REPORT TITLE
Efficacy of 050515-3-A-SNE Nature-Cide Insecticidal Dust

STUDY
Product Development 15

TRIALS
CTECFE / RHIPSA / CIMXLE / BLTTGE / MONOPH / MUSCDO / SOLEIN

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April 23, 2015

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September 2015

SPONSOR
Nature-Cide

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STUDY OBJECTIVE(S):

To determine the mortality and repellency associated with the 050515-3-A-SNE insecticidal dust

TEST SUBSTANCE INFORMATION:

1. Controls - Untreated
2. 050515-3-A-SNE Insecticidal Dust
3. Sentry Natural Defense® Natural Flea & Tick Carpet Powder (1.00% Peppermint Oil, 1.50% Cinnamon Oil, 1.50% Lemon Grass Oil, 1.70% Clove Oil, 1.70% Thyme Oil), 1582MSP101,(030713-1-A-SNE)

TEST SYSTEM INFORMATION:

Mortality Evaluation:

Trial	Test System	Strain	Stage/Age	Source
CTECFE	Cat Flea (*Ctenocephalides felis*)	Lab	Eggs, Larvae, & Adults	Purchased
RHIPSA	Brown Dog Tick (*Rhipicephalus sanguineus*)	Lab	Nymphs & Adults	Purchased
CIMXLE	Bed Bugs (*Cimex lectularius*)	"Cooper 2" Wild	Adults	Collected/Reared
BLTTGE	German Cockroach (*Blattella germanica*)	Lab	80% Mixed Nymphs, 10% Adult Males, 10% Non-Gravid Adult Females	Purchased / Lab Reared
MONOPH	Pharoah's Ants (*Monomorium pharaonis*)	Field	Workers	Collected/Lab Reared
MUSCDO	House Fly (*Musca domestica*)	Lab	Adults (2-3 days old)	Purchased
SOLEIN	Red Imported Fire Ant (*Solenopsis invicta*)	Field	Workers	Field

Flea Repellency Evaluation:

Trial	Test System	Strain	Stage/Age	Source
CTECFE	Cat Flea (*Ctenocephalides felis*)	Lab	Adults	Purchased

Tick Repellency Evaluation:

Trial	Test System	Strain	Stage/Age	Source
RHIPSA	Brown Dog Tick (*Rhipicephalus sanguineus*)	Lab	Adults	Purchased

MATERIALS AND METHODS:

The following is the Standardized Testing Method for evaluating the efficacy of pesticides when applied as residual applications against various arthropod species. Further details related to this specific study are described following the test method summary. Select action items and illustrations have been removed from this standardized test method in an effort to make the report more precise and accurate to the study conducted. Any details removed from this test method were deemed irrelevant to the study conducted in this report.

Mortality Evaluation:

310.1 Materials:

Test Arena Information:

310.1.1 Test Arenas: Petri dishes and/or SOLO cups were used as the test arenas.

310.1.2 Post-Treatment Arenas: Various containers were used per species. The Post-Treatment arenas were used to contain the test systems in a clean environment after exposure to the test substance(s).

310.1.3 Food/Moisture: Various food and moisture items were used per species.

Test Equipment:

310.1.4 CO_2 and Regulator: A standard 20 pound CO_2 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.

310.1.5 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.

310.1.6 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.

310.2 Methods:

Test Substance Preparation & Applications:

310.2.1 The test substance container(s) were clearly labeled with the test substance name.

310.2.2 The applications were conducted by applying a minimum of 0.05-0.25 grams (amount used was dependent on the test system) into each test arena. Care was taken to make sure each replicate was coated evenly and with similar amounts of dust.

310.2.3 The test substances were evaluated immediately after the applications under ambient laboratory conditions.

Test Design:

310.2.4 The evaluations of this study followed the photographs in Appendix A: Photograph section of the report.

310.2.5 Prior to applications, each Surface Type 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 Surface Types and/or 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).

310.2.6 The test systems were sorted onto the surfaces using the appropriate methods based on the species type.

310.2.7 All of the test systems were confirmed to be of "good vigor" (alive) prior to exposure to the surfaces.

310.2.7.1 Only live test systems were selected for use in the study.

310.2.7.2 After all test systems were transferred onto the surfaces, they were confirmed to be alive and exhibiting normal behavior before continuing with the study.

