STILLMEADOW
INCORPORATED

TITLE

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

TEST GUIDELINE

OECD 213

AUTHOR

Cole Younger, PhD

STUDY COMPLETION DATE

25 May 2022

PERFORMING LABORATORY

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

LABORATORY STUDY ID

25414-22

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: 25 May 22
Cole Younger, PhD
STILLMEADOW, Inc.

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SUMMARY

This study was designed to assess the acute oral toxicity potential of the test substance, MITEXSTREAM, when administered in the diet of the adult worker honey bee, *Apis mellifera,* at a dose rate of 1 oz/gallon. The test substance and toxic standard were administered orally in a 50% sucrose solution to honey bees for four hours, with a control group fed only sucrose solution. Observations for mortality and clinical signs of toxicity were made at approximately 4, 24 and 48 hours after dosing in the control group, test group, and positive control groups (0.01, 0.1 and 1.0 µg/bee dimethoate).

The estimated Median Lethal Dose (LD_{50}) for dimethoate at 24 hours was 0.032 µg/bee.

At 48 hours, mortality for the control group, test group, and positive control groups (0.01, 0.1 and 1.0 µg/bee dimethoate), was 2%, 0%, 0%, 92% and 100%, respectively. Three bees in the test group and 14 bees in the positive control groups were moribund at hour 4. The control and test groups consumed an average of 0.37 g and 0.29 g per replicate, respectively, from the 100 µL offered of the appropriate substance during the study. There were no significant differences in food consumption between the control group and the treated groups. Based on these results, the LD_{50} of the test substance, MITEXSTREAM, is considered greater than the dose rate of 1 oz/gallon and was non-toxic to honey bees.

INTRODUCTION

The objective of this study was to assess the acute oral toxicity potential of the test substance, MITEXSTREAM, when administered orally to adult worker honey bees as part of their diet. The 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 original protocol, raw data, this report, any amendment(s), and a sample of the test substance are archived at STILLMEADOW, Inc., for 15 years. The study was initiated on 01 Apr 22, and the laboratory portion of the study was conducted from 25 - 27 Apr 2022.

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 2022; 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 SUBSTANCE

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)

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 and Age on Day 0:	500 bees (100 bees per group in five replicates of 20 bees each); Young adult worker bees
Identification:	Numbered cups with treatment identification
Acclimation:	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
Housing:	16-ounce cardboard cup with screen lid
Environmental Controls Set to Maintain:	· Incubator temperature at times of observation: 33 ± 5°C · Incubator relative humidity at times of observation: 50 - 70% · Lighting dark except when dosed or observations were made
Measured Incubator Temperature and Relative Humidity:	32°C / 53 - 63%
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 / Source:	50:50 w/v sucrose: dechlorinated (DC) water solution fed *ad libitum* after dosing period (Sucrose: Mfr: Sigma-Aldrich, 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 Test Bees

Bees were fasted for approximately two hours before dosing.

Preparation of Test Substance

The test substance dosing solution was prepared per Sponsor provided label instructions. The label instructions stated the dilution rate was 1 oz/gallon. One hundred milliliters of the test substance was prepared for the dosing solution by mixing 0.79 mL of the test substance with 99.21 mL of 50% sucrose solution. The solution was stirred for approximately 5 minutes.

Preparation of Sucrose Solution

The 50% sucrose solution was prepared by dissolving 500 g of sucrose in 500 mL of sterile DC water. Solution was prepared in the same manner as needed to be provided *ad libitum*.

Preparation of Positive Control

On day 0, three dose levels (0.01, 0.1, and 1.0 µg/bee) of the toxic standard, dimethoate, were prepared by dissolving 0.002 g of dimethoate in 10 mL 50% sucrose solution to make a 0.2 mg/mL solution, which was serially diluted (1:10) to make 0.02 mg/mL and 0.002 mg/mL solutions. The positive control solutions were dosed at 100 µL per 20 bees.

Test and Control Substance Administration

On day 0, the honey bees were immobilized using CO_2, randomly taken from the test container and divided into five groups with five cups of 20 bees in each group. Group I was provided only the 50% sucrose solution and served as the untreated control group. Bees in Group II were given the test substance at 100 µL per cup. Bees in Groups III - V were dosed with the toxic standard, dimethoate, at 0.01, 0.1, and 1.0 µg/bee, respectively, and served as the positive control.

