

VOLUME 3

Title

**Spartan Mosquito Eradicator Pro Tech
EPA Reg. No. 93813-R
Laboratory 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

Michael S. Bonner, Ph.D.

Prepared by

Technology Sciences Group, Inc.
1150 18th 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

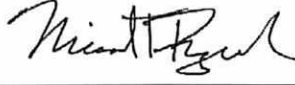
Study ID

AC2T-20190729-3

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No information is claimed confidential on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B) or (C).

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

Company Agent: 
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:**



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

Date: July 29, 2019

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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.

The study presented herein demonstrates effectiveness against specific mosquito species important for arbovirus vector control, including *Culex quinquefasciatus* and *Aedes aegypti*. Several boric acid formulations that represented diluted versions of the Spartan Mosquito Eradicator Pro Tech product were evaluated. A 1.0% boric acid in 10% sugar/water solution was tested to represent an overly diluted product used in the field, and a 3.0% boric acid in 10% sugar/water solution was tested to represent an evaporated product used in the field along with a 10% sugar/water solution to serve as control.

With the control and treatment groups, allowance was made for mosquitoes that may have died during shipment from the supplier; therefore, initial mortality counts were taken prior to exposure to the test material and the adjusted control mosquito counts are reflected in the enclosed report. Mosquito mortality data were collected over the course of a 14-day test period, the results of which are included on pages 7-8 of this volume. Both test concentrations of boric acid (1.0% and 3.0%) yielded very effective outcomes. For *Culex quinquefasciatus* mosquitoes, 100% control was achieved within 3 days for both test groups; and for *Aedes aegypti*, 100% control was achieved within three days for the 1.0% group with greater than 95% control being achieved within three days followed by 100% control within six days for the 3.0% group.

The report that follows presents, in more detail, the summarized results above. In addition, there is enclosed a Standard Operating Procedure used by the registrant and its testing laboratory to enumerate mosquito counts during the conduct of the study.



SPARTAN MOSQUITO ERADICATOR TRIALS

Culex quinquefasciatus and *Aedes aegypti*

January 20, 2018

Introduction:

Spartan Mosquito with home offices in Hattiesburg and Laurel Mississippi is the manufacturer and marketer of a novel patent pending mosquito eradicating device. The device has been shown reduce mosquito populations by up to 95% for up to 30 days. One of the salient features of this unique product is the patent pending cap design which allows mosquitos to enter but excludes larger insects such as honey bees. Another cap design feature is the "fluid level control vent" architecture which combines a convex cap top and one optimized water entry port. This feature effectively allows an adequate amount of rain water to enter the device thus replacing evaporative losses. The company has retained Bonner Analytical Testing Company to perform analytical trials on various aspects of the product.

Experimental:

The purpose of this study is to determine the range of lethal concentration for boric acid/sugar mixtures. This was done by subjecting ~200 male/female *Culex* mosquitoes and ~100 male/female *Aedes* mosquitoes, per aquarium, to various controls and concentrations of boric acid/sugar solutions. The test was conducted for 15 days starting on 01/20/2018 and ending on 02/04/2018. The temperature range was maintained at 73 to 80 °F for the duration of the study.

This study also investigated the effectiveness of the mosquito entry ports.

Preparation of material:

Two different concentrations of boric acid in 10% sugar were prepared in multiple tubes and tested at 1.0% and 3.0% boric acid in solution. The purpose for these concentrations is to provide field conditions in which an eradicator could potentially be evaporated or diluted. Controls were prepared, containing 10% sugar solution only. All test chambers also included a non-toxic food source option for the animals, which was provided in the shipment containers.

*Note: When referring to a two tube aquarium, this indicates that one tube has a black cap with entry ports and the other tube has no top. A one tube aquarium indicates a single tube with a black cap and entry portals.

The following is a list of aquariums, number of tubes, concentrations for each tube and the counts. Because there was some mortality when the animals arrived at the laboratory an initial "dead count" was recorded. The dead count from 01/20/2018 was taken after 30 minutes being released into the test environment.

Control 10% Sugar Solution:

| | Aquarium #1 Control - Culex quinquefasciatus | Aquarium #4 Control - Aedes Aegypti |
|-------------------------------|---|--|
| | Two Tubes | One Tube |
| | Control 10% Sugar | Control 10% Sugar |
| Initial Count | ~200 | ~100 |
| Total Dead Count 1/20/2018 | Immediately - 14 | Immediately - 8 |
| 23-Jan | 17 | 9 |
| 26-Jan | 18 | 10 |
| 30-Jan | 19 | 14 |
| 1-Feb | 20 | 16 |
| 4-Feb | 22 | 18 |

The Control #1 accumulated 8 dead over the course of 2 weeks, on the bottom of the aquarium. 5 of the 8 were identified as males (males typically survive seven days). Control #4 had 10 dead over a 2-week period. 7 of 10 were identified as males.

~1.0% & 3.0 % Boric Concentration applied to Culex:

| | Aquarium 15 - Culex quinquefasciatus | | Aquarium 16 - Culex quinquefasciatus | |
|-------------------------|--------------------------------------|------------|--------------------------------------|------------|
| | Tube ID | Boric Acid | Tube ID | Boric Acid |
| | Tube 15A | 1.0% | Tube 16A | 3.0% |
| | Tube 15B | 1.0% | | |
| Initial Count | ~200 | | ~200 | |
| Dead Count 1/20/2018 | Immediately - 11 | | Immediately - 5 | |
| 23-Jan | All Dead | | All Dead | |
| 26-Jan | | | | |
| 30-Jan | | | | |
| 1-Feb | | | | |
| 4-Feb | | | | |

Both concentrations provided a 100% kill rate within 3 days.