310.2.8 The number of replicates conducted per test substance and the number of test systems evaluated per replicate were conducted using 3 replicates of 10 specimens per replicate (30 specimens per test substance) with most trials. The total number of specimens used per trial was dependent on the availability of the test systems, and therefore for certain trials, the replicates and number per replicate may have been slightly more or less than 30 per test substance.

Observation Methods:

310.2.9 The number of "Alive", "Knockdown (KD)", and "Dead" test systems per arena was recorded prior to surface exposure (Pre-trt), and at hourly observations after exposure to the dusts, and then daily as needed after exposure.

310.2.10 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.

310.2.11 The test systems were held in the test arenas with the dust for 4-24 hours (time was dependent on the test systems) and then they were transferred into clean post-treatment arenas.

310.2.12 Definitions of "Alive", "Knockdown (KD)", and "Dead":

310.2.12.1 Alive – Test System exhibited normal forward motion and/or the ability to fly.

310.2.12.2 Knockdown (KD) – Test System exhibited some movement, but could not crawl and/or fly.

310.2.12.3 Dead - Test System exhibited no movement, even when stimulated.

Environmental Conditions:

310.2.13 The test systems were tested under ambient laboratory conditions.

Flea Repellency Evaluation:

322.1 Materials:

Test Arena Information:

322.1.1 Treatment Arena for housing the treatment replicates: 150mm Petri dishes.

322.1.2 Water Moat Tubs: 22"L x 15"W x 5"H plastic tubs with lids.

322.1.3 Testing Surfaces: Builder grade carpet.

322.1.4 Filter Paper: 55mm filter paper used to cover the fleas after they were introduced into the test arenas.

Test Equipment:

322.1.5 Digital Balance(s): Balances were used as needed in preparing and/or weighing the test substance canisters before and after applications.

322.1.6 CO_2 and Regulator: A standard 20 pound CO_2 cylinder with regulator was used to anesthetize the test systems and sort them into the test arenas (prior to exposure to the test substances).

322.1.7 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.

322.1.8 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.

322.2 Methods:

Test Design:

322.2.1 The Water Moat Tubs were placed in the test room and labeled with the appropriate test substance and replicate code.

322.2.2 Each Water Moat Tub was filled with approximately 2 inches of water.

322.2.3 Drops of liquid detergent were added to the water as a surfactant to aid in the drowning of fleas that left the treatment surfaces and landed in the moat.

322.2.4 The Petri's were placed on top of a 2" x 4" wood block in the center of the Water Moat Tubs.

322.2.5 The carpets were cut so that they fit "tightly" inside the Petri dishes (1 per Petri).

322.2.6 This setup was conducted using 4 replicates per test substance.

Test Substance Preparation & Applications:

322.2.7 The applications were conducted by treating each carpet with the following application rates:

322.2.7.1 050515-3-A-SNE - 12.8oz/500ft^2

322.2.7.2 050515-3-A-SNE - 5.5oz/500ft^2.

322.2.7.3 Sentry - 10oz/500ft^2 (Sentry's label recommends 10oz to cover 500-1000ft^2.)

Test System Introduction:

322.2.8 The fleas (10 per replicate) were sorted by the appropriate means into intermediate containers.

322.2.9 The fleas were introduced into the center of each arena and were covered with the filter paper and the lids of the tubs were closed. (This signaled the start of the test).

322.2.10 The test room door was closed and the lights were turned "off" for the duration of the test.

Observations:

322.2.11 After 4 hours, the lids were removed from the tubs and the Petri dishes were immediately removed from the tubs and covered.

322.2.12 The number of fleas observed in the water moats was counted and were considered "repelled".

Environmental Conditions:

322.2.13 The test systems were tested under ambient laboratory conditions.

Tick Repellency Evaluation:

316.1 Materials:

Test Arena Information:

316.1.1 Petri Dishes: 150mm and 90mm Petri dishes.

316.1.2 Filter Paper: 4 cm. x 8 cm. papers

Test Equipment:

316.1.3 Digital Balance(s): Balances were used as needed in preparing and/or weighing the test substance canisters before and after applications.

