# VOLUME 4

# Title

# Spartan Mosquito Eradicator Pro Tech EPA Reg. No. 93813-R Field Efficacy Evaluation Against Mosquitoes

# Data Requirements

Invertebrate Control Agent Product Performance Testing Guidelines (OCSPP Guideline 810.3400) Mosquito, Black Fly, and Biting Midge (Sand Fly) Treatments

#### Authors

Jeremy Hirsch Chris Bonner Michael S. Bonner, Ph.D.

# Prepared by

Technology Sciences Group, Inc. 1150 18<sup>th</sup> Street NW, Suite 1000 Washington, DC 20036

#### Conducting Laboratory

Bonner Analytical Testing Company 2703 Oak Grove Road Hattiesburg, Mississippi 39402

#### **Date Completed**

July 29, 2019

# Sponsored/Submitted by

AC2T dba Spartan Mosquito P.O. Box 18556 Hattiesburg, Mississippi 39404

# Study ID

AC2T-20190729-4

# STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No information is claimed confidential on the basis of its falling within the scope of FIFRA (d)(1)(A), (B) or (C).

Company:

Company Agent:

AC2T dba Spartan Mosquito P.O. Box 18556 Hattiesburg, Mississippi 39404

hinthal

Micah T. Reynolds, Regulatory Consultant to AC2T dba Spartan Mosquito Technology Sciences Group, Inc.

Date:

July 29, 2019

# NOTICE

This report is the property of AC2T dba Spartan Mosquito and, as such, is considered to be confidential for all purposes other than compliance with FIFRA Section 10. Submission of this report in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

# GOOD LABORATORY PRACTICES STATEMENT

This study was not conducted in full compliance with Good Laboratory Practices as outlined in 40 CFR 160; however, the data reported herein were collected using sound scientific principles, trained staff persons, and robust procedures.

Sponsor / Submitter:

Mintheal

Micah T. Reynolds, Regulatory Consultant to AC2T dba Spartan Mosquito Technology Sciences Group, Inc.

Date:

July 29, 2019

# **Table of Contents**

Study Title Page1
Statement Of No Data Confidentiality Claims2
Good Laboratory Practices Statement
Table of Contents4
Executive Summary5
Rural Area Study; Project No. BA041317
Residential Area Study; Project No. BA07191715
Residential Area Study; Project No. BA07201719
Field Trial Efficacy Study Conducted at Ocean Springs, MS; Project No. BT08132018-0324
Standard Operating Procedures: Mosquito Counts using a CDC Light Trap for Boric Acid Active Ingredient (Revision 1.0)
Standard Operating Procedures: Mosquito Counts using a CDC Light Trap for Boric Acid Active Ingredient (Revision 1.1)
Standard Operating Procedures: Mosquito Counts using a CDC Light Trap for Spartan Mosquito Pro Tech (Revision 2.1)

# **Executive Summary**

The Spartan Mosquito Eradicator Pro Tech is a unique pesticide product for the control and suppression of mosquito populations. It is an attractive insecticide bait product containing 9.04% boric acid as the toxicant; however, in-use diluted concentration is 2.3% boric acid once warm water is added to the product. Other inert ingredients in the product are used to attract mosquitoes and once the product is ingested, boric acid will kill the target pest. Mosquito populations will begin to decline and, within two weeks, effective control will last for 30 days. Prior to placement of the product tubes along the perimeter of the area to be treated, warm water is added to the product to initiate attractancy. A specialized cap is placed onto the tube with openings large enough only for mosquitoes to gain access and enter but small enough that other non-target organisms, such as honeybees, butterflies, or hummingbirds, cannot access the product. This specialized cap also affords the ability for the product tube to naturally replenish with rain water over the life of the product as initial water levels may decrease due to evaporative losses.

Field efficacy trials were conducted at various rural, residential, and coastal sites to evaluate longterm effectiveness of the Spartan Mosquito Eradicator Pro Tech product. Based on testing across several sites, each with different geographical features (i.e. wooded areas and proximity to water bodies or inland waterways), the Spartan Mosquito Eradicator Pro Tech remains effective for 90 days after placement and begins to substantially reduce mosquito populations within two weeks after placement. It is important to note that while data presented herein support product effectiveness for up to 90 days, the registrant is seeking only a 30 day claim of effectiveness on the label.

# Rural Area Study: Project No. BA041317

In this rural area study in New Augusta, Mississippi, several test sites were selected where two product tubes were placed per acre. This evaluation, conducted in mid-spring to early-summer, allowed for the evaluation of the use of 2 product tubes per acre when mosquitoes were emerging early in the season.

The first test site represented a wooded area and comprised 3 square acres with a total of 6 product tubes spaced less than 180 feet apart around the perimeter of the treatment area. The second test site represented an area near a water body and comprised 4 square acres with a total of 8 product tubes spaced less than 180 feet apart around the perimeter of the treatment area. In each test site, product tubes were placed in well-shaded areas and hung approximately 6 feet above the ground. A third site served as a control and was both heavily wooded and was near a water body. The control site provided consistent mosquito pressure for the duration of the 90-day study period. Results for both test sites indicated a significant reduction of mosquito population, compared to control, by day 14 of the study with near 100% control being achieved. Mosquito counts remained low at both test sites through study day 90 providing on or near 100% control for the duration of the study period.

Results of the study demonstrate very effective control of mosquitoes using the application rate of 2 product tubes per acre spaced along the perimeter of the treatment area.

# Residential Area Study: Project No. BA071917

In this first residential area study in Hattiesburg, Mississippi, a residential test site was selected where four product tubes were placed per acre. This evaluation, conducted in early-summer to early-fall, allowed for the evaluation of the use of 4 product tubes per acre when mosquito population pressures were at their highest.

The test site represented a semi-wooded residential area and comprised 1 square acre with a total of 4 product tubes spaced less than 180 feet apart around the perimeter of the treatment area. Product tubes were placed in well-shaded areas and hung approximately 6 feet above the ground. A second site served as a control site. The control site provided consistent mosquito pressure for the duration of the 90-day study period. Results from the test site indicated a significant reduction of mosquito population, compared to control, by day 14 of the study with near 100% control being achieved. Control remained at a level of 95% or greater for the duration of the study period.