For dosing, a micropipette tip containing 100 µL of the appropriate treatment was inserted in a hole in the screen of each container. The tops of the micropipette tips were covered with parafilm to prevent evaporation. The tips were weighed prior to dosing. After four hours, the micropipettes were removed and reweighed to determine the amount consumed, and the honey bees were fed 50% sucrose *ad libitum* for the remainder of the study.

PROCEDURES (cont.)

Observations

Bee Groups I - V 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.). Any dead bees were not removed from the paper cup until the end of the study, and all bees were frozen and disposed of at termination of study. Relative humidity and temperature were recorded at the first daily 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.

Statistical Analysis

Statistical analysis of food consumption in the treated groups compared to the control group was performed using a one-way parametric analysis of variance (ANOVA), with Dunnett's Multiple Comparisons Post Test if significance was indicated ($p < 0.05$). The analysis was calculated by GraphPad InStat version 3.10 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com. Statistical analysis of mortality between the test and control groups could not be performed because the test group's standard deviation was zero.

Evaluation of Results

If test group mortality did not exceed control mortality and not more than 10% of control bees died during the test, the LD_{50} was considered greater than the dose rate of 1 oz/gallon.

RESULTS AND DISCUSSION

Control, Test substance and Toxic Standard Consumption

The amount of test substance, sucrose solution or toxic standard consumed during the 4-hour dosing period by the bees in each cup is presented in Table 3 and summarized in Table 1. The control and test groups consumed an average of 0.37 g and 0.29 g per replicate, respectively, from the 100 µL offered of the appropriate substance during the study. The toxic standard groups, at 0.01, 0.1 and 1.0 µg/bee, consumed an average of 0.43 g, 0.37 g and 0.28 g per replicate, respectively, from 100 µL of toxic standard offered. There were no significant differences in food consumption between the control group and the treated groups.

Table 1 - Honey Bee Mean Food Consumption Summary

Group	Mean Food Consumption per Cup[a]
	4 Hours Post-Dose
Control	0.3719 a
MITEXSTREAM	0.2870 a
Dimethoate 0.01 µg/bee	0.4282 a
Dimethoate 0.1 µg/bee	0.3694 a
Dimethoate 1.0 µg/bee	0.2793 a
p value	*0.2458*

[a] Different letters within the same column indicate significance compared to control at $p < 0.05$.

LD_{50} for Positive Control

The estimated Median Lethal Dose (LD_{50}) for dimethoate at 24 hours was 0.032 µg/bee. 95% confidence levels were unable to be obtained. A printout of the LD_{50} analysis is included as Appendix D.

RESULTS AND DISCUSSION (cont.)

Clinical Signs of Toxicity, Mortality Observations and Evaluation

Clinical signs of toxicity and mortality observations are presented in Table 4. Statistical comparisons could not be performed as the test group's standard deviation was zero.

At 48 hours, mortality in the control and test groups was 2% and 0%, respectively (Table 2). Three bees in the test group and 14 bees in the positive control groups were moribund at hour 4. Based on these results, the LD_{50} of the test substance, MITEXSTREAM, is considered greater than the dose rate of 1 oz/gallon.

Table 2 - Cumulative Mean and Percent Mortality Summary

Group[a]	Mean Dead / % Mortality Time Post-Dose		
	4 Hours	**24 Hours**	**48 Hours**
Control	0.0 / 0.0	0.0 / 0.0	0.4 / 2.0
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	2.8 / 14.0	18.4 / 92.0	18.4 / 92.0
Dimethoate 1.0 µg/bee	19.6 / 98.0	20.0 / 100.0	20.0 / 100.0

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

CONCLUSION

This study was designed to assess the acute oral toxicity potential of the test substance, MITEXSTREAM, when administered in the diet of the adult worker honey bee, *Apis mellifera,* at a dose rate of 1 oz/gallon.

The estimated Median Lethal Dose (LD_{50}) for dimethoate at 24 hours was 0.032 µg/bee.

At 48 hours, mortality for the control group, test group, and positive control groups (0.01, 0.1 and 1.0 µg/bee dimethoate), was 2%, 0%, 0%, 92% and 100%, respectively. Three bees in the test group and 14 bees in the positive control groups were moribund at hour 4. The control and test groups consumed an average of 0.37 g and 0.29 g per replicate, respectively, from the 100 µL offered of the appropriate substance during the study. There were no significant differences in food consumption between the control group and the treated groups. Based on these results, the LD_{50} of the test substance, MITEXSTREAM, is considered greater than the dose rate of 1 oz/gallon and was non-toxic to honey bees.

