCrop1

C. DATA MANAGEMENT

1. Records:

The following records will be maintained during the study and archived at STILLMEADOW, Inc. upon study termination.

a. Protocol and Protocol Amendments (if any)
b. Final report and amendments (if any)
c. Study correspondence
d. Bee procurement data
e. Test substance receipt, identification as supplied by the Sponsor, preparation, administration and disposition
f. Test insect information: number, species and source
g. Daily clinical signs and mortality, if any
h. Other pertinent data

2. Data Storage:

All raw data, original protocol, original final report, any amendment(s), and a reserve test substance sample will be sent to the following address per Sponsor request for archiving:
Raquel Hale
PureCrop1
[redacted]
707-391-7399

3. Data Reporting:

The final report will include all data as described in the Good Laboratory Practice Standards, including:

a. Statement from the Quality Assurance Unit
b. Signature of the Study Director
c. A GLP Compliance Statement signed by the Study Director
d. Names of scientific personnel involved in the study
e. Dates of study initiation and termination
f. Identification, label information, description, preparation and storage of the test substance and vehicle information.
g. All pertinent honey bee information and observation methods
h. Description of the test procedures
i. Daily observations for mortality and clinical signs of toxicity
j. Mortality and sublethal effects percentage calculations
k. Dose response curve, slope and LD_{50}, as applicable and if possible
l. A copy of this Protocol
m. Any deviations and the impact, if any, on the study

4. Report Generation:

A final report will be generated after completion of the laboratory portion of the study.

Appendix B - Surfactant Certificate of Analysis

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	Polysorbate 80 tested according to Ph Eur
Product Number:	59924
Batch Number:	BCCF2192
Brand:	Sigma-Aldrich
CAS Number:	9005-65-6
Formula:	
Formula Weight:	
Quality Release Date:	02 MAR 2021
Recommended Retest Date:	MAR 2022

TEST	SPECIFICATION	RESULT
PHARMACOPOEA TESTS	CORRESPONDS TO REQUIREMENTS	TESTED ACCORDING TO PH.EUR.10.4
IDENTIFICATION A	IR	CORRESPONDS
IDENTIFICATION D	COMPOSITION OF FATTY ACIDS	CORRESPONDS
ACID VALUE	MAX. 2.0	0.3
HYDROXYL VALUE	65 - 80	74
PEROXIDE VALUE	MAX. 10.0	1.4
SAPONIFICATION VALUE	45 - 55	49
RESIDUAL SOLVENTS	CORRESPONDS	CORRESPONDS
DIOXAN	MAX. 10 PPM	< 10 PPM
ETHYLENE OXIDE	MAX. 1 PPM	< 1 PPM
COMPOSITION OF FATTY ACIDS	CORRESPONDS (GC)	CORRESPONDS
HEAVY METALS	CORRESPOND TO REQUIREMENTS	ELEMENTAL IMPURITIES ACCORDING TO ICH Q3D ARE NOT LIKELY TO BE PRESENT
TOTAL ASH	MAX. 0.25 %	< 0.01 %
WATER	MAX. 3.0 %	2.5 %
Fatty acid (C14:0)	≤5.0 %	0.1 %
Fatty acid (C16:0)	≤16.0 %	11.6 %
Fatty acid (C16:1)	≤8.0 %	0.99 %
Fatty acid (C18:0)	≤6.0 %	3.2 %
Fatty acid (C18:1)	≥58.0 %	67.5 %

Appendix B - Surfactant Certificate of Analysis (cont.)

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Fatty acid (C18:2)	≤18.0 %	0.14 %
Fatty acid (C18:3)	≤4.0 %	< 0.1 %

Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Appendix C - Positive Control Certificate of Analysis

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

Dimethoate

CATALOG NUMBER	N-11758-100MG
LOT NUMBER	8401400
DATE CERTIFIED	04/04/19
EXPIRATION DATE	04/30/22
CAS NUMBER	60-51-5
MOLECULAR FORMULA	C5H12NO3PS2
MOLECULAR WEIGHT	229.27
STORAGE	Store under refrigeration.
HANDLING	See Safety Data Sheet
INTENDED USE	For laboratory use only.
ISO GUIDE 34 CERTIFIED	[ ]

Analytical Test	Value
FT-IR SPECTROSCOPY	CONFORMS TO STRUCTURE
% PURITY (HPLC)	99.4

Chem Service, Inc. guarantees the purity to be +/- 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Certified By:

Mary Beth O'Donnell

Mary Beth O'Donnell
CSM/TC

COA Form
Revision 3 (3/2015)

Appendix C - Positive Control Certificate of Analysis (cont.)

