



NON-GLP STUDY REPORT

Date: June 2017

Sponsor: Sprinkler Magician
Study: Direct Spray 17
Trial: AEDSAE
Sponsor Code: N/A
Test Method: 311

REPORT TITLE

Evaluation of the Mosquito Magician (2017 Formula) as a Direct Spray against Yellow Fever Mosquitoes (*Aedes aegypti*)

STUDY

Direct Spray 17

TRIAL

AEDSAE

SPONSOR CODE

N/A

EXPERIMENTAL START DATE

April 14, 2017

EXPERIMENTAL COMPLETION DATE

April 20, 2017

REPORT DATE

June 06, 2017

TEST FACILITY/AUTHORS

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SPONSOR

Sprinkler Magician



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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA §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: _____

Typed Name of Signer: _____

Typed Name of Company: Sprinkler Magician



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COMPLIANCE STATEMENT

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

Test Facility Management:

Eric J. Snell
Snell Scientifics, LLC.

Date: 06/06/16

Study Director:

Todd Smith
Snell Scientifics, LLC.

Date: 06/06/16

Sponsor:

Date: _____

Submitter:

Date: _____



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STUDY OBJECTIVE(S):

To evaluate the toxicity of the test substance when applied as a direct spray application against yellow fever mosquitoes (*Aedes aegypti*).

TEST SUBSTANCE INFORMATION:

#	Test Substance	Dilution Rate	Active Ingredients	EPA Reg. No. and/or Est. No.	Lot and/or Batch #	Snell Receipt Code
1	Controls - Untreated	N/A	N/A	N/A	N/A	N/A
2	Mosquito Magician 2017 Formula	4% sample:96% water (8mL:192mL water)	N/A	N/A	N/A	040317-1-A-SPR

TEST SYSTEM INFORMATION:

Trial	Test System	Strain	Stage/Age	Source
AEDSAE	Yellow Fever Mosquito (<i>Aedes aegypti</i>)	Lab	Adults Females	Purchased/Lab Reared



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MATERIALS AND METHODS:

The following is the Snell Scientifics Standardized Testing Method for evaluating the efficacy of pesticides when applied as direct spray applications against various arthropod species.

311.1 **Materials:**

Test Arena Information:

- 311.1.1 Treatment Arenas: 1.75" CPVC Mesh Cartridge (BioQuip 7250NSW mesh). The Test Arenas were used to contain the test systems during the test substance applications.
- 311.1.2 Post-Treatment Arenas: 20oz SOLO cup with mesh lid. The Post-Treatment arenas were used to contain the test systems in a clean environment after exposure to the test substance(s).
- 311.1.3 Food/Moisture: 10% sucrose soaked cotton swabs.

Test Equipment:

- 311.1.4 Volumetric Measuring Equipment: Graduated cylinders and/or beakers were used as needed in preparing and/or measuring the flow rates of the test substance(s).
- 311.1.5 Digital Balance(s): Balances were used as needed in preparing and/or weighing the test substance canisters before and after applications.
- 311.1.6 CO₂ and Regulator: A standard 20 pound CO₂ cylinder with regulator was used to anesthetize the test systems and sort them into the test arenas (prior to exposure to the test substances). The test systems were allowed to adequately recover from anesthetizing before being exposed to the test substance(s), and they were not anesthetized at any point following exposure to the test substance(s). Any additional transfers required after exposure to the test substances was conducted using methods that did not involve anesthetizing.
- 311.1.7 Intermediate Sorting/Transfer Containers: Additional sorting and transfer containers were used to aid in moving the test systems from the primary rearing/collection containers and into the treatment and/or post-treatment arenas.
- 311.1.8 Metronome/Timing Equipment: A metronome and/or other timing equipment were used as needed to assist in the timing when conducting the applications and/or when collecting the observations.

Application Equipment:

- 311.1.9 Application Equipment: Snell Sci. Trigger Sprayer.