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

25 May 22
Date

STUDY PERSONNEL

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

Technical Writer
Monica Dunn, BS

Table 3 - Food Consumption
Honey Bee, *Apis mellifera,* Acute Oral Toxicity Limit Test
Test Substance: MITEXSTREAM

VEHICLE CONTROL

Cup	Amount of Solution (g)		
	Day 0	**4 Hours**	***0-4 Hours***
	Given	Remaining	*Consumed*
1	1.5166	1.0866	*0.4300*
2	1.4669	1.0424	*0.4245*
3	1.4650	1.3290	*0.1360*
4	1.4852	1.0494	*0.4358*
5	1.4977	1.0646	*0.4331*
		Mean per cup	***0.3719***
		SD	***0.1319***
		SE	***0.0590***

MITEXSTREAM

Cup	Amount of Solution (g)		
	Day 0	**4 Hours**	***0-4 Hours***
	Given	Remaining	*Consumed*
1	1.4733	1.0611	*0.4122*
2	1.4934	1.0633	*0.4301*
3	1.4780	1.3814	*0.0966*
4	1.4709	1.3775	*0.0934*
5	1.4641	1.0616	*0.4025*
		Mean per cup	***0.2870***
		SD	***0.1755***
		SE	***0.0785***

DIMETHOATE 0.01 µg/bee

Cup	Amount of Solution (g)		
	Day 0	**4 Hours**	***0-4 Hours***
	Given	Remaining	*Consumed*
1	1.4638	1.0440	*0.4198*
2	1.5154	1.0853	*0.4301*
3	1.5198	1.0826	*0.4372*
4	1.4539	1.0257	*0.4282*
5	1.4435	1.0178	*0.4257*
		Mean per cup	***0.4282***
		SD	***0.0064***
		SE	***0.0028***

DIMETHOATE 0.1 µg/bee

Cup	Amount of Solution (g)		
	Day 0	**4 Hours**	***0-4 Hours***
	Given	Remaining	*Consumed*
1	1.5115	1.3221	*0.1894*
2	1.5170	1.1309	*0.3861*
3	1.5350	1.1098	*0.4252*
4	1.5284	1.1017	*0.4267*
5	1.5319	1.1121	*0.4198*
		Mean per cup	***0.3694***
		SD	***0.1020***
		SE	***0.0456***

DIMETHOATE 1.0 µg/bee

Cup	Amount of Solution (g)		
	Day 0	**4 Hours**	***0-4 Hours***
	Given	Remaining	*Consumed*
1	1.5295	1.3419	*0.1876*
2	1.5860	1.3750	*0.2110*
3	1.5308	1.1866	*0.3442*
4	1.5408	1.2924	*0.2484*
5	1.4829	1.0776	*0.4053*
		Mean per cup	***0.2793***
		SD	***0.0924***
		SE	***0.0413***

Note: Beginning number of bees on day 0: 20 per container (100 bees per group)

Table 4 - Mortality, Observations and Percent Mortality
Honey Bee, *Apis mellifera,* Acute Oral Toxicity Limit Test
Test Substance: MITEXSTREAM

Group I - Vehicle Control			
Cup	Time Post Dose (Hours)		
	4	24	48
1	0	0	0
2	0	0	0
3	0	0	1
4	0	0	0
5	0	0	1
Mean	**0.0**	**0.0**	**0.4**
SD	**0.0**	**0.0**	**0.5**
SE	**0.0**	**0.0**	**0.2**
Total Dead	**0**	**0**	**2**
Percent Mortality	**0.0**	**0.0**	**2.0**
Total Live	**100**	**100**	**98**
Observations	**NOA**	**NOA**	**Rest NOA**

Group II - MITEXSTREAM			
Cup	Time Post Dose (Hours)		
	4	24	48
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
Mean	**0.0**	**0.0**	**0.0**
SD	**0.0**	**0.0**	**0.0**
SE	**0.0**	**0.0**	**0.0**
Total Dead	**0**	**0**	**0**
Percent Mortality	**0.0**	**0.0**	**0.0**
Total Live	**100**	**100**	**100**
Observations	**1 moribund (Cup 3), 2 moribund (Cup 4)**	**NOA**	**NOA**

Note: Digits indicate number of dead bees per cup unless otherwise noted. Mortality is cumulative.
Beginning number of bees on Day 0: 20 per cup (100 bees per group).
SD, standard deviation; SE, standard error; NOA, no observable abnormalities

Table 4 - Mortality, Observations and Percent Mortality (cont.)
Honey Bee, *Apis mellifera,* Acute Oral Toxicity Limit Test
Test Substance: MITEXSTREAM