~1.0% & 3.0 % Boric Concentration introduced to Aedes:

| | Aquarium 18 - Aedes aegypti | | Aquarium 19 - Aedes aegypti | |
|-------------------------|-----------------------------|------------|-----------------------------|------------|
| | Tube ID | Boric Acid | Tube ID | Boric Acid |
| | Tube 18A | 1.0% | Tube 19A | 3.0% |
| | Tube 18B | 1.0% | | |
| Initial Count | ~100 | | ~100 | |
| Dead Count 1/20/2018 | Immediately - 16 | | Immediately - 2 | |
| 23-Jan | All Dead | | 1 alive | |
| 26-Jan | | | All Dead | |
| 30-Jan | | | | |
| 1-Feb | | | | |
| 4-Feb | | | | |


Both concentrations showed nearly 100% kill rate after 3 days.

Observations:

The January 2018 trials resulted in four statistically valid experiments which showed 100% mortality to both the Culex and Aedes at two different concentrations.

Additionally, there was no significant difference between the mortality rates of the single tube experiment and the dual tube experiment with one open tube. This shows that the cap entry ports which exclude desirable insects such as honey bees are as effective in attracting mosquitos as an open container.

Certified by:


Michael S. Bonner, Ph.D.
Chairman of the Board

Spartan Mosquito

STANDARD OPERATING PROCEDURES

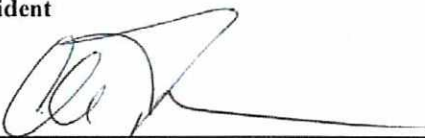
TITLE: Mosquito Counts for In-house Testing

Revision 1.0

Total Number of Pages 4



President



Vice-President

1.0 Scope and Application

The purpose of this document is to provide guidance on how to conduct mosquito counts in a controlled laboratory setting.

2.0 Summary of Method

This SOP list steps to conduct mosquito counts while using Spartan Mosquito devices.

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. No one is allowed to enter the testing area without written approval.

4.0 Personnel Qualifications

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

5.0 Apparatus & Materials**5.1 Spartan Mosquito Device**

5.2 Aquarium or another habitat which is fully encapsulated. It must have the ability to add additional food or blood source if needed and a screened area to allow air to naturally move through the housing. ALL HOUSING/AQUARIUMS WILL BE REFERED TO AS HAQ GOING FORWARD. See definitions.

5.3 Ability to measure, capture and store the volumes or weights (accurately +/- three sig figs) at 1, 10 100 and 1000 ml and/or grams, of product in various states

5.4 Analytical Sample - Mosquitoes – Female only if available, at a minimum of 200 per HAQ.

5.5 Food Source - Sugar Water wick for feeding.

5.6 Temperature controlled habitat between 75 to 85 °F

5.7 Major Instrumentation and testing.

***All sub-sampling and testing methodologies will be agreed upon between client and Bonner Analytical before any efficacy data or analysis is conducted.

5.7.1 ICP-AES – The ability to measure the amount of elemental inorganic active ingredients by ICP/AES.

5.7.2 pH Meter – meter must provide two decimal place readings.

- 5.7.3 Plate counts – colony count of microbial growth for a 24 hour incubation count.
- 5.7.4 GC/MS(or FID) – The ability to measure the organic content.
- 5.7.5 Analytical Balance – four decimal places.
- 5.7.6 Conductivity Meter

6.0 Definitions

- 6.1 HAQ – a habitat area enclosed with netting and/or glass, allowing air flow, daily MAX and MIN monitored controlled temperature, food source and testing product, where determined.
- 6.2 Batch – testing a defined species of mosquito, at a concentration of active/inactive ingredients, in a consistent environment.
- 6.3 Quality Assurance – (per batch)
 - 6.3.1 Control - A minimum of two HAQ with food source.
- 6.4 Analytical Sample – a minimum of 2 replicate concentrations of product, which follow and/or contain all the same variables within HAQ, Batch and Quality Assurance criteria
- 6.5 Food Source - Sugar Water (or other approved food) wick for feeding.

7.0 Procedure

- 7.1 All batches within the HAQ will contain a Quality Assurance package. The following is what would be required to carry out the efficacy testing on day 0.
 - A. All products for testing will have an identification number which will be used for tracking each tube.
 - B. Individual tube's contents will be weighed to 2 decimal places.
 - C. Inoculate each device with hot/warm distilled water and then follow directions. Make an indication on the bag where the meniscus of the water level is.
 - D. Retain 250mLs of water that was used for filling the tubes. This will be used for establishing baselines for designated analyses.
 - E. 30 to 60 minutes after Step C, collect 10 milliliters from the top of the product. This will be used for verifying the elemental concentration.
 - F. Prepare to release a minimum of 100 mosquitoes into each HAQ

- 7.2 At day 0, after 30 minutes of releasing the mosquitoes, conduct a dead mosquito count within each habitat.
- 7.3 Continue mosquito counts daily, as in section 7.2, until there are no more living mosquitoes observed in the analytical samples.
- 7.3.1 Document the MAX and MIN temperature and relative humidity for each 24 hours cycle.
- 7.4 Once the test is complete, mark and label meniscus of the tube, collect 10 milliliters of sample for testing, if needed. Remove device, seal and archive at 4 °C until all data has been approved.

8.0 Documentation

All information must be collected and recorded in a bound logbook. The initial entry should include detailed information as discussed above. 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.