MRID 498001-10: Evaluation of Residual Activity

The study objective was to evaluate the long-term residual efficacy of Aprehend spray applications on six different surface types (quilted fabric, polyester, vinyl base board, unpainted wood, water-based painted wood, and water-based painted drywall) over three months. Treatments consisted of a combination of type of substrate and time intervals since time of application. Specifically, efficacy of the sprayed surfaces was evaluated on day 1, 32, 35, 70, and 97 post- spraying. The product was sprayed as specified on the product label. A spray gun attached to a mini air compressor running at 3-5 psi was used with a flow rate of 14.26 mL/min. A 2" spray band was applied by holding the gun approximately 4" from the surface, moving at a speed of 3-4 seconds/linear meter. Four linear spray swaths with a volume each of 1.6 ± 0.4 µL/cm2 were prepared for each surface type, resulting in an average concentration of 4.3 ± 2.1 x 106 µL conidia/cm2. Four replicates were prepared for each treatment and sub-divided into five separate areas prior to exposure. In this experimental design, 'time since application' was replicated once only for each surface type treatment (4 total). Different groups of 10 adult bed bugs (mixed sex) were randomly exposed to each treatment for 15 minutes on different surfaces treated at different time points (on day 1, and then subsequently at day 32, day 35, day 70 and day 97 post spray application (3 months)). At the end of the exposure time, each group of bed bugs was removed from the test surface, placed in individual containers provided with a piece of untreated filter paper folded as harborage and kept in a dark incubator at 23 ± 1 ° C, 50 ± 5 % RH. Bed bugs were checked daily for mortality for a period of 14 days. Daily mortality data were analyzed using Kaplan-Meier Survival Analysis with 95% confidence intervals for estimated means and median survival times. The Log-rank test was used for post-hoc analysis to determine whether survival distributions of populations were statistically different from one another. Mycosis was confirmed from 3 sub-samples of bed bug cadavers taken from each surface by age treatment combination. The cadavers, labeled according to treatment replicate and date of death, were incubated at 100 % humidity. A standard germination test (SOP 6) was employed to determine the viability of conidia over time. Bed bugs used in this study were a combination of field strain bed bugs from a lab colony maintained at Penn State University since 2005 and a lab colony of Harlan strain bed bugs maintained at NC State University since 2008. All bed bugs were maintained under standard conditions of 23 ± 1 ^{0}C; 50 ± 5 % relative humidity (RH); photoperiod: 14:10h (L:D), and fed weekly on blood meal (Human, O positive) via an artificial feeding system as recommended by OCSPP 810.3900 guidelines, *Laboratory Testing Methods for Bed Bugs Pesticide Products*. Mean Survival Times (MST) were similar for both bed bug strains on all surfaces; lower and upper bound confidence intervals overlapped in all but the painted wood treatment. Results on residual activity of the product on different surfaces at different exposure times are tabulated below. MSTs varied for different surfaces treated at different application times (refer to tables 1 to 6 below). Percent mortality of 92% or greater was achieved after 14 days post exposure to box spring fabric (quilted cotton fabric), unpainted wood, water base painted wood, and drywall treated with the product 3 months prior to exposure to bed bugs. These results show that, independent of percentage mycosis recorded from bed bug cadavers collected from different treatments (See Table 7 under Study Summary below), it was the type of substrate that determined the residual efficacy of the product on bed bug mortality over time. Results from 3 month residual activity on polyester and vinyl base board do not support the label claim that the product was effective up to 3 months in controlling bed bugs. This efficacy claim is only supported by data from cotton fabric, unpainted wood, water-base painted wood, and drywall. Conidial viability was observed to decline over time as measured by direct germination counts on box spring cover, dust cover, vinyl baseboard, unpainted wood, painted wood and dry wall. Germination results show an overall decline in conidial viability, and no apparent relationship between conidial viability and percent mortality was apparent over time. Even at ≈20% conidial viability, the efficacy of the spray residue remained higher as measured by bug mortality after 3 months on cotton fabric and unpainted wood (Tables 1 and 4) than that on polyester, vinyl and drywall substrates (Tables 2, 3 and 6). The researcher's explanation that surface type played a role in facilitating /inhibiting conidial transfer to bed bug did not explain this lack of correspondence between residual conidial viability and percent mortality on different surfaces.

Table 1. Mean survival time and total mortality of bed bug populations following 15 minutes exposure to box spring fabric treated with Aprehend at recommended label application rate. Exposures were conducted 1, 32, 35, 70 and 97 days after spray application. Residual activity on	**Mean survival time (days)**	SEM	95% CI Lower Bound	95% CI Upper bound	**Total % mortality**	% [conidial] Viability spray residue
Box spring fabric (quilted cotton fabric)						
Day 1 (field strain)	**4.04**	0.07	3.90	4.18	**100**	86.84±1.39
Day 32 (field strain)	**4.7**	0.14	4.44	4.96	**100**	86.54±0.68
Day 35 (Harlan strain)	**4.32**	0.07	4.19	4.45	**100**	
Day 70 (Harlan strain)	**8.8**	0.60	7.63	9.97	**72**	45.41±1.92
Day 97 (Harlan strain)	**7.64**	0.50	6.66	8.62	**92**	22.49±0.83

