STILLMEADOW
INCORPORATED

TITLE

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

TEST GUIDELINE

OCSPP 850.3020

AUTHOR

Cole Younger, PhD

STUDY COMPLETION DATE

13 May 2022

PERFORMING LABORATORY

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

LABORATORY STUDY ID

25413-22

REGULATORY COMPLIANCE STATEMENT

This study was conducted in the spirit of compliance with Good Laboratory Practice Standards.

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

Study Director:_____ Date: 13 May 22
Cole Younger, PhD
STILLMEADOW, Inc.

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SUMMARY

In a 48-hour acute contact toxicity study, honey bees, *Apis mellifera,* were exposed to the test substance, MITEXSTREAM, by direct topical application to their thorax at a dose rate of 1 oz/gallon with a dose of 2 µL per 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 at a dose rate of 1 oz/gallon. A group of 100 bees remained untreated and served as the untreated control group. Another group of 100 bees was dosed with the vehicle only and served as the vehicle control group. 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.

The estimated Median Lethal Dose (LD_{50}) for dimethoate at 24 hours was 1.194 µg/bee with 95% confidence levels of 0.9819 - 1.4543 µg/bee.

Percent mortality at 48 hours in the untreated control, vehicle control, test substance, and two positive control groups (0.01 and 0.1 µg/bee of dimethoate) was 0%. Percent mortality at 48 hours in the 1.0 µg/bee of dimethoate positive control group was 43%. With a mortality of 0% after 48 hours, the LD_{50} for the test substance, MITEXSTREAM, is considered to be greater than the nominal dose rate of 1 oz/gallon with a dose of 2 µL per bee and was non-toxic when administered by contact to honey bees.

INTRODUCTION

The objective of this study was to assess the acute contact toxicity potential of the test substance, MITEXSTREAM, when administered topically to adult honey bees. This study was conducted according to the approved protocol (included as Appendix A) and STILLMEADOW, Inc. SOPs. There were no deviations from the protocol that affected the quality or outcome of the study. The protocol, raw data, this report, any amendment(s) and a sample of test substance are archived at STILLMEADOW, Inc. for 15 years. The study was initiated on 01 Apr 22 was conducted from 20 - 22 Apr 22.

SPONSOR INFORMATION

Company Name:	Black Bird Potentials Inc.
Address:	3505 Yucca Drive, Suite 104 Flower Mound, TX 75028

TEST SUBSTANCE

Label Identification:	MITEXSTREAM RTU
Date and Quantity Received:	08 Mar 22; 130.6 g and 117.5 g (GW)
Physical Description:	Slightly yellow
Storage Conditions:	Room temperature
Purity & Composition:	0.23% geraniol and 0.21% citronellol per Sponsor provided label
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. A copy of the Sponsor provided label is included as report Appendix B.

CONTROL SUBSTANCES

Vehicle control:	Deionized water with 1 mL of Polysorbate 80 Polysorbate 80 (Mfr: Sigma-Aldrich, Lot: BCCF2192, Exp: Jan 2023) A copy of the manufacturer's Certificate of Analysis for the polysorbate 80 is included as report Appendix C.
Positive control (toxic standard):	Dimethoate (0.01, 0.1 and 1.0 µg/bee) CAS# 60-51-5 (Mfr: Chem Service, Lot: 12567700, Exp: 31 May 2024) A copy of the manufacturer's Certificate of Analysis for the positive control is included as report Appendix D.

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 and Age:	600 bees (20 bees per replicate; 5 replicates per treatment group) Young adult worker bees
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:	31°C / 63 - 72%
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: SLCL2154, Exp: Apr 2027)

No contaminants were expected to have been present that would have interfered with or affected the results of the study.

PROCEDURES

Preparation of Dosing Solutions

The vehicle was prepared by mixing 500 mL of deionized water with 1 mL of Polysorbate 80.

The test substance dosing solution was prepared per Sponsor provided label instructions. The label instructions stated 1 oz of test substance should be diluted with 127 oz of water. One hundred milliliters of the test substance was prepared for the dosing solution by bringing 0.79 mL of the test substance to volume with 100 mL of the vehicle. The solution was then placed on a stir plate.

The positive control (dimethoate) solution at 1.0 µg/bee was prepared by mixing 0.005 g of dimethoate with 10 mL of deionized water. This solution was then serially diluted to prepare the 0.1 and 0.01 µg/bee positive control dosing solutions. All solutions were dosed at 2 µL per bee.