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311.2 Methods:*Test Design:*

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

# Reps per Substance	# Systems per Rep	# Systems per Substance	# Test Substances	Total # Systems	# Test Arenas
4	10	40	2	80	8

Test Substance Preparation & Applications:

- 311.2.6 The test substance(s) were adequately shaken prior to applications.
- 311.2.7 The applications were conducted by spraying each replicate until wet using 1 pull from an approximate 12-inch distance using the mist setting that best covered the treatment area.
- 311.2.8 The average flow rate and/or pre-and post-application weights of the test substance(s) were measured during the study:

Test Substance:	Flow Rate (mL/pull)	Pre-Weight (g.)	Post-Weight (g.)	Weight Loss (g.)	Number of Sprays	Average Loss (g/spray)
Mosquito Magician 2017 Formula	1.0	235.6	230.3	5.3	5	1.1



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Observation Methods:

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

Environmental Conditions:

- 311.2.13 The test systems were tested under ambient laboratory conditions.
- 311.2.14 Average environmental conditions recorded during the study:
- 311.2.14.1 Applications: Temperature: 55°F Humidity: 80%
 - 311.2.14.2 Laboratory: Temperature: 70°F Humidity: 46%



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RESULTS / DISCUSSION:

The results of this study are shown in Table 1, which illustrates the percent mortality for the yellow fever mosquitoes (*Aedes aegypti*). In addition to the percent mortality that is shown in Table 1, the mortality rates that were recorded following the test substance applications were statistically analyzed using a t test for independent samples. The analysis was conducted using a one-tailed distribution and probability value of $p \leq 0.05$ to evaluate if any significant differences in mortality were recorded between the control and the test substance populations.

The Mosquito Magician 2017 formulation recorded 93% mortality within 24 hours after the applications and reached 98% mortality by day 5 of the study. The untreated control population only recorded 5% mortality during the 6-day study. The specimens that were treated with the Mosquito Magician 2017 formulation recorded mortality rates that were significantly higher than the untreated control population from the 24-hour observation until study completion (6-days).

CONCLUSION:

The results of the study indicate that an 8:192 dilution of the Mosquito Magician 2017 formulation has the ability to kill yellow fever mosquitoes (*Aedes aegypti*) when used as a direct spray application.

Disclaimer: Snell Scientifics does not intend for the data or conclusions contained in this report to be construed as an endorsement of any of the test substances evaluated in this study. Unofficial changes made to the report, such as changing the test substance information, sponsor of the study, or the data will result in the invalidation of this report.



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TABLES:

Table 1.

Yellow Fever Mosquito - % Mortality											
Test Substance:	Pre-trt	30 min	1 hr	2 hr	4 hr	24 hr	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT
Controls - Untreated	0%	0%	0%	0%	0%	0%	0%	0%	3%	3%	5%
Mosquito Magician 2017 Formula (8mL:192mL water)	0%	0%	0%	0%	10%	93%	95%	95%	95%	98%	98%



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APPENDIX A: PHOTOGRAPHS

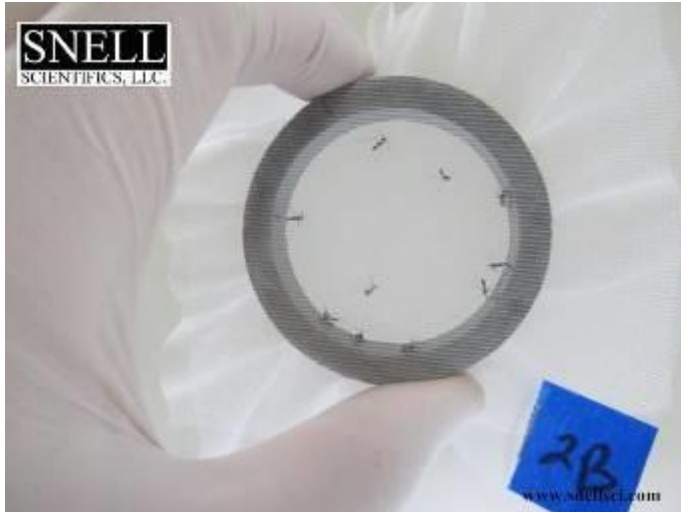
Photograph 1. Test Substance



Photograph 2. Test Arenas after Treatment



Photograph 3. Test Systems after Treatment



Photograph 4. Test Systems in Post-Treatment Arena (Control Population)





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Photograph 5. Test Systems in Post Treatment after Treatment





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APPENDIX B: RAW DATA



NON-GLP STUDY PROTOCOL
Sponsor: Sprinkler Magician
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Trial: AEDSAE
Sponsor Code: N/A
Test Method: 311

Date: April 4, 2017

Sponsor Representative: Peter Olt

Data File Info: Data File Name: SprinklerMagicianMosqDirect17
Worksheet Name: AEDSAE Direct
Report File Name: SprinklerMagicianMosqDirect17-AEDSAE

STUDY OBJECTIVE(S):

The objective of the study is to evaluate the toxicity of the test substance(s) when applied as direct spray applications against yellow fever mosquitoes (*Aedes aegypti*).