316.1.4 CO_2 and Regulator: A standard 20 pound CO_2 cylinder with regulator was used to anesthetize the test systems to sort into sets of 10.

316.1.5 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.

316.1.6 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.

316.2 Methods:

Test Substance Preparation & Applications:

316.2.1 200mg of the test substance was placed inside a fine mesh bag.

316.2.2 The test substances were aged under ambient laboratory conditions and evaluated weekly.

Test Design:

316.2.3 The Petri dishes were assembled by placing one base inside one another. Water was poured into the outer dish to act as a moat for stopping the ticks from escaping.

316.2.4 The paper was cut to 4 cm (width) x 8 cm (length).

316.2.5 Lines were drawn at 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, and 6 cm from the bottom of the paper.

316.2.6 The 1 cm line at the bottom of the filter paper was folded over until it reached the 2 cm line to create a “basket” to introduce the test systems into.

316.2.7 The test substance/mesh bag was attached to the top 2 cm of the paper (6 cm line to 8 cm top of paper).

316.2.8 This setup was conducted using 4 replicates per test substance and per aged evaluation.

Test System Introduction:

316.2.9 Once the appropriate dry time was reached, the paper was suspended slightly above the 90mm Petri dish and the ticks (10 per replicate) were introduced into the "basket" of each paper.

Observations:

316.2.1 The location of the ticks on the paper (2 cm (basket), 3 cm, 4 cm, 5 cm, 6 cm, on treatment (test substance), or dropped off the paper) was recorded at 3, 5, and 10 minutes after the ticks were placed in the basket.

316.2.2 The degree of repellency was calculated by considering the ticks that remained in the 2 cm/basket section and/or that dropped off the paper as 100% repelled from the formula. If the ticks crawled up towards the treatment and into the 3 cm section they were considered 80% repelled, ticks in the 4 cm section were 60% repelled, ticks in the 5 cm section were 40% repelled, ticks in the 6 cm section were 20% repelled, and ticks that contacted the treated area were 0% repelled. The percent repellency for each replicate was calculating by multiplying the total number of ticks in each section by the appropriate repellency percentage for the section and then taking the sum of all percentages for that replicate. An average of the percent repellency for each replicate was calculated to determine the final repellency percentage.

316.2.3 Ticks that crawled completely up the paper and onto the treated area were removed to prevent them from returning to the lower un-treated portions and providing a false reading.

Environmental Conditions:

316.2.4 The test systems were tested under ambient laboratory conditions.

RESULTS / DISCUSSION:

Mortality Evaluations:
The results from the mortality evaluations are shown in Tables 1-2. Table 1 shows the percent mortality for the motile test systems and Table 2 shows the percent eclosion of the cat flea eggs. In addition to the percent mortality that is shown in the tables, the mortality rates for each trial (test system) were statistically analyzed using a t test for independent samples. The analysis was conducted using a one- tailed distribution and probability value of p=0.05 to evaluate if any significant differences in mortality were recorded between the un-treated controls and the test substances. For any trials that had competitive formulations tested, a second analysis was conducted using a two-tailed distribution and probability value of p=0.05 to evaluate if any significant differences in mortality were recorded between the test substances.

All of the completed trials recorded 100% mortality with the 050515-3-A-SNE treated specimens. Significant differences in the mortality rates between the specimens treated with the 050515-3-A-SNE formulation and the un-treated controls were recorded with all of the evaluated species. The 050515-3- A-SNE formulation also outperformed the competitive product during several species evaluations.

Flea Repellency Evaluation:
The results from the cat flea repellency evaluation are shown in Table 3. The fleas that were exposed to the carpets that were treated with the 050515-3-A-SNE formulation recorded 80% (12.8oz/500ft^2) and 70% (5.5oz/500ft^2) repellency, compared to 78% repellency with the specimens that were exposed to the Sentry Natural Defense® carpet powder. The untreated control population recorded 30% repellency.

Tick Repellency Evaluation:
The results from the tick repellency evaluation are shown in Table 4, which illustrates the percent repellency of the brown dog ticks during each weekly aged evaluation. The ticks that were tested with the 050515-3-A-SNE formulation recorded 36-91% repellency during the 0-11 week aged evaluations, with an average of 65% repellency during the study. In comparison, the controls recorded 24-66%, with an average of 43% repellency.