Results of the study demonstrate very effective control of mosquitoes using the application rate of 4 product tubes per acre spaced along the perimeter of the treatment area during times when mosquito pressure is well established and at its highest.

# Residential Area Study: Project No. BA072017

In this second residential area study in Hattiesburg, Mississippi, a residential test site was selected where five product tubes were placed on a test site comprising 1.5 acres. This evaluation, conducted in early-summer to early-fall, allowed for the evaluation of the use of 5 product tubes per acre when mosquito population pressures were at their highest. Product tubes were placed in well-shaded areas and hung approximately 6 feet above the ground. A second site served as a control site. The control site provided consistent mosquito pressure for the duration of the 90-day study period. Results from the test site indicated a significant reduction of mosquito population, compared to control, by day 16 of the study (day 14 provided a rain out with no measurements) with near 100% control being achieved. Control remained at a level of 95% or greater from study day 16 through the duration of the study period.

Results of the study demonstrate very effective control of mosquitoes using the application rate of 5 product tubes per 1.5 acres spaced along the perimeter of the treatment area during times when mosquito pressure is well established and at its highest.

# Field Trial Efficacy Study Conducted at Ocean Springs, MS: Project No. BT08132018-03

In this coastal area study in Ocean Springs, Mississippi, conducted in later-summer to mid-autumn, allowed for the evaluation of the use of 4 product tubes per acre when mosquito population pressure remained high near a coastal region. The test site comprised 2 acres and represented a wooded area in a coastal region with high prevalence for mosquito habitat. A total of 8 product tubes (4 tubes per acre) were placed at 180 feet apart around the perimeter of the treatment area. In the test site, product tubes were placed in well-shaded areas and hung approximately 6 feet above the ground. A second site served as a control and was heavily wooded and was also located near the coastal area. The control site provided consistent mosquito pressure for the duration of the 80-day study period. Results for the test site indicated a significant reduction of mosquito population, compared to control, by day 14 of the study with near 100% control being achieved. Control continued to be at or near 100%; however, the meteorological influence of two successive hurricanes (Hurricane Gordon in September 2018 and Hurricane Nate in October 2018) impacted the effectiveness of the product. It is important to note that the first hurricane incident did not impact efficacy of the product; however, the second hurricane did negatively impact product performance. Mosquito population suppression was again achieved within approximately two weeks after the second hurricane event.

Results of the study demonstrate effective control of mosquitoes using the application rate of 4 product tubes per acre in a coastal region and also demonstrate that the product can continue to perform after major meteorological impacts.

# Summary

Results from these field trials under diverse geographical locations and meteorological impacts demonstrate that the Spartan Mosquito Eradicator Pro Tech product will perform under a broad range of scenarios including all areas in which mosquito habitats are prevalent and population is high. The product performs consistently across all of these geographic areas, and when impacted by a major meteorological event, will regain a high level of efficacy in suppressing mosquito populations over a treatment area.

The different evaluations consistently indicate that approximately within two weeks after placement of the product, greater than 95% reduction in mosquito population is observed in the treated area. It is also observed that an application rate of 2 product tubes per acre are sufficient to control mosquitoes in early season emergence and that 4 product tubes per acre can control mosquito populations in areas or at times where pest pressure is greater. Considering the above, the registrant considers the data supportive of a label claim of product effectiveness for 30 days.

The reports that follow present the various field trial results in more detail. In addition, there are enclosed Standard Operating Procedures used by the registrant and its testing laboratory to enumerate mosquito counts using the CDC Light Trap apparatus employed for mosquito collection during the field trials. Each SOP revision is substantially similar to the previous with minor revisions. The first three field trials, conducted in 2017, used the SOP Revision 1.0 while the field trial conducted in August 2018 used the SOP Revision 1.1. SOP Revision 2.1 is the most current and is included only for reference to provide an illustration of placement of Spartan Mosquito Eradicator Pro Tech products deployed under field use situations and the location of the CDC Light Trap collection unit.

Rural Area Study Project Number: BA041317 Study Initiation: April 13, 2017 Study Completion: July 12, 2017 SPARTAN



WWW.SPARTANMOSQUITO COM

Company Number: 93813

EPA Registration Number: Pending

Rural Area Study

Project Number: BA041317

Test Solution Boric Acid Active Ingredient

Effects on Mississippi Mosquito Population over a ninety-day period

Conducted by Jeremy Hirsch, President -Spartan Mosquito

Location of Test Site: 458 Buck Creek Road, New Augusta, MS; GPS Coordinates (per Google Maps) 31.283906, -89.057296

#### EFFICACY STUDY SUMMARY:

STUDY INITIATION DATE: 04/13/17

STUDY COMPLETION DATE: 07/12/17

2 Test and 1 Control areas: Test Area #1, 3 Sq. Acres at 31.287236, -89.052198, Test Area #2, 4 Sq. Acres at 31.287970, -89061962 DESCRIPTION: 2 Tubes per acre spaced at less than 180ft apart, in well-shaded areas, at 6ft above ground level.

DRY WEIGHT ACTIVE INGREDIENT: Boric Acid 9.0%, INERT INGREDIENTS Sucrose 90.8%, Yeast 0.2%

IN USE INGREDIENT: Warm water added to the fill line on the tube resulting in final concentrations of Sucrose 23.3%, Boric Acid 2.3%, Yeast 0.05%.

WATER: Warm tap water approved for consumption above 80 degrees Fahrenheit.

#### TEST RESULTS:

Mosquito Counts conducted using a CDC Light Trap and SOP Trap Counts\_Rev1.0 Conducted by Jeremy Hirsch.