Group III - Dimethoate 0.01 µg/bee			
Cup	**Time Post Dose (Hours)**		
	4	**24**	**48**
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
Mean	**0.0**	**0.0**	**0.0**
SD	**0.0**	**0.0**	**0.0**
SE	**0.0**	**0.0**	**0.0**
Total Dead	**0**	**0**	**0**
Percent Mortality	**0.0**	**0.0**	**0.0**
Total Live	**100**	**100**	**100**
Observations	**NOA**	**NOA**	**NOA**

Group IV - Dimethoate 0.1 µg/bee			
Cup	**Time Post Dose (Hours)**		
	4	**24**	**48**
1	2	12	12
2	3	20	20
3	0	20	20
4	6	20	20
5	3	20	20
Mean	**2.8**	**18.4**	**18.4**
SD	**2.2**	**3.6**	**3.6**
SE	**1.0**	**1.6**	**1.6**
Total Dead	**14**	**92**	**92**
Percent Mortality	**14.0**	**92.0**	**92.0**
Total Live	**86**	**8**	**8**
Observations	**2 moribund (Cups 1 & 4), 5 moribund (Cup 3), 6 moribund (Cup 5)**	**Rest NOA**	**Rest NOA**

Note: Digits indicate number of dead bees per cup unless otherwise noted. Mortality is cumulative.
Beginning number of bees on Day 0: 20 per cup (100 bees per group).
SD, standard deviation; SE, standard error; NOA, no observable abnormalities

Table 4 - Mortality, Observations and Percent Mortality (cont.)
Honey Bee, *Apis mellifera,* Acute Oral Toxicity Limit Test
Test Substance: MITEXSTREAM

Group V - Dimethoate 1.0 µg/bee		
Cup	**Time Post Dose (Hours)**	
	4	**24**
1	20	20
2	20	20
3	20	20
4	18	20
5	20	20
Mean	**19.6**	**20.0**
SD	**0.9**	**0.0**
SE	**0.4**	**0.0**
Total Dead	**98**	**100**
Percent Mortality	**98.0**	**100.0**
Total Live	**2**	**0**
Observations	**1 moribund (Cup 4), Rest NOA**	**All Found Dead**

Note: Digits indicate number of dead bees per cup unless otherwise noted. Mortality is cumulative.
Beginning number of bees on Day 0: 20 per cup (100 bees per group).
SD, standard deviation; SE, standard error; NOA, no observable abnormalities

Appendix A - Signed Protocol

STILLMEADOW
INCORPORATED

PROTOCOL FOR STUDY 25414-22

Study Title: Honey Bee, *Apis mellifera*, Acute Oral Toxicity Limit Test (OECD 213)

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

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

Appendix A - Signed Protocol (cont.)

PROTOCOL FOR STUDY 25414-22

A. GENERAL

1.	Study Title:	Honey Bee, *Apis mellifera,* Acute Oral Toxicity Limit Test
2.	Purpose:	To assess the acute oral toxicity potential of the test substance when administered to adult worker honey bees.
3.	Methods Guidelines:	The study will be conducted according to OECD Guideline 213.
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 purity, stability, identity, synthesis methods and location of documentation.
6.	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:	The test substance, and serial dilution concentrations of the toxic standard will be administered orally to honey bees in a 50% sucrose solution for four hours and the amount consumed will be recorded. A group of bees will be fed the 50% sucrose solution alone for comparison. 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.
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*, worker; STILLMEADOW, Inc. bee colony, 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:	500 bees; 100 bees per group in five replicates of 20 bees each. Five groups: Control, Test and three toxic standard levels.
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 bee hive
b. Indoor Chambers:	Disposable cardboard containers with a screened lid
c. Food:	50:50 w/v sucrose:dechlorinated (DC) water solution; available *ad libitum* after test dose is removed.
d. Environment:	Incubator temperature: 33 ± 5°C. Target relative humidity: 50-70% 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.

3. Group Allocation and Randomization:	Honey bees will be immobilized once using CO_2, randomly taken from the collection container and placed in one of five groups.
4. Preparation of Test Bees:	Bees will be fasted for approximately two hours before dosing. Any bees found moribund during this time will be rejected and replaced by healthy bees fasted concurrently before starting test.

Appendix A - Signed Protocol (cont.)

B. EXPERIMENTAL DESIGN (cont.)

5. Dose Preparation: To prepare the dosing solution, 1 oz of test substance will be diluted in 127 oz of 50% sucrose solution. The solution will be stirred for at least 30 seconds.