Test Substance and Control Administration

A limit test with a dose rate of 1 oz/gallon was conducted with the test substance administered in the vehicle. On day 0, the bees in the holding chambers were immobilized using CO_2, then randomly divided into six groups, each group consisting of 100 bees placed in five 16-oz. paper cups (20 bees per cup). A single dose of the test substance, the vehicle control (water with Polysorbate 80) or the positive control (dimethoate at 0.01, 0.1 or 1.0 µg/bee) was applied to each bee's thorax via a microapplicator, the bees being dosed topically on the dorsal side of their thorax with 2 µL of the appropriate solution. An untreated control was conducted concurrently.

PROCEDURES (cont.)

Observations

All bees were observed at ~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.

LD_{50} for Positive Control

The LD_{50} for dimethoate was determined by Rosiello, Essignmann and Wogan: Rapid and Accurate Determination of the Median Lethal Dose and its Error with a Small Computer, Journal Toxic Environ Health, 797-809, 1977 Computed on Microsoft Office 97 Visual Basic copyright 1997. A printout of the LD_{50} analysis is presented in Appendix E.

Statistical Analysis

Statistical analysis of mortality at ~4, 24 and 48 hours in the treatment groups compared to the untreated control group could not be conducted due to all groups, except for the 1.0 µg/bee dimethoate group, having a standard deviation of 0. Results were also evaluated by comparing percent mortality using the following formula:

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

Evaluation of Results

Results were evaluated by comparing mortality between the treated and untreated control groups. For the test to be valid, no more than 20% of the bees in the untreated or vehicle control groups can be dead at the end of the test.

RESULTS AND DISCUSSION

LD_{50} for Positive Control

The estimated LD_{50} for dimethoate at 24 hours was 1.194 µg/bee with 95% confidence levels of 0.9819 - 1.4543 µg/bee.

Mortality, Observations and Percent Mortality

Percent mortality at 48 hours in the untreated control, vehicle control, test substance, and two of the positive control groups (0.01 and 0.1 µg/bee of dimethoate) was 0%. Percent mortality at 48 hours in the 1.0 µg/bee of dimethoate positive control group was 43% (Table 1). Three bees in the 1.0 µg/bee of dimethoate positive control group were found moribund at hour 4.

With a mortality of 0% after 48 hours, the LD_{50} for the test substance, MITEXSTREAM, is considered to be greater than the nominal dose rate of 1 oz/gallon with a dose of 2 µL per bee and was non-toxic when administered by contact to honey bees.

Table 1 - Cumulative Mean and Percent Mortality Summary

Group [b]	Mean Dead[a] / % Mortality		
	4 Hours	24 Hours	48 Hours
Untreated Control	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
Vehicle Control	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
MITEXSTREAM	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
Dimethoate 0.01 µg/bee	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
Dimethoate 0.1 µg/bee	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
Dimethoate 1.0 µg/bee	2.0 / 10.0	8.2 / 41.0	8.6 / 43.0

[a] Statistical analysis could not be conducted due to at least one group having a standard deviation of 0.

[b] Each group began with 100 honey bees on day 0.

CONCLUSION

In a 48-hour acute contact toxicity study, honey bees, *Apis mellifera,* were exposed to the test substance, MITEXSTREAM, by direct topical application to their thorax at a dose rate of 1 oz/gallon with a dose of 2 µL per bee.

The estimated Median Lethal Dose (LD_{50}) for dimethoate at 24 hours was 1.194 µg/bee with 95% confidence levels of 0.9819 - 1.4543 µg/bee.

Percent mortality at 48 hours in the untreated control, vehicle control, test substance, and two of the positive control groups (0.01 and 0.1 µg/bee of dimethoate) was 0%. Percent mortality at 48 hours in the 1.0 µg/bee of dimethoate positive control group was 43%. With a mortality of 0% after 48 hours, the LD_{50} for the test substance, MITEXSTREAM, is considered to be greater than the nominal dose rate of 1 oz/gallon with a dose of 2 µL per bee and was non-toxic when administered by contact to honey bees.

