Report Title:
Evaluation of SafeRest Premium Mattress Encasement in Preventing Bed Bug *(Cimex lectularius)* Penetration and Feeding

Study:
Bed Bug Prevention of Mattress Covers 2010

Trial:
Bed Bug Fabric Feeding Prevention

Experimental Start Date:
November 5, 2010

Experimental Completion Date:
November 11, 2010

Report Date:
December 2010

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Objective(s):

To evaluate SafeRest Premium Mattress Encasement in preventing bed bug *(Cimex lectularius)* penetration and feeding.

Test Substances:

1. SafeRest Premium Mattress Encasement (Snell Code: 110110-1-D-BBS)
   1. Terry Cloth Fabric
   2. Zipper Closure

Materials and Methods:

The following is the Snell Scientifics Standardized Testing Method for evaluating the efficacy of fabric and closures as barriers to hematophagic arthropods. 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.

314.1 Materials:

314.1.1 Glass jars – pint size jars w/ screw on lids
314.1.2 8" Dia. PVC Pipe
314.1.3 Cardboard – harborage inserts inside jars
314.1.4 Fabrics- test fabrics and zipper enclosures.
314.1.5 Dark surface used to evaluate eggs or debris from the shake through method.
314.1.6 Feeding attractant – Human subject to attract bed bugs.
314.1.7 CO2 and regulator – standard 20 pound cylinders and gas regulator - used for anesthetizing the insects.
314.1.8 Intermediate transfer/holding chambers – used for housing the insects after they were removed from their primary breeding housing. Intermediate chambers were used to anesthetize the insects and sort them into jars.
314.1.9 Count down timer – used to accurately measure exposure times.

314.2 Methods:

314.2.1 Pint size jars were equipped with the test fabrics by:
- Placing the fabric over the open end of the jar and securing the outer screw-on lid over the fabric.

314.2.2 Pint jars were equipped with cardboard inserts that provided harborage for the bed bugs and also allowed access for the bed bugs to travel from the bottom of the jar to the lid/fabric area of the jar.

314.2.3 8" PVC pipes were equipped with the test fabrics by:
- Zipper closures were sealed onto the pipe end with clear silicon and an outer metal clamp.

314.2.4 Each jar contained approximately ~500 various size bed bugs (1^{st} instars – adults), eggs, and debris.

314.2.5 For evaluating the zipper or seam areas of the enclosures, ~500 bed bugs (1^{st} instars – adults), eggs, and debris were placed inside the PVC pipe.

314.2.6 Various sized bed bugs allowed for evaluating the possibility of different sized mouth parts feeding through the test fabrics.

314.2.7 Bed bugs used for feed through tests were starved for at least 7 days prior to testing.

314.2.8 To evaluate for feed through ability, the fabrics were held to human body parts for at least 15 minutes.

314.2.9 Following the 15 minute feeding exposure, the bed bugs were removed from the pint jars and inspected for signs of feeding.

314.2.10 The Zipper or seam enclosures were held to human body parts to evaluate the ability of the bed bugs to feed through the zippers.

314.2.11 Following feed-through method, the 8" PVC was shaken over a black surface (construction paper) for approximately 30 seconds.

314.2.12 Debris collected on the black surface was evaluated under a microscope to confirm if eggs were able to pass through.

314.2.13 If feeding through the zipper or seam enclosure was suspected, the bed bugs were inspected under a microscope for visual signs of feeding.

314.2.14 Feeding through the test fabrics was documented as yes/no or # fed during the 15 minute exposure.

314.2.15 Zipper and seam enclosures were documented as the ability for bed bugs to feed through the zippers/seams (yes/no), # fed, and quantitative amounts of debris shaken through the zipper/seams enclosures (ex. # 1^{st} instars, # eggs, etc.).

314.2.16 Additional Testing Details Not Fully Described in Standard Protocols:

Test Set-Up: The evaluations in this study followed Test Photographs 1-9.

Test System Information:

Test System	Strain	Stage/Age	# Replicates per Substance	# Specimens per Replicate
Bed Bugs *(Cimex lectularius)*	Susceptible	Mixed	1	~500

Source of Test Systems: Test specimens were laboratory reared prior to testing.

Environmental Conditions:

Conditions in Laboratory: Temperature: 77° F Humidity: 51%

Method Used to Evaluate Testing Results:

The insects were examined to ascertain if feeding had taken place. The measure of feeding was any additional color or swelling of the insect's abdomen**.**

Results / Discussion:

The results of this study are tabulated in Tables 1-3. Table 1 illustrates the number of bed bugs (*Cimex lectularius*) that fed through the fabric onto the human host, the number that escaped, and the number of blood spots that were observed on the fabric after the 15 minute evaluation. Table 2 illustrates the results from the "Shake-Through" method to evaluate if any debris could pass through the SafeRest zipper closure. Table 3 illustrates the number of bed bugs that fed through and escaped from the zipper closure.

The SafeRest terry cloth fabric and the zipper enclosure were effective in preventing bed bugs of all life stages from feeding on the human subject. The fabric and zipper closure also prevented all bed bug stages from escaping during the evaluations. Based on the results of this study, the SafeRest mattress encasement (terry cloth fabric and zipper closure) is effective for preventing penetration and feeding of bed bugs (*Cimex lectularius*) when exposed to a human subject.

Tables:

Table 1.

Fabric Feed Through Method: Terry Cloth Fabric						
Rep	Exposure Time	Bed Bug Stage	Approx #	# Fed	# Escaped	# Blood Spots on Fabric
A	15 min	Mixed	~ 500	0	0	0

Table 2.