Control Area:

			New	w Augus	sta, MS	458 Bu	ck Cree	k Road					
Days	Control Area - 31.278286, -89.061033												
	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts		
0	04/13/17	1430	54	85	0.00 In	04/14/17	1520	55	83	0.00 In	33		
8	04/20/17	1637	63	86	0.02 In	04/21/17	1545	59	85	0.00 In	21		
14	04/26/17	1255	56	83	0.03 In	04/27/17	1242	60	82	0.18 In	47		
30	05/13/17	1010	63	78	0.66 In	05/14/17	1032	57	87	0.00 In	Rain out		
31	05/14/17	1207	57	87	0.00 In	05/15/17	1155	61	89	0.00 In	37		
60	06/12/17	1918	71	88	0.03 In	06/13/17	1955	73	87	0.00 In	19		
90	07/12/17	1523	72	91	0.00 In	07/13/17	1537	73	91	0.10 In	42		

Test Area #1:

			New	w Augus	sta, MS	458 Bu	ck Cree	k Road						
Days		Test Area #1 - 31.287236, -89.052198												
	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts			
0	04/13/17	1350	54	85	0.00 In	04/14/17	1320	55	83	0.00 ln	44			
8	04/20/17	1557	63	86	0.02 In	04/21/17	1502	59	85	0.00 In	13			
14	04/26/17	1202	56	83	0.03 In	04/27/17	1150	60	82	0.18 ln	2			
30	05/13/17	0925	63	78	0.66 In	05/14/17	0950	57	87	0.00 In	Rain out			
31	05/14/17	1125	57	87	0.00 In	05/15/17	1107	61	89	0.00 In	0			
60	06/12/17	1835	71	88	0.03 In	06/13/17	1900	73	87	0.00 In	2			
90	07/12/17	1440	72	91	0.00 In	07/13/17	1445	73	91	0.10 In	1			

CONCLUSION: A 2.3% solution of boric acid in a 23 % sucrose solution achieved 95% reduction within 14 days after application and 100 % mosquito reduction on day 31. On days 60 and 90 mosquitoes where encountered but less than 95%.

#### Test Area #2

			New	w Augus	sta, MS	458 Bu	ck Cree	ek Road						
D		Test Area #2 - 31.287970, -89.061962												
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts			
0	04/13/17	1410	54	85	0.00 In	04/14/17	1440	55	83	0.00 In	53			
8	04/20/17	1620	63	86	0.02 In	04/21/17	1525	59	85	0.00 In	21			
14	04/26/17	1235	56	83	0.03 In	04/27/17	1215	60	82	0.18 In	0			
30	05/13/17	0948	63	78	0.66 In	05/14/17	1010	57	87	0.00 In	Rain out			
31	05/14/17	1148	57	87	0.00 In	05/15/17	1132	61	89	0.00 In	1			
60	06/12/17	1900	71	88	0.03 In	06/13/17	1932	73	87	0.00 In	0			
90	07/12/17	1500	72	91	0.00 In	07/13/17	1508	73	91	0.10 In	4			

CONCLUSION: A 2.3% solution of boric acid in a 23 % sucrose solution achieved 100% reduction within 14 days after application and 98 % mosquito reduction on day 31. On day 90 4 mosquitoes where trapped, units may have ended.



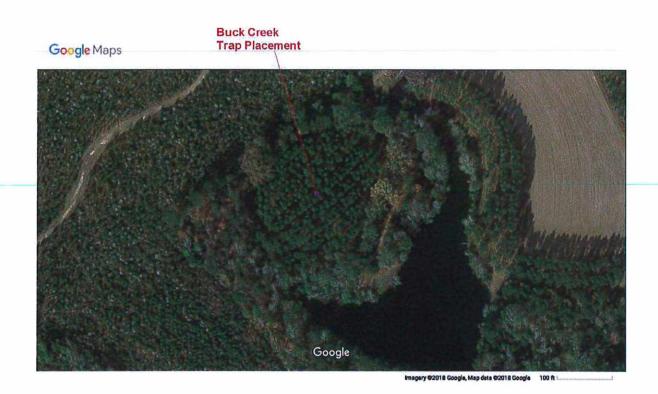
Imagery 62018 Google, Map data 62018 Google 500 ft

Google Maps



Imagery ©2018 Google, Map data ©2018 Google 100 ft





Volume IV – Field Efficacy Evaluation Against Mosquitoes – Spartan Mosquito Eradicator Pro Tech Page 14 of 44 Residential Area Study Project Number: BA071917 Study Initiation: July 19, 2017 Study Completion: October 19, 2017 **SPARTAN** 



WWW SPARTANMOSQUITO COM

EPA Registration Number: Pending

Residential Area Study

Company Number: 93813

Project Number: B A071917 Test Solution Boric Acid Active Ingredient Effects on Mississippi Mosquito Population over a ninety-day period Conducted by Chris Bonner Vice-President-Spartan Mosquito Location of Test Site: 33 Franklin Place, Hattiesburg MS 39402; GPS Coordinates (per Google Maps) 31.281971, -89.441252

#### EFFICACY STUDY SUMMARY:

STUDY INITIATION DATE: 07/19/17

STUDY COMPLETION DATE: 10/19/17

1 Acres square at 31.281971, -89.441252

DESCRIPTION: 4 Tubes per acre spaced at 180ft apart, in well-shaded areas, at 6ft above ground level. 4 Total Tubes deployed. DRY WEIGHT ACTIVE INGREDIENT: Boric Acid 9.0%, INERT INGREDIENTS Sucrose 90.8%, Yeast 0.2 %

IN USE INGREDIENT: Warm water added to the fill line on the tube resulting in final concentrations of Sucrose 23.3%, Boric Acid 2.3%, Yeast 0.05%.

WATER: Warm tap water approved for consumption above 80 degrees Fahrenheit.

#### TEST RESULTS:

Mosquito Counts conducted using a CDC Light Trap and SOP Trap Counts\_Rev1.0 Conducted by Chris Bonner.