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

13 May 22
Date

STUDY PERSONNEL

Technical Staff
Stephen Balestrier, BS
Hugo Martinez

Technical Writer
Monica Dunn, BS

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

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%**	All NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
Total % Mortality	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**				

VEHICLE CONTROL - Deionized Water and Polysorbate 80

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%**	All NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
Total % Mortality	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**				

MITEXSTREAM

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%**	All NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
Total % Mortality	**0.0%**	**0.0%**	**0.0%**	**0.0%**	**0.0%**				

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: MITEXSTREAM

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%**	All NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	All 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%**	All NOA
24 Hours	0	0	0	0	0	0.0	0	**0.0%**	All NOA
48 Hours	0	0	0	0	0	0.0	0	**0.0%**	All 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	0	3	1	2	4	2.0	10	**10.0%**	1 moribund (Cups 2, 4 and 5), Rest NOA
24 Hours	8	9	8	7	9	8.2	41	**41.0%**	Rest NOA
48 Hours	9	10	8	7	9	8.6	43	**43.0%**	Rest NOA
Total % Mortality	**45.0%**	**50.0%**	**40.0%**	**35.0%**	**45.0%**				

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 - Signed Protocol

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PROTOCOL FOR STUDY 25413-22

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

Test Substance: MITEXSTREAM

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

Approved: _____ 01 Apr 22
Cole Younger, PhD
Study Director
STILLMEADOW, Inc.
Date

Sponsor: Black Bird Potentials Inc.
3505 Yucca Drive, Suite 104
Flower Mound, TX 75028
940-367-6154
eric@newlanpllc.com

Attention: Eric Newlan
Black Bird Potentials Inc.
2201 Long Prairie Road, Suite 107-762
Flower Mound, TX 75022

Approved: _____ 4/1/22
Eric Newlan
Vice President
Date

Appendix A - Signed Protocol (cont.)

PROTOCOL FOR STUDY 25413-22

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 the spirit of compliance with Good Laboratory Practice Standards: All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOPs).
5.	Test Substance:	MITEXSTREAM. 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.
6.	Positive Control Substance:	Dimethoate (CAS# 60-51-5).
7.	Proposed Schedule:	Proposed Experimental Start Date: 25 Apr 22 Proposed Experimental End Date: 30 May 22
8.	Study Director:	Cole Younger, PhD
9.	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 at a dose rate of 1 oz/gallon with a dose of 2 µL per 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.
10.	Protocol Amendments:	Any alteration in the Protocol will be justified, approved by the Study Director and recorded in writing.
11.	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.

Appendix A - Signed Protocol (cont.)

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.

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 with a dose rate of 1 oz/gallon will be conducted with the test substance being administered with the appropriate vehicle as a single dose level of 2 µL per bee to five replicates of 20 bees.

Appendix A - Signed Protocol (cont.)

B. EXPERIMENTAL DESIGN (cont.)

4. Vehicle Selection: Acetone is the preferred vehicle, although other organic solvents may be used, if needed. If the test substance is not soluble in a suitable organic solvent, then deionized (DI) water may be used as the vehicle. 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 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.

   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 1 oz/gallon
      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.

Appendix A - Signed Protocol (cont.)

B. EXPERIMENTAL DESIGN (cont.)

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.

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 - Signed Protocol (cont.)

