Sponsor:Essante OrganicsStudy:Mosquito Repellency 16Trial:AEDSAESponsorCode:N/ATest Method:411-5

REPORT TITLE

Repellent Efficacy Study of Don't Bug Me! Bug Spray & Repellent against Yellow Fever Mosquitoes (*Aedes aegypti AKA Zika Mosquito*)

STUDY

Mosquito Repellency 16

AEDSAE

SPONSOR CODE N/A

EXPERIMENTAL START DATE September 22, 2016

EXPERIMENTAL COMPLETION DATE October 05, 2016

REPORT DATE

TEST FACILITY/AUTHORS

Eric S., Todd S., Joshua S., and Randy H. Insect Testers and Authors Facility Name on File Facility Location Meansville, GA. 30256

SPONSOR

Essante Organics

NON-GLP STUDY REPORT								
Sponsor:	Essante Organics							
Study:	Mosquito Repellency 16							
Trial Sponsor	AEDE							
Code: Test	N/A							
Method:	411-5							

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

<u>No</u> 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(1)(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: Essante Organics

NON-GLP STUDY REPORTSponsor:Essante OrganicsStudy:Mosquito Repellency 16Trial:AEDSAESponsorCode:N/ATest Method:411-5

Date: October 2016

COMPLIANCE STATEMENT

This study was <u>NOT</u> 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.

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Test Facility Management:

Eric J.S.

Date: 10/27/16

St

Date: 10/27/16

Study Director:

Todd S.

NON-GLP STU	DY REPORT
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Study:	Mosquito Repellency 16
Trial:	AEDSAE
SponsorCode:	N/A
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STUDY OBJECTIVE(S):

To measure the repellency associated with Essante Orgnaics Don't Bug Me! formulation against yellow fever mosquitoes (*Aedes aegypti AKA Zika Mosquito*).

TEST SUBSTANCE INFORMATION:

#	Test Substance	Active Ingredients	EPA Reg. No. and/or Est. No.	Lot and/or Batch #	Receipt Code
1	Control Untroated	NI/A	N/A	N/A	N/A
1	Control - Ontreated	IVA	IN/A	IN/A	IN/A
2	Don't Bug Me! Spray	40.0% Soybean Oil, 10.0% Castor Oil, 3.65% Lemongrass Oil, 3.4% Citronella Oil, 1.3% Cedar Oil, 1.25% Rosemary Oil, 1.0% Peppermint Oil, 0.2% Geranium Oil	N/A	N/A	Product #210

TEST SYSTEM INFORMATION:

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

TEST DESIGN (per aged evaluation):

# Reps per Substance	# Systems per Rep	# Systems per Substance
2 - controls; 4 - test substance	25	50-100

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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 finger pump 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-minute aged evaluation were air dried and then evaluated. The membranes used for the additional aged evaluations were allowed to air dry for 15-minutes after the applications and then they were placed on top of water moistened paper towels (lightly moistened) to maintain a "skin like" consistency by preventing complete moisture loss from the membranes while aging. The membranes were placed on the paper towels with the untreated side in contact with the paper towels and water was added to the paper towels as needed to maintain moisture during the aging process.

Aged Evaluations:

7. The controls and each test substance was evaluated at 15-minutes, 2 hours, and 4 hours after the applications. Each evaluation consisted of 2 replicates for the controls and 4 replicates for the test substances.

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 researcher's arm above the membrane. Mesh was placed on the top side of the membrane to prevent direct contact of the membrane with the researcher's arm and disposable wood spacers were placed on top of the

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- 10. An untreated pre-treatment evaluation was conducted prior to testing the treated membrane by counting the number of mosquitoes that landed and probed on an untreated membrane during a 5-minute time period. Only cages that had pre-treatment activity of at least 3 probes were used for testing.
- 11. The treated membrane was evaluated in the same manner as the untreated membrane 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.
- 12. The study was conducted using two different researchers (attractant) for each test substance and aged evaluation.

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: % repellency = (1 - (treatment avg./pre-treatment avg.)).

Don't Bug Me! formulation provided high mosquito repellency during each aged evaluation (15-minute, 2-hours, and 4-hours). The Don't Bug Me! formulation recorded 100% landing and probing repellency during the 15-minute and 2-hour evaluation, and recorded 99% landing and 100% probing repellency during the 4-hour evaluation. The untreated control populations showed an increase in landing and probing activity during all three aged evaluations.

CONCLUSION:

The results of the study indicate that Essante Organics Don't Bug Me! formulation is capable of providing high repellency against yellow fever mosquitoes (*Aedes aegypti*) within 15-minutes, 2-hours, and 4-hours after the applications.

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TABLES:

Table 1.

Yellow Fever Mosquitoes (<i>Aedes aegypt</i> i)												
Average Number of Landings & Probes												
Pre-treatmentTreatmentPre-treatmentTreatmentActivityActivityActivityActivityActivity								Treat Acti	ment vity			
	15 min		15 min		2 hr		2 hr		4 hr		4 hr	
Test Substance:	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes
Controls - Untreated	30	27	31	28	25	21	31	27	20	17	27	23
Don't Bug Me! Spray	27	23	0	0	36	32	0	0	28	25	0	0

Table 2.