#### Control Area:

				Hatties	burg M	1S - Can	neron R	oad					
Deve	Control Area - 31.282774, -89.444512												
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts		
0	07/19/17	1555	73	95	0.00 In	07/20/17	1522	74	96	0.00 In	61		
8	07/27/17	1800	73	95	0.00 In	07/28/17	1815	71	94	0.00 In	42		
14	08/02/17	1655	69	87	0.00 In	08/03/17	1648	72	83	0.03 In	57		
30	08/18/17	1720	76	94	0.00 In	08/19/17	1735	73	94	0.02 In	26		
33	08/21/17	1900	74	93	0.00 ln	08/22/17	1845	74	93	0.00 In	47		
52	09/09/17	0915	58	84	0.00 In	09/10/17	0900	58	84	0.00 In	54		
62	09/19/17	0715	71	91	0.03 ln	09/20/17	0705	71	90	1.19 ln	Rain Out		
72	09/29/17	0813	68	90	0.00 ln	09/30/17	0803	64	90	0.00 In	32		
92	10/19/17	1535	49	82	0.00 ln	10/20/17	1703	54	81	0.00 In	16		

Test Area:

			H	lattiesb	urg, MS	5 - 33 Fr	anklin	Place						
		Test Area - 31.281971, -89441252												
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts			
0	07/19/17	1535	73	95	0.00 ln	07/20/17	1502	74	96	0.00 In	72			
8	07/27/17	1742	73	95	0.00 In	07/28/17	1802	71	94	0.00 In	15			
14	08/02/17	1630	69	87	0.00 In	08/03/17	1620	72	83	0.03 In	3			
30	08/18/17	1745	76	94	0.00 In	08/19/17	1720	73	94	0.02 In	2			
33	08/21/17	1845	74	93	0.00 In	08/22/17	1823	74	93	0.00 In	0			
52	09/09/17	0933	58	84	0.00 In	09/10/17	0917	58	84	0.00 In	0			
62	09/19/17	0720	71	91	0.03 In	09/20/17	0732	71	90	1.19 ln	Rain Out			
72	09/29/17	0800	68	90	0.00 In	09/30/17	0745	64	90	0.00 In	0			
92	10/19/17	1551	49	82	0.00 In	10/20/17	1642	54	81	0.00 In	1			

CONCLUSION: A 2.3% solution of boric acid in a 23 % sucrose solution achieved 96% reduction within 14 days after application and 97 % mosquito reduction within 30 days. On day 33 there was 100% eradication.

Google Maps



Imagery @2018 Google, Map data @2018 Google 100 ft

Residential Area Study Project Number: BA072017 Study Initiation: July 20, 2017 Study Completion: October 18, 2017 SPARTAN



WWW SPARTANMOSQUITO COM

Company Number: 93813

EPA Registration Number: Pending

Residential Area Study

Project Number: BA072017 Test Solution Boric Acid Active Ingredient

Effects on Mississippi Mosquito Population over a ninety-day period

Conducted by Chris Bonner Vice-President-Spartan Mosquito

Location of Test Site: 2711 Oak Grove Road, Hattiesburg MS 39402; GPS Coordinates (per Google Maps) 31.270913, -89.440314

EFFICACY STUDY SUMMARY:

STUDY INITIATION DATE: 07/20/17

STUDY COMPLETION DATE: 10/18/17

1.5 Acres square at 31.270913, -89.440314

DESCRIPTION: 4 Tubes per acre spaced at less than 180ft apart, in well-shaded areas, at 6ft above ground level. 5 Total Tubes deployed.

DRY WEIGHT ACTIVE INGREDIENT: Boric Acid 9.0%, INERT INGREDIENTS Sucrose 90.8%, Yeast 0.2 %

IN USE INGREDIENT: Warm water added to the fill line on the tube resulting in final concentrations of Sucrose 23.3%, Boric Acid 2.3%, Yeast 0.05%.

WATER: Warm tap water approved for consumption above 80 degrees Fahrenheit.

TEST RESULTS:

Mosquito Counts conducted using a CDC Light Trap and SOP Trap Counts\_Rev1.0 Conducted by Chris Bonner.

Control Area:

				Hatties	burg N	1S - Can	neron R	oad						
		Control Area - 31.282774, -89.444512												
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts			
0	07/19/17	1555	73	95	0.00 ln	07/20/17	1522	74	96	0.00 In	61			
8	07/27/17	1800	73	95	0.00 In	07/28/17	1815	71	94	0.00 In	42			
14	08/02/17	1655	69	87	0.00 In	08/03/17	1648	72	83	0.03 In	57			
30	08/18/17	1720	76	94	0.00 In	08/19/17	1735	73	94	0.02 In	26			
33	08/21/17	1900	74	93	0.00 In	08/22/17	1845	74	93	0.00 In	47			
52	09/09/17	0915	58	84	0.00 In	09/10/17	0900	58	84	0.00 In	54			
62	09/19/17	0715	71	91	0.03 In	09/20/17	0705	71	90	1.19 ln	Rain Ou			
72	09/29/17	0813	68	90	0.00 In	09/30/17	0803	64	90	0.00 In	32			
92	10/19/17	1535	49	82	0.00 In	10/20/17	1703	54	81	0.00 In	16			

Test Area:

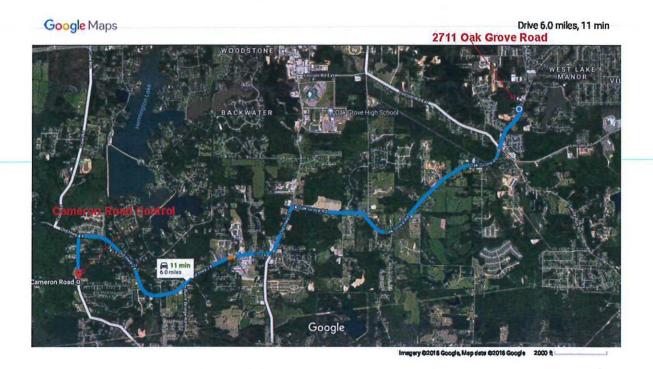
			Hat	tiesbur	g, MS -	2711 0	ak Grov	ve Road							
Deve		Test Area - 31.270913, -89.440314													
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts				
0	07/20/17	1708	74	96	0.00 In	07/21/17	1715	75	95	0.00 In	42				
8	07/28/17	1842	71	94	0.00 In	07/29/17	1830	79	90	0.67 ln	13				
14	.08/03/17	1745	72	83	0.03 In	08/04/17	1802	76	90	0.67 ln	Rain Out				
16	08/05/17	1130	76	91	0.02 In	08/06/17	1215	75	87	0.00 In	1				
30	08/19/17	1825	73	94	0.02 In	08/20/19	1822	70	94	0.78 In	Rain Out				
33	08/22/18	1925	74	93	0.00 In	08/23/18	1915	75	91	0.00 In	2				
50	09/08/17	0630	55	82	0.00 In	09/09/17	0615	58	84	0.00 In	0				
60	09/18/17	0530	71	91	0.00 In	09/19/17	0600	71	91	0.03 In	1				
90	10/18/17	0815	47	77	0.00 In	10/19/17	0803	49	82	0.00 In	0				