6. Dose Level: A limit test with a dose rate of 1 oz/gallon will be conducted with the test substance being administered for four hours as a single dose level to five replicates of 20 bees each. The bees will be observed for mortality and clinical signs of toxicity at ~4, 24 and 48 hours.

7. Vehicle Selection: 50% sucrose will be used as the vehicle.

8. Test Substance Administration: On Day 0, each container of bees will be randomly assigned to either the test substance, toxic standard or control sucrose solution group. For dosing, a micropipette tip containing 100 µL of the appropriate solution will be inserted in a hole in the screen for each container. The top of the micropipette tips will be covered with parafilm to prevent evaporation. The tips will be weighed prior to dosing and at four hours to determine the amount consumed. After four hours, honey bees will be fed 50% sucrose and DC water *ad libitum*.

9. Untreated Control: An untreated control group will be conducted concurrently. The untreated sucrose solution will be administered to five replicates of 20 bees each as a single dose level equivalent to the amount given to the test group.

10. Toxic Standard: A toxic standard (positive control) will be conducted concurrently. Three dose levels (0.01, 0.1, and 1.0 µg/bee) of the toxic standard, dimethoate, will be administered to five replicates of at least 20 bees. Toxic standard groups will receive the dimethoate in the same manner as test and controls.

11. 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 mortality in the test group is greater than 10% at 48 hours and the untreated control mortality is ≤10%, observations will be extended to 72 and 96 hours. Any dead bees will not be removed until the end of the study and all bees will be frozen and disposed of at the end of the study. Relative humidity and temperature will be recorded at the first daily observation time.

Appendix A - Signed Protocol (cont.)

B. EXPERIMENTAL DESIGN (cont.)

12. Test Validity: For the test to be considered valid, control mortality cannot exceed 10% at the end of the test.

13. Evaluation of Results: If mortality does not exceed the controls and not more than 10% of control bees die during the test, the LD_{50} will be considered greater than 1 oz/gallon.

14. 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 Teflon-lined caps.

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

16. 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, source and hive number.
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 and storage of the test substance, and identification of the vehicle used
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. A copy of this Protocol
i. 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

MITE XSTREAM™

For control of mites, mold, and mildew on agricultural crops and ornamental plants.

Harnessing the Power of Water™

ACTIVE INGREDIENTS:
Geraniol (2-trans-3,7-Dimethyl-2,6-octadien-1-ol)..0.23%
Citronellol (3,7-Dimethyl-6-octen-1-ol).....0.21%
INERT INGREDIENTS.....99.66%
TOTAL.....100.00%

KEEP OUT OF REACH OF CHILDREN

CAUTION

See additional Precautionary Statements on side panel.

Net Weight: 8.7lbs/gal or 2.298 kg/L
Net Contents: 1 gal (3.78L) Batch Code:____

EPA Reg. No.: 95366-1-99731
EPA Establishment No.: 10508-TX-001

Manufactured by: Black Bird Potentials Inc. 3505 Yucca Drive, Suite 104
Flower Mound, Texas 75028 Telephone: 833-223-4204

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 - Positive Control Certificate of Analysis

Dimethoate

25414-22/B-118
exact copy 8/27/2022

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	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.

PJLA Testing

PJLA Reference Material Producer

COA Form
Revision 3 (3/2015)

Print Date: 11/01/21

Appendix D - LD_{50} Analysis for Positive Control

Positive Control at 24 Hours

LD_{50} Analysis*
Honey Bee, *Apis mellifera,* Acute Oral Toxicity Limit Test
Study Number: 25414-22
Dimethoate

24 Hours

Dose Level µg/bee	Number Dead	Number Treated	Mortality
1	100	100	100%
0.1	92	100	92%
0.01	0	100	0%

LD_1	0.004 µg/bee
LD_5	0.007 µg/bee
LD_{10}	0.010 µg/bee
LD_{16}	0.013 µg/bee
LD_{50}	**0.032 µg/bee**
LD_{84}	0.0826 µg/bee
LD_{90}	0.1086 µg/bee
LD_{95}	0.1524 µg/bee
LD_{99}	0.2892 µg/bee

95% confidence levels were unable to be obtained.

Slope function (s) = 2.27 with 95% confidence limits of 2.164 to 2.401.
Variance of Slope = 1.21

Calculated X^2 = 2.634 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
Computed on Microsoft Office 97 Visual Basic copyright 1997

original: 10May22 MD calc check: 10May22

review: 10May22