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

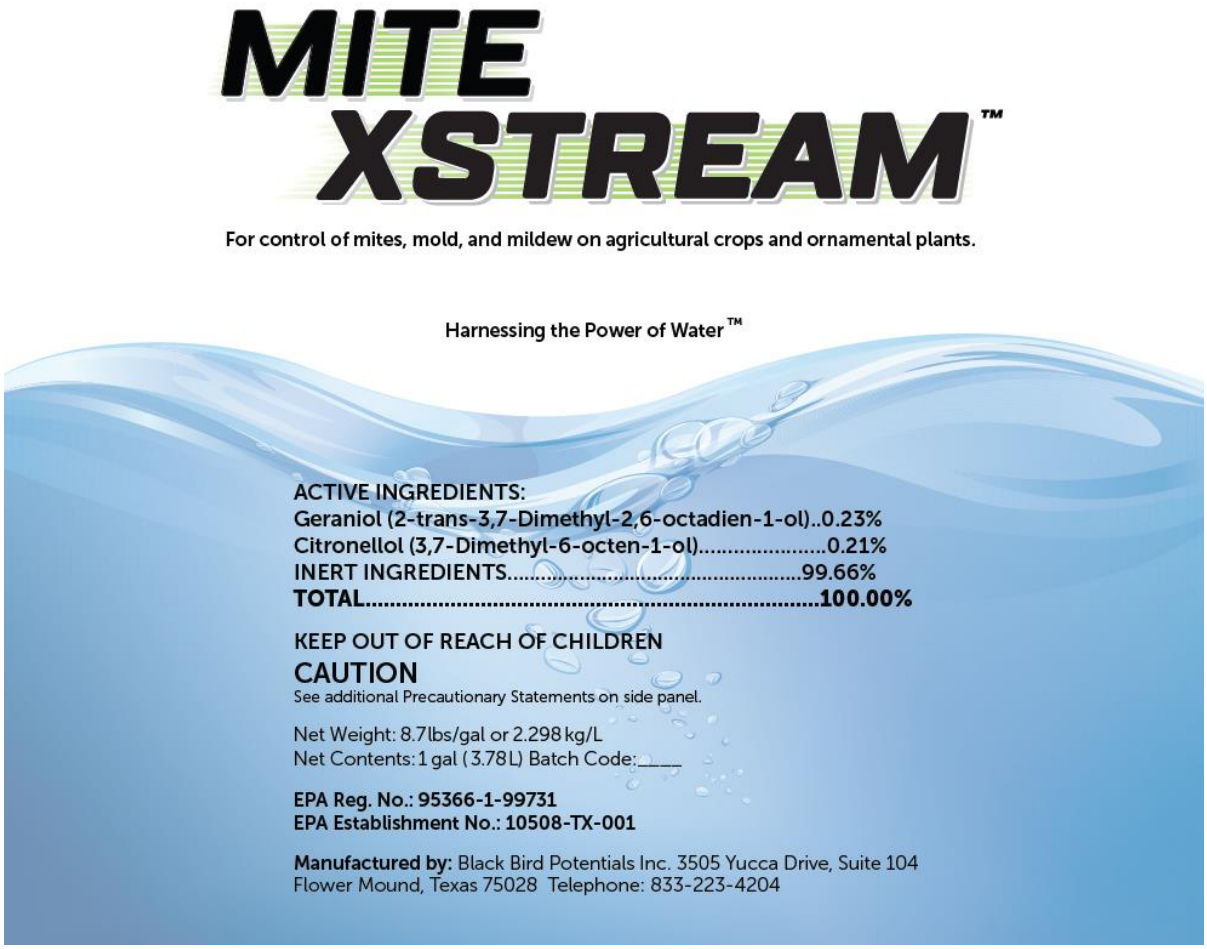
2. Data Storage: All raw data, original protocol, original final report, any amendment(s), and a retained test substance sample will be archived at STILLMEADOW, Inc. for a period of 15 years.

3. Data Reporting: The final report will include:

   a. Signature of the Study Director
   b. Names of scientific personnel involved in the study
   c. Dates of study initiation and termination
   d. Identification, label information, description, preparation and storage of the test substance and vehicle information.
   e. All pertinent honey bee information and observation methods
   f. Description of the test procedures
   g. Daily observations for mortality and clinical signs of toxicity
   h. Mortality and sublethal effects percentage calculations
   i. Dose response curve, slope and LD_{50}, as applicable and if possible
   j. A copy of this Protocol
   k. 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 – Sponsor Provided Label



Appendix B - Sponsor Provided Label (cont.)

GENERAL INFORMATION

MITEXSTREAM is biochemical miticide that controls gray mold, powdery mildew, downy mildew and mites, Eotetranychus spp.,Tetranychus spp. and Panonychus spp.,including spider mite, two-spotted mites, pacific mite, willamette mite, citrus rust mite, broad mite and the European red mite. This product is ideal for mite control in integrated pest management (IPM). Use **MITEXSTREAM** alone or in rotation with other miticides.

READ ALL DIRECTIONS FOR USE BEFORE APPLYING THIS PRODUCT.

DIRECTIONS FOR USE:
It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during applications. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CRF Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted-entry interval, and notification to workers.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 12 hours. The REI is 72 hours in outdoor areas where average annual rainfall is less than 25 inches a year.

PPE required for early entry to treated areas (that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water), is:

- coveralls over long-sleeved shirt and long pants
- shoes plus socks
- protective eyewear (goggles, face shield or safety glasses)
- chemical-resistant gloves (made of waterproof material)

Notify workers of the application by warning them orally and by posting warning signs at entrances to treated areas.