CONCLUSION: A 2.3% solution of boric acid in a 23 % sucrose solution achieved 97% reduction within 16 days after application and 95 % mosquito reduction on day 33. On day 50 through 90 there was one mosquito caught.

Google Maps



Imagery \$2018 Google, Map data \$2018 Google 100 ft



Field Trial Efficacy Study Conducted at Ocean Springs, Mississippi Project Number: BT08132018-03 Study Initiation: August 13, 2018 Study Completion: October 31, 2018



# **Case Narrative**

#### SPARTAN MOSQUITO ERADICATOR

Ninety Day Field Trial EFFICACY STUDY Conducted At 207 LaSalle Court Ocean Springs, MS

Performed By

Michael S. Bonner, Ph.D. Bonner Analytical Testing Company

August 13, 2018 - October 31, 2018

Project Number: BT08132018 - 03

#### INTRODUCTION:

Spartan Mosquito retained Bonner Analytical Testing Company to evaluate the effectiveness of their "Spartan Mosquito Eradicator" as a mosquito control device. This ninety day Efficacy study was conducted August 13, 2018 through October 31, 2018. The field trial was conducted on a two acre parcel located at 207 LaSalle Ct., Ocean Springs, MS; a control site was located 0.51 miles north of the test site off Lovers Lane.

Bonner Analytical Testing Co. 2703 Oak Grove Road, Hattiesburg, MS 39402 Phone No.: 601-264-9965 Fax No.: 601-268-7084 www.bateo.com

#### EFFICACY STUDY SUMMARY:

Test Site Location: A two acre parcel located at 207 LaSalle Ct, Ocean Springs, MS (30.420941° N 88.842199 ° W) See Figure 1 below.

Control Site Location: Vacant wooded parcel located 0.51 miles north of the test site location off Lovers Lane (30.413338°N 88.840151°W) See Figure 1 below.

Study Initiation Date: 08/13/18

Study Completion Date: 10/31/18

Description and Placement of Spartan Tubes: 4 Tubes per acre spaced at 180ft apart, in well-shaded areas, at 6ft above ground level. 8 Total Tubes deployed. See Figure 1 for placement.

Dry Weight of Ingredients: Boric Acid 9.0%, INERT INGREDIENTS Sucrose 90.8%, Yeast 0.2 %

In Use Concentration of Ingredients: Warm water added to the fill line on the tube resulting in final concentrations of Sucrose 23.3%, Boric Acid 2.3%, Yeast 0.05%.

Water: Warm tap water approved for consumption 80 - 110 degrees Fahrenheit.

#### **RESULTS AND DISCUSSION:**

A total of eight Spartan Eradicator tubes were deployed at the two acre test site located at 207 LaSalle Ct. on August 13, 2018. Mosquito counts at the test site and the control site were performed utilizing the Model 512 CDC Miniature Light Trap. Traps were deployed at each site for 24 hours then returned to the laboratory for keying, sexing and counting.

On day zero, 63 mosquitoes were trapped at the control site and 73 at the test site. See Tables 1 and 2 below.

#### Table 1

Cor	trol Total Mosquit	o Count = 63
Gender	Culex salinarius	Anopheles crucian complex
Male	8	5
Female	47	3

#### Table 2

Test	Area Total Mosqu	ito Count = 73
Gender	Culex salinarius	Anopheles crucian complex
Male	18	4
Female	39	12

Bonner Analytical Testing Co. 2703 Oak Grove Road, Hattiesburg, MS 39402 Phone No.: 601-264-9965 Fax No.: 601-268-7084 www.bateo.com

#### CONCLUSION:

A 2.3% solution of boric acid in a 23 % sucrose solution achieved 97% reduction within 14 days after application and 100 % mosquito reduction within 30 days. On 9/3/18, the Spartan devices where taken down due to Hurricane Gordon. On 9/7/18 the devices taken down on 9/3/18 where redeployed. On day 60, one mosquito was caught in the CDC trap at the test site. On 10/7/18 the devices were taken down again due to Hurricane Nate making land fall on 10/8/18. The devices were redeployed on 10/9/18. On day 69, 23 mosquitoes were trapped and on day 88 the mosquito population had reduced back down to 6. See Tables 3 and 4.

		£	-
Та	b	e	÷.
	U.	-	-

			207 I	LaSalle	e Ct. O	cean Sp	rings	, MS			
	1		_	Test Are	ea - 30.42	0941°N 88	.842199	9°W			
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts
0	08/13/18	1603	74	90	0.00 In	08/14/18	1615	76	91	0.00 In	73
7	08/20/18	1335	77	88	0.00 In	08/21/18	1305	78	90	0.00 In	18
14	08/27/18	1548	77	89	0.00 In	08/28/18	1635	77	89	0.18 ln	2
30	09/02/18	1105	75	87	0.00 In	09/03/18	1124	75	88	0.00 ln	0
				Hu	ricane Go	rdon 9/4/1	8				
60	10/03/18	1732	74	89	0.00 ln	10/04/18	1749	73	89	0.00	in 1
				Hu	rricane Na	ate 10/8/18					
69	10/12/18	1900	57	79	0.00 In	10/13/18	1932	60	82	0.00	ln 23
88	10/30/18	0803	66	78	0.00 In	10/31/18	0842	73	82	0.00	In 6