Table 2. Mean survival time and total mortality of bed bug populations following 15 minutes exposure to dust cover fabric treated with Aprehend™ at recommended label application rate. Exposures were conducted 1, 32, 35, 70 and 97 days after spray application. Residual activity on	**Mean survival time (days)**	SEM	95% CI Lower Bound	95% CI Upper bound	**Total % mortality**	% Viability spray residue
Dust cover fabric (polyester)						
Day 1 (field strain)	**8.24**	0.53	7.19	9.29	**92**	85.80±0.59
Day 32 (field strain)	**7.1**	0.47	6.17	8.03	**86**	90.43±0.91
Day 35 (Harlan strain)	**8.06**	0.48	7.11	9.01	**88**	
Day 70 (Harlan strain)	**6.72**	0.42	5.9	7.54	**94**	52.66±1.12
Day 97 (Harlan strain)	**9.6**	0.58	8.43	10.69	**68**	43.70±0.99

Table 3. Mean survival time and total mortality of bed bug populations following 15 minutes exposure to vinyl baseboard treated with Aprehend at recommended label application rate. Exposures were conducted 1, 32, 35, 70 and 97 days after spray application.

6 Residual activity on **Vinyl baseboard**	**Mean survival time (days)**	SEM	95% CI Lower Bound	95% CI Upper bound	**Total % mortality**	% Viability spray residue
Day 1 (field strain)	**3.26**	0.01	3.06	3.46	**100**	84.40±1.41
Day 32 (field strain)	**6.66**	0.47	5.73	7.59	**88**	86.38±0.90
Day 35 (Harlan strain)	**5.88**	0.25	5.39	6.38	**98**	
Day 70 (Harlan strain)	**8.22**	0.51	7.21	9.23	**80**	28.26±0.87
Day 97 (Harlan strain)	**7.84**	0.54	6.79	8.90	**80**	40.57±1.92

Table 4. Mean survival time and total mortality of bed bug populations following 15 minutes exposure to unpainted wood treated with Aprehen at recommended label application rate. Exposures were conducted 1, 32, 35, 70 and 97 days after spray application. Residual activity on	**Mean survival time (days)**	SEM	95% CI Lower Bound	95% CI Upper bound	**Total % mortality**	% Viability spray residue
Unpainted wood						
Day 1 (field strain)	**8.8**	0.48	7.85	9.45	**76**	ND*
Day 32 (field strain)	**6.76**	0.50	5.78	7.74	**86**	60.26±3.70
Day 35 (Harlan strain)	**6.7**	0.38	5.96	7.44	**94**	
Day 70 (Harlan strain)	**8.38**	0.531	7.34	9.42	**76**	34.04±3.22
Day 97 (Harlan strain)	**6.14**	0.33	5.49	6.79	**96**	27.39±1.08

Table 5. Mean survival time and total mortality of bed bug populations following 15 minutes exposure to painted wood treated with Aprehend at recommended label application rate. Exposures were conducted 1, 32, 35, 70 and 97 days after spray application. Residual activity on	**Mean survival time (days)**	SEM	95% CI Lower Bound	95% CI Upper bound	**Total % mortality**	% Viability spray residue
Painted Wood						
Day 1 (field strain)	**5.22**	0.16	4.92	5.52	**100**	ND*
Day 32 (field strain)	**5.4**	0.13	5.16	5.64	**100**	51.96±0.89
Day 35 (Harlan strain)	**4.66**	0.07	4.52	4.80	**100**	
Day 70 (Harlan strain)	**4.12**	0.14	3.84	4.40	**100**	58.19±2.17
Day 97 (Harlan strain)	**5.32**	0.21	4.92	5.34	**100**	60.14±4.58

Table 6. Mean survival time and total mortality of bed bug populations following 15 minutes exposure to drywall treated with Aprehend at recommended label application rate. Exposures were conducted 70 and 95 days after spray application.

7 Residual activity on **Drywall**	**Mean survival time (days)**	SEM	95% CI Lower Bound	95% CI Upper bound	**Total % mortality**	% Viability spray residue
Day 1 (field strain)	**N.D.***	-	-	-	**N.D.**	N.D.
Day 32 (field strain)	**N.D.**	-	-	-	**N.D.**	N.D.
Day 35 (field strain)	**N.D.**	-	-	-	**N.D.**	
Day 70 (Harlan strain)	**5.48**	0.39	4.71	6.25	**100**	46.54±1.11
Day 97 (Harlan strain)	**5.04**	0.15	4.74	5.34	**100**	45.81±1.32

Table 7. Percentage mycosis of bed bug cadavers following incubation at 100 % humidity for 3 days at room temperature.

Surfaces Day Box spring cover fabric	Dust cover fabric	Vinyl baseboard	Unpainted wood	Painted wood	Drywall	Control	
1	100	100	100	100	100	ND*	0.00
32	94.00	86.04	93.18	83.72	98.00	ND*	0.00
35	100	100	100	100	100	ND*	0.00
70	100	100	100	97.37	96.00	100	76.92**
97	97.56	97.06	97.50	100	100	100	0.00