NON-AGRICULTURAL USE REQUIREMENTS

The requirements in this box apply to uses of this product that are NOT within the scope of the Worker Protection Standard for agricultural pesticides (40 CFR Part 170). The WPS applies when this product is used to produce agricultural plants on farms, forests, nurseries, or greenhouses.

Keep children and pets out of the treated area until sprays have dried.

MIXING INSTRUCTIONS:
MITEXSTREAM has been found to be compatible with most commonly used pesticides and fertilizers. Test for compatibility before using this product in a tank mix with other pesticides or with fertilizers. To test for compatibility, mix a small amount of each product, in the proportions indicated in each product's instructions, in a small jar.

USE INSTRUCTIONS:

- Do not apply with surfactants.
- Do not apply this product through any type of irrigation system.

USE RECOMMENDATIONS:

- **MITEXSTREAM** has been evaluated for phytotoxicity on a wide range of crops and ornamentals. However, since testing on all varieties of all crops and ornamentals is not feasible, test a small portion of the area to be treated for phytotoxicity before treating the entire area. Further, all possible combinations or sequences of pesticide sprays, including fertilizers, surfactants, adjuvants and other pesticides, have not been tested, thus testing for phytotoxicity of spray mixtures is recommended.
- **MITEXSTREAM** is effective in all temperatures above freezing (32°F, 0°C)
- **MITEXSTREAM** is most effective on low to moderate infestations.

USE RATES:
Add **MITEXSTREAM** to an empty spray tank followed by water to the required amount. Stir/agitate the mixture thoroughly. Thorough mixing is necessary for uniform coverage. Apply **MITEXSTREAM** as soon mites are identified on the plants, or when conditions favor mite outbreaks and/or mold and mildew outbreaks.

Appendix B - Sponsor Provided Label (cont.)

CROPS	DILUTION RATE	SPRAY VOLUME (gallons per acre)
Hemp	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Hops	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Strawberries	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Coffee	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Soybeans	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Cucurbits: balsam apple, balsam pear, bitter melon, butternut squash, calabaza, cantaloupe, casaba, chayote, chinese cucumber, chinese waxgourd, citron melon, crenshaw melon, crookneck squash, cucumber, gherkin, edible gourd, golden pershaw melon, honeydew melon, honey balls, hubbard sqhash, mango melon, Persian melon, pineapple melon, pumpkin, Santa Claus melon, scallop squash, straightneck squash, snake melon, vegetable marrow, watermelon, zucchini	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate

Appendix B - Sponsor Provided Label (cont.)

CROPS	DILUTION RATE	SPRAY VOLUME (gallons per acre)
Stone Fruit: apricot, cherry (sweet or tart), nectarine, peach, plum, plumcot, prune (fresh)	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Pome Fruit: apple, crabapple, loquat, mayhaw, pear, quince	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate
Ornamental Plants and Nursery Stock: bareroot, container, bedding and flowering stock, field grown cut flowers, vegetable transplants, nursery and landscape plants	1 fl. oz. / 1 Gal (1 Gal / 128 Gal)	0.5 – 2 Gal concentrate per acre at proper dilution rate

If in eyes:	FIRST AID
	• Hold eye open and rinse slowly and gently with water for 15-20 minutes. • Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. • Call a poison control center or doctor for treatment advice.

HOTLINE NUMBER

- Have product container or label with you when calling a poison control center or doctor or going for treatment.
- For medical emergencies, call the poison control center at 1-800-222-1222.
- For emergency information concerning this product, contact the National Pesticide Information Center (NPIC) at h800-858-7378, Monday through Friday, 8 AM to 12 PM PST or at http://npic.orst.edu.

Appendix B - Sponsor Provided Label (cont.)

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS

CAUTION. Causes moderate eye irritation. Avoid contact with eyes or clothing. Wear protective eyewear (goggles, face shield or safety glasses). Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco, or using the toilet.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers whom may be exposed to the dilute through application or other tasks must wear long-sleeved shirts and long pants, socks, shoes and protective eyewear (goggles, face shield or safety glasses). Follow the manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

USER SAFETY RECOMMENDATIONS

Users should:

- remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing

ENVIRONMENTAL HAZARDS

For terrestrial uses: do not apply directly to water, or to areas where the surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment washwaters or rinsate.

Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage and disposal.
STORAGE: Store in a cool place and out of direct sunlight.
PESTICIDE DISPOSAL: To avoid wastes, use all of the material in this container by application according to label directions. If waste cannot be avoided, offer remaining product to a waste disposal facility or pesticide disposal program (often such programs are run by state or local governments or by industry).

CONTAINER HANDLING: Nonrefillable container. Do not reuse or refill this container. Clean container promptly after emptying. Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow beings to drip. Triple Rinse as follows: Fill container ¼ full with water and recap. For 5 gallons or less guidelines: Shake for 10 seconds. Drain for 10 seconds after the flow begins to drip. Follow Pesticide Disposal instructions for rinsate. Repeat procedure two more times.

WARRANTY STATEMENT

This material conforms to the description on the label and is reasonably fit for the purposes referred to in the directions for use. Timing, unfavorable temperatures, water conditions, presence of other materials, method of application, weather, watering practices, nature of soil, disease, problems, condition of the crop, incompatibility with other chemicals, pre-existing conditions and other conditions influencing the use of this product are beyond the control of the seller. To the extent consistent with applicable law, buyer assumes all risks associated with the use, storage and handling of this material not in strict accordance with the directions given herein.

TO THE EXTENT CONSISTENT WITH APPLICABLE LAW, NO OTHER EXPRESS OR IMPLIED WARRANTY OF FITNESS OR MERCHANTABILITY IS MADE.

Appendix C - Polysorbate 80 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

Study # 25413-22/B-118

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 %

printed: 08/01/MAY 22

Appendix C - Polysorbate 80 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 D - 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

25413-22
exact copy 08/26/22

CERTIFICATE OF ANALYSIS

Dimethoate

CATALOG NUMBER	N-11758-100MG
LOT NUMBER	12567700
DATE CERTIFIED	05/18/21
EXPIRATION DATE	05/31/24
CAS NUMBER	60-51-5
MOLECULAR FORMULA	C5H12NO3PS2
MOLECULAR WEIGHT	229.27
STORAGE	Refrigerator storage (2 - 8 °C)
HANDLING	See Safety Data Sheet
INTENDED USE	For laboratory use only.

Analytical Test	Value
FT-IR SPECTROSCOPY	CONFORMS TO STRUCTURE
% PURITY (HPLC)	99.5

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

Chem Service, Inc. is accredited to ISO 17034:2016, ISO/IEC 17025:2017 and certified to ISO 9001:2015

COA Form
Revision 3 (3/2015)

Print Date: 11/01/21

Appendix E - LD_{50} Analysis for Positive Control

24 Hours

LD_{50} Analysis*
Honey Bee, *Apis mellifera,* Acute Contact Toxicity Limit Test
Study Number: 25413-22
Positive Control: Dimethoate

24 Hours

Dose Level µg/bee	Number Dead	Number Treated	Mortality
0.01	0.25	100	0%
0.1	0.25	100	0%
1.00	41	100	41%

Data was transformed according to Ghosh. In statistical analysis. Fundamentals of Experimental Pharmacology, 2nd edition, Scientific Book Agency Calcutta,1984,187-9. "…..the corrected % formula for 0 and 100% mortality; For 0% dead: 100(0.25/n), For 100% dead: 100((n-0.25)/n), Where n is the number of organisms/animals per group used in the experiment."

LD_1 0.195 µg/bee
LD_5 0.331 µg/bee
LD_{10} 0.438 µg/bee
LD_{16} 0.550 µg/bee

95% lower confidence limit: 0.9819 µg/bee
LD_{50} 1.194 µg/bee
95% upper confidence limit: 1.4543 µg/bee

LD_{84} 2.5917 µg/bee
LD_{90} 3.2517 µg/bee
LD_{95} 4.3053 µg/bee
LD_{99} 7.3211 µg/bee

Slope function (s) = 2.18 with 95% confidence limits of 2.263 to 2.101.
Variance of Slope = 1.28

Calculated X^2 = 8.464 with 1 degrees of freedom.
Values for P = 0.5 T = 12.7 X^2 = 3.84
*Rosiello, Essignmann and Wogan: Rapid and Accurate Determination of the Median Lethal Dose and its Error with a Small Computer, Journal Toxic Environ Health, 797-809, 1977
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original: 02May22 MD calc check: bb 09May22

review: 09May22