#### Table 4

			Cont	rol Ar	ea - O	cean S	pring	s, MS			
			(	Control A	rea - 30	.413338°N	88.840	151°W			
Days	Start Date	Start Time	Low Temp	High Temp	Rainfall	Stop Date	Stop Time	Low Temp	High Temp	Rainfall	Counts
0	08/13/18	1533	74	90	0.00 In	08/14/18	1559	76	91	0.00 In	63
7	08/20/18	1223	77	88	0.00 ln	08/21/18	1242	78	90	0.00 In	88
14	08/27/18	1613	77	89	0.00 ln	08/28/18	1658	77	89	0.18 ln	59
30	09/02/18	1115	75	87	0.00 In	09/03/18	1137	75	88	0.00 In	72
				Hu	rricane G	ordon 9/4/	18				
60	10/03/18	1747	74	89	0.00 In	10/04/18	1802	73	89	0.00 In	48
	~			Нι	irricane M	late 10/8/	18				
69	10/12/18	1837	57	79	0.00 In	10/13/18	1906	60	82	0.00 In	31
88	10/30/18	0817	66	78	0.00 In	10/31/18	0830	73	82	0.00 In	44

Bonner Analytical Testing Co. 2703 Oak Grove Road, Hattiesburg, MS 3940? Phone No.: 601-264-9965 Fax No.: 601-268 7084 www.batco.com

Figure 1: Test Site at 207 LaSalle Ct., Ocean Springs, MS with Spartan Mosquito Eradicator and Mosquito Trap Placement

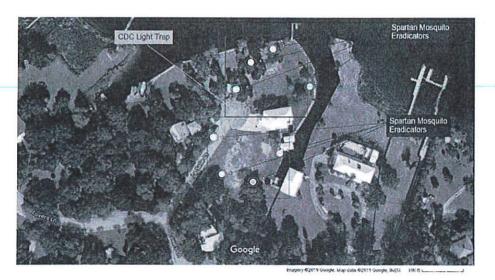
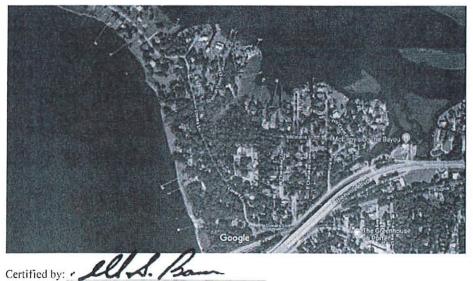


Figure 2: Test Site (207 LaSalle Ct.) and Control Site Locations



Michael S. Bonner Ph.D.

Bonner Analytical Testing Co. 2703 Oak Grove Road, Hattiesburg, MS 39402 Phone No : 601-264-9965 Fax No.: 601-268-7084 www.batco.com

# Standard Operating Procedures: Mosquito Counts Using CDC Light Trap for Boric Acid Active Ingredient (Revision 1.0) (Revision 1.1) (Revision 2.1)

Standard Operating Procedures Spartan Mosquito Mosquito Counts March 4 2017

Spartan Mosquito

STANDARD OPERATING PROCEDURES

TITLE: Mosquito Counts using a CDC Light Trap

for Boric Acid Active Ingredient

**Revision 1.0** 

Total Number of Pages 5

l.h President

Vice-President

Standard	Operating	Procedures	
Spartan Moso	uito		

#### 1.0 Scope and Application

The purpose of this document is to provide guidance on how to conduct mosquito counts in what has been shown to provide real life situations.

#### 2.0 Summary of Method

This SOP list steps to conduct mosquito counts using a CDC Light Trap (further referred to as "Trap") while using the Spartan Mosquito delivery system with boric acid as active ingredient.

#### 3.0 Health and Safety

It must be understood that subjecting one's self to this procedure can increase the possibility to contracting mosquito vectored illnesses. Unless you have signed documentation removing the liability from AC2T and its subsidiaries you are not allowed to proceed with this procedure.

\*\*\*While setting up the trap, take all precautionary steps to avoid getting bit.

#### 4.0 Personnel Qualifications

The President, Vice-President and those that have been educated on the potential health risk of carrying out this study.

#### 5.0 Apparatus & Materials

5.1 Spartan Mosquito+. The number of units is directed by acreage and time of season.

- 5.2 Laboratory notebook.
- 5.3 CDC Light Trap
- 5.4 Battery
- 5.5 String
- 5.6 CO2 emitter for Trap A Spartan Mosquito tube containing ~10% sugar ~1% Dry Brewer's Yeast in solution (40 grams granular sugar and 4 grams of Brewer's Yeast). Warm/hot tap water or distilled water maybe used to fill and activate the tube.

#### 6.0 Procedure

- 6.1 What to know before starting.
  - 6.1.1 Test areas should be as far removed from any other mosquito control agents. The optimum locations are going to be in rural areas.

6.1.2 At least two areas must be used simultaneously when tested, one or more for the eradicators and another for a control (no eradicators product used). The areas should be between ½ to 5 miles of each other and exude similar foliage, terrain, sunlight and available water (creek, swamp, and/or pond).

In addition, if there are mosquito control agents are being used in the area, be sure the control and test area in similar proximity of their application.

- 6.1.3 Counts are considered mosquitoes contained in the trap after the test is complete.
- 6.1.4 For areas to be viable for testing, the initial counts (0-days) should contain a minimum of 10 to 20 mosquitoes.
- 6.1.5 Traps should be set out to run for 24 hours. Different species of mosquitoes are active at different times of the day. If the analyst is evaluating a particular species with a specified activity time then a lesser run time can be use. If this is done take great care to consider sunset and sunrise when setting the traps.
- 6.1.6 Counts must be taken at a minimum of 0, 14, 30, 60 and 90 days or as close to these days if weather prevents you from conducting counts within your predetermined time frame.

NOTE: It is extremely important to be as consistent as possible when conducting counts.

#### 6.2 Setting up and conducting 0-day counts

6.2.1 Choose a destination for the trap that will be in the middle of the test areas. For each area, control and test sites, use the same location each time. When setting the trap up, use a 10% sugar/ 1% yeast (referred to as 10/1Y) tube for CO2 emission.