Yellow Fever Mosquitoes (Aedes aegypti)										
Percent Repellency of Landings & Probes Based on Pre-Treatment Activity (negative numbers represent an increase in activity)										
	15 min 2 hr 4 hr									
Test Substance:	Landings	Probes	Landings	Probes	Landings	Probes				
Controls - Untreated	-2%	-6%	-27%	-26%	-38%	-32%				
Don't Bug Me! Spray	100%	100%	100%	100%	99%	100%				

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APPENDIX A: PHOTOGRAPHS

Photograph 1. Test Substance



Photograph 2. Treated Collagen Membrane



Photograph 3. Mosquitoes on Untreated Collagen Membrane



Photograph 4. Test Cage with Membrane



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Study:	Mosquito	Repellency	16					Trial:	AEDSAE						
Test Are Treatme	test Arena Info: reatment Arena: 1'x1' mosquito cages with 25 female mosquitoes. 1'x1' wooden board with 2.5" x 6" opening placed over top of arena, collagen membrane across opening.							Test Syste Strain/Stag Exposure 1 Exposure 1	am: pe/Age: Time/Date: Duration:	n: Yellow Fever Mosquito /Age: Lab/Adult Females/Purchased-Lab me/Date: <u>Sea</u> Bellow wation: 5 min					
Test Substance Info: Mix Time/Date: <u>N/A</u> Treatment Time/Date: <u>Sub</u> Dry Time/Evaluation: <u>Sub</u>			N/A - Astervisk Anto: See Balano												
Start Da Rep II's: Rep II's: Rep II's:	A-B C-D A-D4	nental Cond Date(s): Date(s): Date(s):	itions: on/r on/a elas	12/16 6/14 1/14	Temp (F): Temp (F): Temp (F):	Appli 10 10	RH%: RH%: RH%: RH%:	48 48 72	Temp (F): Temp (F): Temp (F):	Labor 72 72 72 72	atory: RH%: RH%: RH%:	41 50 52			
Test Sub	stance/Mix	Rate:	Controls - I	Delsamu							-				
Applicati	ion Details:		N/A												
	1		Pro-treatment Activity (time after applicatio					ins) Treatment Activity (time after ap					plications)		
	Sta	t Time /	15 min		2 hr		46		15	min	2	hr	44		
Rep	Rea	earcher:	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	
A	12:50 AA	(Jul (A)	31	25	24	20	32	20	28	25	31	21	31	29	
В	(0:00 Am	Real, (1)	21	28	25	22	17	M	33	31	38	34	40	16	
Initials:	iy —		4 AH	4-44	ALH	ARH	41 64	A KH	44-64	AV KH	AL AH	4 64	APRIL	44 5-11	
Date:	_		012416	011116	01/32/16	14/35 110	N/IS/IL	MISSIN	Set > No	India lie	0/01/16	06164164	A 165 / 16	11/10/19	
Test Sul	hetance/Max	Date:	Don't Ru	a Mal											
Applicat	ion Details	- variati	Collagen g	g mot	sprayed with	h test subs	lance using	finger pum	o spraver un	nil wet, then	gently rubb	ed into the	membrane		
	1		P	re-treatme	ont Activity	time after	application	(5)	I	Treatment	Activity (ti	me after ap	plications)	
8	Sta	t Time /	15	min	2	hr	1 1	4	15	5 min		hr	46	-	
Rep	Res	earcher:	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	Landings	Probes	
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C	1072544	Reals(P)	24	25	28	37	P.M	24	0	0	0	0	0	a	
D	150044	Rundale	ac	24	33	30	25	23	0	0	0	0	0	0	
initials:			AL RH	AT KH	4-64	14 R.4	At AH	Ar 64	11-65	Ar CH	A KH	Ar Kit	ARH RH	114	
Date:			1-1 22-14	0-22-14	05/22/16	ca)zehL	NISIA	phan	astering	\$A20 14	05/28/16	ONIMIL	10/05/12	1/2/15A	

Research Name:	Taken	S	tr_s	gnature:	John	St	Date(s):	Ales the Role:	Print Tow
Name:	fandy	K	dys	gnature _	18	All	Date(s):	O122/11 Role:	Ch s

Date: October 2016

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Essante Organics Mosquito Repellency 16 AEDSAE

EXEMPT from EPA registration under FIFRA.

DIRECTIONS: Shake well. Spray directly on skin (and clothing) to be protected. Avoid eyes, mouth, fingers. Face application: Spray on cotton ball, wipe forehead, cheeks, chin. Re-apply as needed.



ACTIVE INGREDIENTS: 40% Organic Soybean Oil 10% Organic Castor Oil 3.65% Organic Castor Oli 3.65% Organic Lemongrass Oil 3.40% Organic Citronella Oil 1.30% Organic Cedar Wood Oil 1.25% Organic Rosemary Oil 1.00% Organic Peppermint Oil 0.20% Organic Geranium Oil **INACTIVE INGREDIENTS:** 39% Deionized Filtered Water 0.20% Organic Lavender Oil

EFFICACY: See website for a FULL LIST OF BUGS and the essential oils they hate the most!

CAUTION: Do not use on pets. Keep out of reach of children. Always supervise application on children. Do not swallow. In case of eye contact generously flush eyes with water. May cause skin irritation in rare cases - test small area of skin first. Wash with soap and water and seek medical attention if skin irritation persists, or if bitten or stung by an insect. Consult physician if pregnant.

EssanteOrganics 11001 N 24th Ave. Phoenix AZ 85029 Certified organic by Baystate Organic Certifiers







to repel mosquitoes & ticks up to 4 hours



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TEST SUBSTANCE STORAGE LOG

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Date	Current	Current	Traceable	Current	Max	Min	Max	Min	Comments	Initials
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pilit.	bb	48	101514696	10' TOAN	LY	LH	54	44		14
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