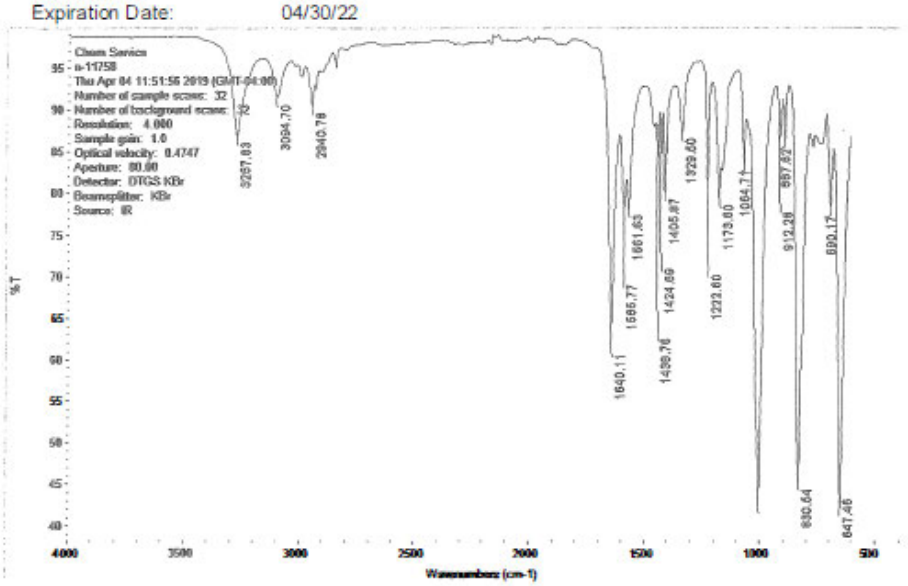
CHEM SERVICE INC

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

Analysis Method: FT-IR Spectroscopy

Catalog Number:	N-11758-100MG
Description:	Dimethoate
Lot Number:	8401400
Expiration Date:	04/30/22



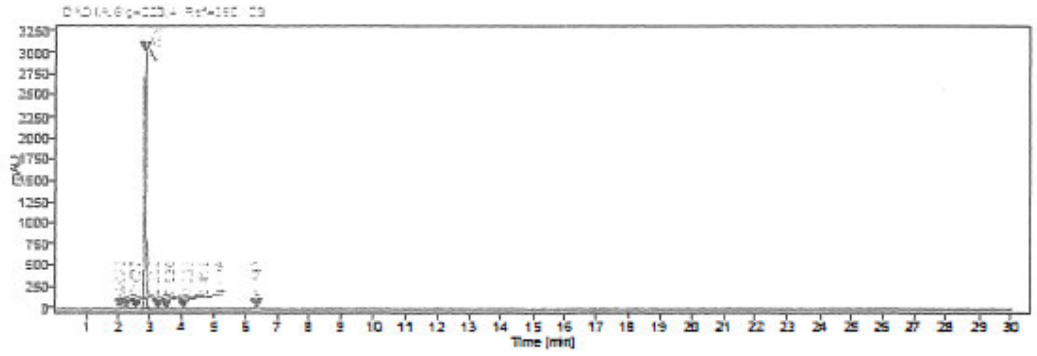
Appendix C - Positive Control Certificate of Analysis (cont.)

CHEMSERVICE INC

660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599
1-800-452-9994 • 1-610-692-3026 • Fax 1-610-692-8729
info@chemservice.com • www.chemservice.com

CERTIFICATE OF ANALYSIS

Data file:	N-11758 151.dx		
Sequence Name:	SingleSample	Project Name:	HPLC4
Sample name:	N-11758	Instrument:	LC4
		Injection date:	2019-04-04 11:28:52-04:00
Inj. volume:	2.000	Location:	P1-A8
Acq. method:	SCREEN.amx	Sample amount:	1.00
Processing method:	*Test2.pmx		



Signal: DAD1A,Sig=220,4 Ref=360,100

RT [min]	Type	Width [min]	Area	Height	Area %
2.023	BV	0.04	2.04	1.17	0.02
2.077	VB	0.14	23.81	7.25	0.21
2.252	BB	0.23	9.13	2.21	0.08
2.536	BB	0.10	1.00	0.36	0.01
2.829	BB	0.48	11358.21	3016.61	99.45
3.223	BB	0.18	10.58	2.70	0.09
3.499	BB	0.21	2.94	0.69	0.03
4.036	BB	0.18	5.40	1.20	0.05
6.343	BB	0.30	7.97	1.44	0.07
		Sum	11421.09		

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