6.2.1.1 Document the times of setting out and pickup of the trap.

6.2.1.2 Document weather related to rain, high winds and temperatures

NOTE: Again, on 0-day, if the trap doesn't yield a minimum of 10 to 20 mosquitoes then the area is not viable for testing.

Once you have completed 0-day trap collection, the following must occur:

- · Deployment must be approved by the President and/or Vice-president
- Verify that all Eradicators are labeled and contain weights of all ingredients and final volume for documentation purposes. This is to confirm the concentration of the boric acid.

- Remove the 10/1Y from all locations.
- · Bring mosquitoes to lab for counting.

#### 6.3 Continued Monitoring and Counting

- 6.3.1 Set traps on days 14, 30, 60 and 90 at a minimum. It is important to position trap in the same location each time.
- 6.3.2 Use a new 10/1Y each time you set out a trap.

#### 7.0 Documentation

All information must be collected and recorded in a bound logbook. The initial entry should include detailed information regarding the locations of the test. Each additional entry (and page) must contain the date, time and initials. Once the entry is completed, a line should be drawn down the remainder of the page.

Each day that counts are taken the following information must be recorded, at a minimum.

- Time when counts start.
- Time when counts stop.
- Temperature
- Mosquitoes caught in trap

Standard Operating Procedures Spartan Mosquito Company Number: 93813

Mosquito Counts July 3, 2018 EPA Registration No.: Pending

Spartan Mosquito

STANDARD OPERATING PROCEDURES

TITLE: Mosquito Counts using a CDC Light Trap

for Boric Acid Active Ingredient

**Revision 1.1** 

Total Number of Pages 5

LL

President

Vice-President

#### Standard Operating Procedures

Spartan Mosquito Company Number: 93813 Mosquito Counts July 3, 2018 EPA Registration No.: Pending

#### 1.0 Scope and Application

The purpose of this document is to provide guidance on how to conduct mosquito counts in what has been shown to provide real life situations.

#### 2.0 Summary of Method

This SOP list steps to conduct mosquito counts using a CDC Light Trap (further referred to as "Trap") while using the Spartan Mosquito delivery system with boric acid as active ingredient.

#### 3.0 Health and Safety

It must be understood that subjecting one's self to this procedure can increase the possibility to contracting mosquito vectored illnesses. Unless you have signed documentation removing the liability from AC2T and its subsidiaries you are not allowed to proceed with this procedure.

\*\*\*While setting up the trap, take all precautionary steps to avoid getting bit.

#### 4.0 Personnel Qualifications

Those that have been educated on the potential health risk of carrying out this study.

#### 5.0 Apparatus & Materials

- 5.1 Spartan Mosquito+. The number of units is directed by acreage and time of season.
- 5.2 Laboratory notebook.
- 5.3 CDC Light Trap
- 5.4 Battery
- 5.5 String
- 5.6 CO2 emitter for Trap A Spartan Mosquito tube containing ~10% sugar ~1% Dry Brewer's Yeast in solution (40 grams granular sugar and 4 grams of Brewer's Yeast). Warm/hot tap water or distilled water maybe used to fill and activate the tube.

#### **6.0** Procedure

- 6.1 What to know before starting.
  - 6.1.1 Test areas should be as far removed from any other mosquito control agents. The optimum locations are going to be in rural areas.

#### Standard Operating Procedures

Spartan Mosquito Company Number: 93813 Mosquito Counts July 3, 2018 EPA Registration No.: Pending

6.1.2 At least two areas must be used simultaneously when tested, one or more for the eradicators and another for a control (no eradicators product used). The areas should be between ½ to 5 miles of each other and exude similar foliage, terrain, sunlight and available water (creek, swamp, and/or pond).

In addition, if there are mosquito control agents are being used in the area, be sure the control and test area in similar proximity of their application.

- 6.1.3 Counts are considered mosquitoes contained in the trap after the test is complete.
- 6.1.4 For areas to be viable for testing, the initial counts (0-days) should contain a minimum of 10 to 20 mosquitoes.
- 6.1.5 Traps should be set out to run for 24 hours. Different species of mosquitoes are active at different times of the day. If the analyst is evaluating a particular species with a specified activity time then a lesser run time can be use. If this is done take great care to consider sunset and sunrise when setting the traps.
- 6.1.6 Counts must be taken at a minimum of 0, 14, 30, 60 and 90 days or as close to these days if weather prevents you from conducting counts within your predetermined time frame.

NOTE: It is extremely important to be as consistent as possible when conducting counts.

#### 6.2 Setting up and conducting 0-day counts

6.2.1 Choose a destination for the trap that will be in the middle of the test areas. For each area, control and test sites, use the same location each time. When setting the trap up, use a 10% sugar/ 1% yeast (referred to as 10/1Y) tube for CO2 emission.

6.2.1.1 Document the times of setting out and pickup of the trap.

6.2.1.2 Document weather related to rain, high winds and temperatures

NOTE: Again, on 0-day, if the trap doesn't yield a minimum of 10 to 20 mosquitoes then the area is not viable for testing.

Once you have completed 0-day trap collection, the following must occur:

- Deployment must be approved by Spartan Mosquito before conducting filed studies.
- Verify that all Eradicators are labeled and contain weights of all ingredients and final volume for documentation purposes. This is to confirm the concentration of the boric acid.

Standard Operating Procedures

Spartan Mosquito Company Number: 93813 Mosquito Counts July 3, 2018 EPA Registration No.: Pending

- Remove the 10/1Y from all locations.
- · Bring mosquitoes to lab for counting and identification.

#### 6.3 Continued Monitoring and Counting

- 6.3.1 Set traps on days 14, 30, 60 and 90 at a minimum. It is important to position trap in the same location each time.
- 6.3.2 Use a new 10/1Y each time you set out a trap.

#### 7.0 Documentation

All information must be collected and recorded in a bound logbook. The initial entry should include detailed information regarding the locations of the test. Each additional entry (and page) must contain the date, time and initials. Once the entry is completed, a line should be drawn down the remainder of the page.