TEST SUBSTANCE INFORMATION:

#	Test Substance	Dilution Rate	Active Ingredients	EPA Reg. No. and/or Est. No.	Lot and/or Batch #	Snell Receipt Code
1	Controls - Untreated	N/A	N/A	N/A	N/A	N/A
2	Mosquito Magician 2017 Formula	4% sample:96% water (8mL:192mL)	N/A	N/A	N/A	040317-1-A-SPR

TEST SYSTEM INFORMATION:

Trial	Test System	Strain	Stage/Age	Source
AEDSAE	Yellow Fever Mosquito (<i>Aedes aegypti</i>)	Lab	Adults Females	Purchased/Lab Reared

TEST DESIGN:

# Reps per Substance	# Systems per Rep	# Systems per Substance	# Test Substances	Total # Systems	# Test Arenas
4	10	40	2	80	8



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NON-GLP STUDY PROTOCOL

Date: April 4, 2017

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Sponsor Code: N/A
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TEST ARENA INFORMATION:

Treatment Arena: 1.75" CPVC Mesh Cartridge (BioQuip 7250NSW mesh).
Post-Treatment Arena: 20oz SOLO cup with mesh lid.
Food/Moisture: 10% sucrose soaked cotton swabs.
Environmental Conditions: The test systems should be tested under ambient laboratory conditions.

TEST SUBSTANCE PREPARATION & APPLICATIONS:

Note:

1. The test substance(s) should be adequately shaken prior to applications.
2. The test substance(s) should be flow rated to determine the amount of volume dispensed per pull.
3. The test substances should be pre and post-weighed to determine the amount of weight loss during the applications.

Application Equipment: Snell Sci. Trigger Sprayer.
Application Rate: 1 trigger pull from 12 inches.
Application Distance: 12 inches.
Application Methods: Apply 1 trigger pull from an approximate 12-inch distance using the spray setting that delivers the majority of the test substance inside the treatment area.

OBSERVATION METHODS:

Observation Times: Observations of the number 'alive', 'knockdown (KD)', and 'dead' specimens should be collected prior to applications (Pre-trt), and at 30 min, 1 hr, 2 hrs, 4 hrs, 24 hrs, and then daily as needed after the applications.

TEST SYSTEM EXPOSURE:

Test System Exposure Duration: The test systems should be transferred from the Treatment Arenas into the clean Post-treatment Arenas 1 hour after the applications.



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EQUIPMENT VERIFICATIONS:

Historical verifications can be used as long as the equipment was verified within 5 days prior to the equipment use date.

Balance Verifications:

Date	Balance Log Page	Balance ID (Model/Serial#)	Certified Weight Mass Range		Actual Balance Reading		Reading Within $\pm 5\%$	Initials
			Lower	Upper	Lower	Upper		
04/10/17	Adam-1	Adam AFA 1001	0.0100g	100.0000g	0.0100g	99.9988g	Yes	RH
04/10/17	ED-1	Symmetry ED2000	1.0g	1000.0g	1.0g	998.6g	Yes	RH

Applicator(s):

Applicator Code	Applicators	Rate Verification
DT	Disposable Trigger Sprayer(s)	x ml/1 pull

Applicator Flow Rate Verifications:

Date	Applicator Code	Equipment Maintenance Log Page (original raw data)	Catch 1	Catch 2	Catch 3	Average Catch	Initials
04/14/17	DT	SPRAD1-03	3.0mL/3pulls	3.0mL/3pulls	3.0mL/3pulls	1.0mL/pull	RH

Applicator Flow Rates and Pre & Post Application Weights:

Test Substances:	Flow Rates (mL/pull)	Pre-Weight (g.)	Post-Weight (g.)	Weight Loss (g.)	Number of Sprays	Average Loss (g/spray)
Mosquito Magician 2017 Formula	1.0mL/pull	235.6	230.3	5.3	5	1.06g/spray

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Date: April 4, 2017

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Study:	Direct Spray 17
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Sponsor Code:	N/A
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STUDY NOTES:

Initials: _____ Date: _____

RH 04/20/17

STUDY PERSONNEL:

Study Director:


Signature

Date: 04/20/17

Principle Investigator:

