REPORT TITLE
Repellency of the Thistle Farms Natural Insect Repellent against Yellow Fever Mosquitoes (Aedes aegypti)

STUDY
Mosquito Repellency 16

TRIAL
AEDSAE

SPONSOR CODE
N/A

EXPERIMENTAL START DATE
April 04, 2016

EXPERIMENTAL COMPLETION DATE
April 05, 2016

REPORT DATE
April 14, 2016

TEST FACILITY/AUTHORS
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SPONSOR
Thistle Farms

This work was conducted under sound scientific principles but does not comply with GLP standards under 40 CFR 160
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).

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 Date: 04/14/16 Snell Scientifics, LLC.

Study Director: Todd Smith Date: 04/14/16 Snell Scientifics, LLC.
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STUDY OBJECTIVE(S):

To measure the repellency associated with the Thistle Farms Natural Insect Repellent against yellow fever mosquitoes (Aedes aegypti).

TEST SUBSTANCE INFORMATION:

<table>
<thead>
<tr>
<th>#</th>
<th>Test Substance</th>
<th>Active Ingredients</th>
<th>EPA Reg. No. and/or Ext. No.</th>
<th>Lot and/or Batch #</th>
<th>Snell Receipt Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control - Untreated</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Thistle Farms Natural Insect Repellent</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>032816-1-A-THI</td>
</tr>
<tr>
<td>3</td>
<td>The Honest Co.® honest bug spray</td>
<td>23% Soybean Oil, 10% Castor Oil, 4% Citronella, 2% Cedar, 2% Lemongrass, 1.5% Rosemary, 1% Geranium, 1% Peppermint</td>
<td>N/A</td>
<td>Lot# 110615F</td>
<td>032816-2-A-THI</td>
</tr>
</tbody>
</table>

TEST SYSTEM INFORMATION:

<table>
<thead>
<tr>
<th>Trial</th>
<th>Test System</th>
<th>Strain</th>
<th>Stage/Age</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEDSAE</td>
<td>Yellow Fever Mosquito (Aedes aegypti)</td>
<td>Lab</td>
<td>Adults Females</td>
<td>Purchased/Lab Reared</td>
</tr>
</tbody>
</table>

TEST DESIGN (per aged evaluation):

<table>
<thead>
<tr>
<th># Reps per Substance</th>
<th># Systems per Rep</th>
<th># Systems per Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS:

Test Surfaces:
1. Collagen membranes were used as a skin analog for testing the test substances.

Application Equipment:
2. The test substances were applied using the product specific trigger sprayers.

Test Substance Preparation & Applications:
3. The collagen membranes were moistened with water and the surface was dried prior to the applications to provide a texture and consistency that was similar to human skin.
4. The applications were conducted to the collagen membranes by shaking the test substances well and then by spraying the membranes until wet, but not to the point of runoff, and then by gently rubbing the test substance into the membrane.
5. The membranes selected for the untreated controls were moistened with water but did not receive any other application.
6. The membranes evaluated during the 15 min. aged evaluation were air dried and then evaluated.

Aged Evaluations:
7. Each test substance was evaluated at 15-minutes after the applications, with 4 replicates evaluated for the controls and each test substance.

Test Design:
8. Twenty-five (25) female mosquitoes were released inside a 1’x1’ cage and the mosquitoes were held without food for at least 2 hours prior to testing.
9. The cage had a 1’x1’ wooden cover on the top of the cage with an approximate 2.5”x6” removable section in its center. The section was removable to provide an opening in the top of the cage for the test surface and for the mosquitoes to try to feed on the test subject’s arm above the test surface. Mesh was placed on the top side of the test surface to prevent direct contact of the test surface with the test subject’s arm and disposable wood spacers were placed on top of the top cover to elevate the test subject’s arm from the test substance and to prevent the mosquitoes from being able to feed on the test subject.
10. An untreated pre-treatment evaluation was conducted prior to testing the treated test surface by counting the number of mosquitoes that landed and probed on an untreated test surface during a 5-minute time period. Only cages that had pre-treatment activity of at least 3 probes were used for testing the treated surface.
11. The treated surface was evaluated in the same manner as the untreated surface and the repellency was calculated by comparing the number of landing and probes during the pre-treatment evaluation to the number of landing and probes during the treatment evaluation.

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12. The study was conducted using two different test subjects for each test substance.

RESULTS / DISCUSSION:

The results of this study are shown in Tables 1 and 2. Table 1 shows the average number of yellow fever mosquito (*Aedes aegypti*) landings and probes that occurred during the pre-treatment and treatment evaluations for each aged evaluation. The results in Table 2 show the percent repellency of the yellow fever mosquitoes, which was calculated by comparing the average number of mosquito landings and probes during the post-treatment evaluation to the average number of landing and probes that occurred during the pre-treatment control evaluation (1 – (treatment avg./pre-treatment avg.)).

The Thistle Farms Natural Insect Repellent recorded 100% landing and probing repellency during the 15-minute aged evaluation, compared to a 97% landing and probing repellency with the Honest Co. bug spray. The untreated control population only recorded 17% (landing) and 10% (probing) repellency rates.

CONCLUSION:

The results of the study indicate that the Thistle Farms Natural Insect Repellent is effective at repelling yellow fever mosquitoes (*Aedes aegypti*) within 15 minutes after the applications are conducted.
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Photograph 5. Forearm Placed above Membrane

[Image of forearm placed above membrane]

Photograph 6. Test Systems on Untreated Membrane

[Image of test systems on membrane]

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APPENDIX B: RAW DATA

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membrane is placed in the opening on top of the cage. The membrane is then covered with a section of mesh to prevent the arm from directly contacting it, then the arm is placed on top of the mesh.

10. The number of mosquitoes that land and probe through the test surface in the 2.5”x6” opening within 5-minutes is documented and the repellency is calculated by comparing the number of landing and probing during the pre-treatment evaluation to the number during the treatment evaluation.

11. The test substances should be re-evaluated as needed at each aged evaluation.

12. The study should be conducted using two (2) or more test subjects for each test substance.

STUDY NOTES:

<table>
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<tr>
<th>Initials</th>
<th>Date</th>
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<tbody>
<tr>
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</tbody>
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STUDY PERSONNEL:

Study Director: [Signature] Date: 01/08/16

Principal Investigator: [Signature] Date: [Redacted]
NON-GLP STUDY REPORT
Date: April 2016
Sponsor: Thistle Farms
Study: Mosquito Repellency 16
Trial: AEDSAE
Sponsor Code: N/A
Test Method: 411-5

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APPENDIX C: TEST SUBSTANCE RECEIVING LOG

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<tr>
<th>Date</th>
<th>Sponsor Code</th>
<th>Study Code</th>
<th>Lot No.</th>
<th>Batch</th>
<th>Shipment Date</th>
<th>Receipt Date</th>
<th>Area</th>
<th>Room</th>
<th>Package Cond.</th>
<th>Specimen</th>
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<th>QA Checker</th>
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### APPENDIX D: TEST SUBSTANCE STORAGE LOG

<table>
<thead>
<tr>
<th>Date</th>
<th>Current Temp (F)</th>
<th>Current Humid (%)</th>
<th>Traceable Serial Number</th>
<th>Current Time</th>
<th>Max Temp (F)</th>
<th>Min Temp (F)</th>
<th>Max Humid (%)</th>
<th>Min Humid (%)</th>
<th>Comments</th>
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<tbody>
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