Each day that counts are taken the following information must be recorded, at a minimum.

- Time when counts start.
- · Time when counts stop.
- Temperature
- · Mosquitoes caught in trap

Standard Operating Procedures Spartan Mosquito Company Number: 93813

Mosquito Counts July 3, 2018 EPA Registration No.: Pending

Attachment #1

Setting of the trap with 10/1Y



Standard Operating Procedures Spartan Mosquito

Mosquito Counts March 23, 2019

Spartan Mosquito

STANDARD OPERATING PROCEDURES

TITLE: Mosquito Counts using a CDC Light Trap

for Spartan Mosquito Pro Tech

**Revision 2.1** 

Total Number of Pages 5

President A, B

Vice-President

Standard Op	erating	Proced	lures
Spartan Mosquito			

Mosquito Counts March 23, 2019

#### 1.0 Scope and Application

The purpose of this document is to provide guidance on how to conduct mosquito counts in what has been shown to provide real life situations.

# 2.0 Summary of Method

This SOP list steps to conduct mosquito counts using a CDC Light Trap (further referred to as "Trap") while using the Spartan Mosquito delivery system with XXXXXX as the active ingredient.

#### 3.0 Health and Safety

It must be understood that subjecting one's self to this procedure can increase the possibility to contracting mosquito vectored illnesses. Unless you have signed documentation removing the liability from AC2T and its subsidiaries you are not allowed to proceed with this procedure.

\*\*\*While setting up the trap, take all precautionary steps to avoid getting bit.

#### 4.0 Personnel Qualifications

The President, Vice-President and those that have been educated on the potential health risk of carrying out this study.

#### 5.0 Apparatus & Materials

- 5.1 Spartan Mosquito Pro Tech. The number of units is directed by acreage and time of season.
- 5.2 Laboratory notebook.
- 5.3 CDC Light Trap
- 5.4 Battery
- 5.5 String
- 5.6 CO2 emitter for Trap A Spartan Mosquito tube containing ~10% sugar ~1% Yeast in solution (40 grams granular sugar and 4 grams of Yeast). Warm/hot tap water or distilled water maybe used to fill and activate the tube.

#### 6.0 Procedure

- 6.1 What to know before starting.
  - 6.1.1 Test areas should be as far removed from any other mosquito control agents. The optimum locations are going to be in rural areas.

Standard Operating Procedures	Mosquito Counts		
Spartan Mosquito	March 23, 2019		

6.1.2 At least six areas must be used simultaneously when testing, three or more for the eradicators and three for controls (no eradicators product used). The areas should be between ½ to 5 miles of each other and exude similar foliage, terrain, sunlight and available water (creek, swamp, and/or pond).

In addition, if there are mosquito control agents are being used in the area, be sure the control and test area in similar proximity of their application.

- 6.1.3 Counts are considered mosquitoes contained in the trap after the test is complete.
- 6.1.4 For areas to be viable for testing, the initial counts (0-days) should contain a minimum of 100 mosquitoes.
- 6.1.5 Traps should be set out to run for 24 hours. Different species of mosquitoes are active at different times of the day. If the analyst is evaluating a particular species with a specified activity time then a lesser run time can be use. If this is done take great care to consider sunset and sunrise when setting the traps.
- 6.1.6 Counts must be taken at a minimum of 0, 14, 30 and 45 days or as close to these days if weather prevents you from conducting counts within your predetermined time frame.

NOTE: It is extremely important to be as consistent as possible when conducting counts.

#### 6.2 Setting up and conducting 0-day counts

6.2.1 Choose a destination for the trap that will be in the middle of the test areas (See Attachment #2 for example). For each area, control and test sites, use the same location each time. When setting the trap up, use a 10% sugar/ 1% yeast (referred to as 10/1Y) tube for CO2 emission.

6.2.1.1 Document the times of setting out and pickup of the trap.

6.2.1.2 Document weather related to rain, high winds and temperatures

NOTE: Again, on 0-day, if the trap doesn't yield a minimum of 100 mosquitoes then the area is not viable for testing.

Once you have completed 0-day trap collection, the following must occur:

- Deployment must be approved by the President and/or Vice-president
- Verify that all Eradicators are labeled and contain weights of all ingredients and final volume for documentation purposes. This is to confirm the concentration of the Active Ingredient.

- · Remove the 10/1Y from all locations.
- Bring mosquitoes to lab for identification and counting.

#### 6.3 Continued Monitoring and Counting

- 6.3.1 Set traps on days 14, 30 and 45 at a minimum. It is important to position trap in the same location each time.
- 6.3.2 Use a new 10/1Y each time you set out a trap.

#### 7.0 Documentation

All information must be collected and recorded in a bound logbook. The initial entry should include detailed information regarding the locations of the test. Each additional entry (and page) must contain the date, time and initials. Once the entry is completed, a line should be drawn down the remainder of the page.

Each day that counts are taken the following information must be recorded, at a minimum.

- Time when counts start.
- Time when counts stop.
- Temperature
- Mosquitoes caught in trap

# Standard Operating Procedures Spartan Mosquito

Mosquito Counts March 23, 2019

Attachment #1

Setting of the trap with 10/1Y

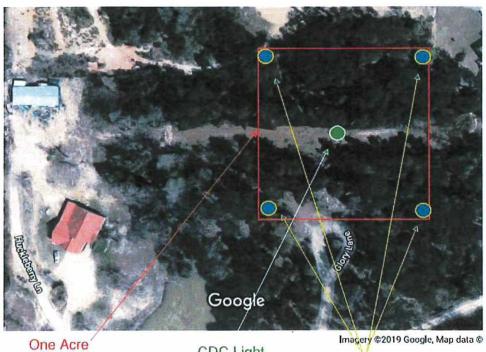


# Standard Operating Procedures Spartan Mosquito

Mosquito Counts March 23, 2019

Attachment #2

Trap and Tube Placement



CDC Light Trap

**Eradicators** 

Volume IV - Field Efficacy Evaluation Against Mosquitoes - Spartan Mosquito Eradicator Pro Tech Page 44 of 44