CONCLUSION:

It is evident from the results of the study that the 050515-3-A-SNE carpet powder provides adequate mortality against egg, larval, and adult stage fleas, nymph and adult stage ticks, and adult stage bed bugs, German cockroaches, Pharoah's ants, house flies, and fire ants. The results also prove that the 050515-3-A-SNE carpet powder provides measureable repellency against cat fleas when used at either a 12.8oz/500ft^2 or a 5.5oz/500ft^2 application rate and against brown dog ticks for up to 11 weeks after applications.

TABLES:

Table 1.

% Mortality									
Test System:	**Test Sub.**	**Pre-trt**	**4 hr**	**24 hr**	**2 DAT**	**3 DAT**	**4 DAT**	**5 DAT**	**6 DAT**
Cat Flea Larvae	Controls - Untreated	0%	0%	0%					
	050515-3-A-SNE	0%	100%	100%					
	Sentry Natural Defense® Natural Flea & Tick	0%	100%	100%					
Cat Flea Adults	Controls - Untreated	0%	0%	0%	3%				
	050515-3-A-SNE	0%	93%	97%	100%				
	Sentry Natural Defense® Natural Flea & Tick	0%	0%	30%	73%				
Brow n Dog Tick Nymphs	Controls - Untreated	0%	0%	0%					
	050515-3-A-SNE	0%	27%	100%					
	Sentry Natural Defense® Natural Flea & Tick	0%	7%	27%					
Bed Bug Adults	Controls - Untreated	0%	0%	0%					
	050515-3-A-SNE	0%	0%	100%					
	Sentry Natural Defense® Natural Flea & Tick	0%	0%	100%					
German Cockroaches	Controls - Untreated	0%	0%	0%	0%				
	050515-3-A-SNE	0%	83%	97%	100%				
Pharoah's Ants	Controls - Untreated	0%	0%	0%	3%				
	050515-3-A-SNE	0%	97%	100%	100%				
House Flies	Controls - Untreated	0%	0%	0%	20%				
	050515-3-A-SNE	0%	57%	90%	100%				
Fire Ants	Controls - Untreated	0%	0%	0%	0%	3%	3%	7%	13%
	050515-3-A-SNE	0%	93%	97%	97%	97%	97%	97%	100%
Brow n Dog Tick Adults	**Test Sub.**	**Pre-trt**	**24 hr**	**4 DAT**	**5 DAT**	**6 DAT**	**9 DAT**	**10 DAT**	
	Controls - Untreated	0%	0%	4%	4%	4%	4%	4%	
	050515-3-A-SNE	0%	21%	63%	71%	92%	96%	100%	
	Sentry Natural Defense® Natural Flea & Tick	0%	17%	33%	29%	33%	50%	63%	

Table 2.

% Egg Eclosion								
Test System:	**Test Sub.**	**Pre-trt**	**4 hr**	**24 hr**	**2 DAT**	**3 DAT**	**4 DAT**	**5 DAT**
	Controls - Untreated	0%	N/A	7%	13%	22%	32%	35%
	050515-3-A-SNE	0%	N/A	0%	0%	0%	0%	0%
Cat Flea Eggs	Sentry Natural Defense® Natural Flea & Tick	0%	N/A	0%	0%	0%	0%	0%

Table 3.

Cat Flea Repellency		
Test Substance	**Application Rate**	**% Repelled**
Control- Untreated	NA	30%
050515-3-A-SNE	12.8oz/500ft2 (0.12g/carpet)	80%
050515-3-A-SNE	5.5oz/500ft2 (0.05g/carpet)	70%
Sentry Natural Defense® Natural Flea & Tick	10oz/500ft2 (0.09g/carpet)	78%

Table 4.

Brown Dog Tick Repellency												
	Weeks											
Test Substance	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**Avg**
Control - untreated	24%	36%	30%	49%	46%	54%	29%	49%	66%	50%	47%	**43%**
050515-3-A-SNE	54%	48%	45%	72%	67%	91%	36%	77%	87%	71%	67%	**65%**

APPENDIX A: PHOTOGRAPHS

Photograph 1. Example of Carpet Powder inside Petri