Signature _____

Date: 04/20/17



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Sponsor: Sprinkler Magician TM#: 311 Page 1 of 1
Study: Direct Spray 17 Trial: AEDSAE
Test Arena Info: 1.75" CPVC cartridge with BioQuip7250NSW mesh
Treatment Arena: 20oz SOLO cup with mesh lid
Post-Trt Arena: Cotton ball soaked in 10% sucrose solution
Food/Moisture: Yellow Fever Mosquito
Strain/Stage/Age: Lab/Adult Females/Purchased/Reared
Exposure Time/Date: 6:40 AM / 04/14/17
Exposure Duration: 1 hr
Test Substance Info: Mix Time/Date: 6:20 AM / 04/14/17
Treatment Time/Date: 6:40 AM / 04/14/17
Dry Time/Evaluation: N/A
Asterisk Info: * =
** =
*** =
Start Date/Environmental Conditions: Application: Laboratory:
Rep #s: A-D Date(s): 04/14/17 Temp (F): 55 RH%: 80 Temp (F): 70 RH%: 46
Rep #s: Date(s): Temp (F): RH%: Temp (F): RH%:

Rep	Cond.	Pre-Trt	30 min	1 hr	2 hr	4 hr	24 hr	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT
A	Alive	10	10	10	10	10	10	10	9	9	9	9
	KD	0	0	0	0	0	0	0	1	0	0	0
	Dead	0	0	0	0	0	0	0	0	1	1	1
B	Alive	10	10	10	10	10	10	10	10	10	10	10
	KD	0	0	0	0	0	0	0	0	0	0	0
	Dead	0	0	0	0	0	0	0	0	0	0	0
C	Alive	10	10	10	10	10	10	10	10	10	10	9
	KD	0	0	0	0	0	0	0	0	0	0	2
	Dead	0	0	0	0	0	0	0	0	0	0	0
D	Alive	10	10	10	10	10	10	10	10	10	10	8
	KD	0	0	0	0	0	0	0	0	0	0	1
	Dead	0	0	0	0	0	0	0	0	0	0	1
Initials		RH	RH	RH	RH	RH	AR	AR	RH	RH	RH	RH
Date:		04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17

Rep	Cond.	Pre-Trt	30 min	1 hr	2 hr	4 hr	24 hr	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT
A	Alive	10	6	5	3	2	2	2	2	2	1	1
	KD	0	4	5	7	8	1	0	0	0	0	0
	Dead	0	0	0	0	0	7	8	8	8	9	9
B	Alive	10	0	0	0	0	0	0	0	0	0	0
	KD	0	10	10	10	9	0	0	0	0	0	0
	Dead	0	0	0	0	1	10	10	10	10	10	10
C	Alive	10	0	0	0	0	0	0	0	0	0	0
	KD	0	10	10	10	10	0	0	0	0	0	0
	Dead	0	0	0	0	0	10	10	10	10	10	10
D	Alive	10	0	0	0	0	0	0	0	0	0	0
	KD	0	10	10	10	7	0	0	0	0	0	0
	Dead	0	0	0	0	3	10	10	10	10	10	10
Initials		RH	RH	RH	RH	RH	AR	AR	RH	RH	RH	RH
Date:		04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17	04/14/17

Researcher(s):
Name: Randy Holliday Signature: Rg Heeg Date(s): 04/14/17 Role: Prin. Inv.
Name: John Smith Signature: John Smith Date(s): 04/14/17 Role: OLC



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APPENDIX C: TEST SUBSTANCE RECEIVING LOG

Snell Scientifics, LLC. Test Substance Receipt Log																
Study Information				Test Substance Information												
Receipt Date	Scanner	Study	Study Code	Test Substance	Arrival Ingt.	GPA Reg. No. and/or EIL No.	Lot and/or Batch #	Snell Receipt Code	Arrst. Rec'd	Cont. Type	Shipper	Package Cont.	Photo Taken (YES)	MECG Receipt Stamp Logged (Y/N)	Storage LEC	Initials
4/23/17	Sprinkler Magician	Direct Spray 17	N/A	Metabolic Magician 2017 Petition	N/A	N/A	N/A	543317-4-8-194	-3204	Btfs	UPS	Cont	Y	N	A	RH



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APPENDIX D: TEST SUBSTANCE STORAGE LOG

Snell Scientifics, LLC
188 Vega Rd. Meansville, GA 30256

Effective Date: F-ENVI01
01Jul08

Temperature and Humidity Log

Building: Small Sciences Room: Chem Storage A Year: 2017

[illegible]

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