Zipper Shake Through Method: Zipper					
Rep	Exposure Time	Bed Bug Stage	Approx #	# Shaken Thru or (Yes/No)	# Escaped
A	30 seconds	Mixed	~500	0	0

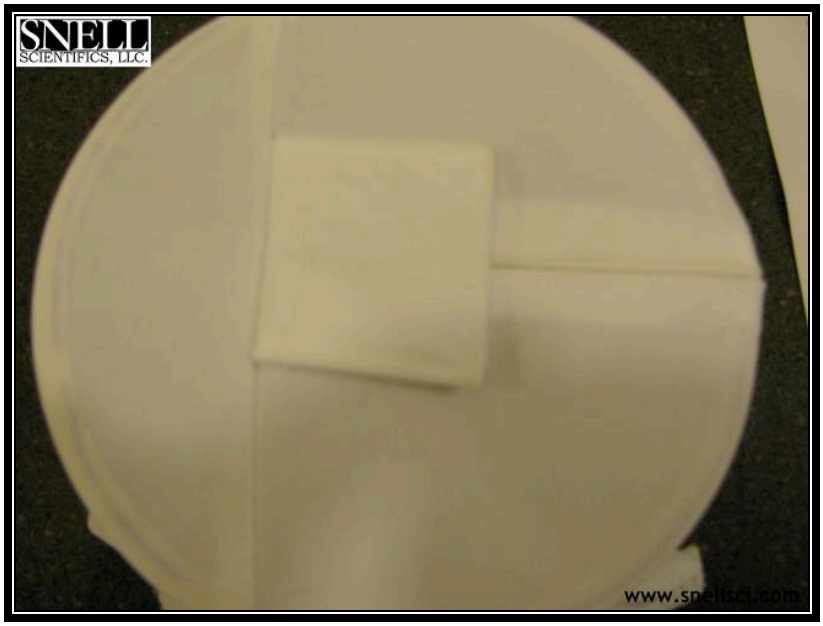
Table 3.

Zipper feed Through Method: Zipper					
Rep	Exposure Time	Bed Bug Stage	Approx #	# Fed	# Escaped
A	15 min	Mixed	~500	0	0

Appendix A: Photographs

Photograph 1. SafeRest Encasement

Photograph 2. Zipper closure on 8” PVC Pipe



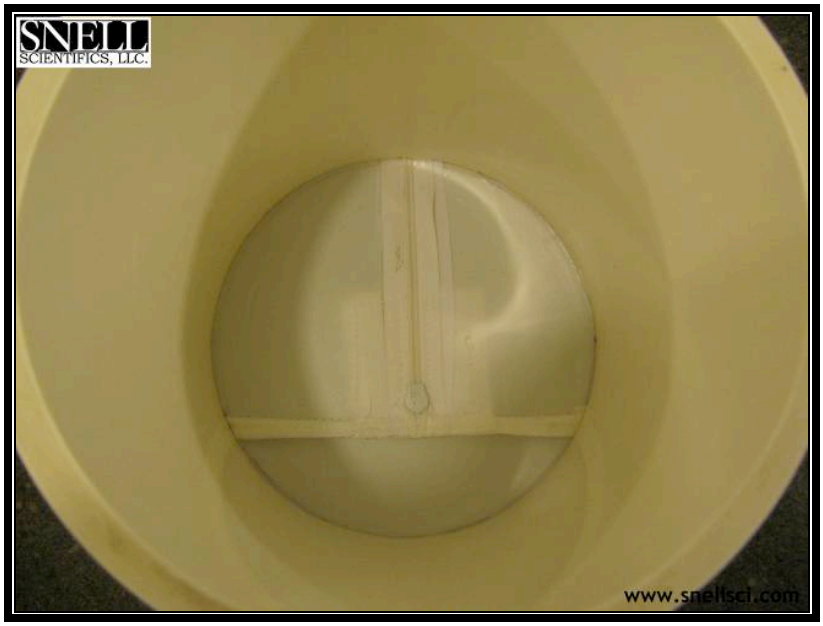
Photograph 3. Terry Cloth Fabric Sealed onto the Pint Jar

Photograph 4. Bed Bugs inside Pint Jar

Photograph 5. Bed Bugs Being Exposed to Human Host

Photograph 6. Zipper Sealed on 8" PVC Pipe

Photograph 7. Inside View of Zipper



Photograph 8. Bed Bugs in 8" PVC Pipe with Zipper

Photograph 9. Zipper Being Held to Human Host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Appendix B: Raw Data

Snell Scientifics LLC: Additional Test Details - Non GLP Tests **11/1/10**

Sponsor: **Bed Bud Supply/SafeRest**
Study: **Bed Bug Prevention of Mattress Covers 2010**

Contact: Mark Sanders

Files: Data File: BBSupplyBBFabric10
Worksheet: BB fabric 10
Report File: BBSupplyBBFabric10

Test Products Received: 11/01/10

The Evaluations in this Study Followed TM#: 314-2.00

Trial/Details: **Bed Bug Fabric Feeding Prevention**

- Fabric sections are to be sealed into pint jar lids
- Enclose unfed bedbugs inside jar (at least 7 day unfed)
- Expose jar to human body part for 15 minutes
- Monitor for any feeding

Prevention of escapes through closure seals (zippers)

- Zipper end sections are to be sealed into a 8" Dia. PVC Pipe end
- Zipper/Fabric is sealed to the pipe end with silicon (to prevent escapes from end of pipe) and an outer ring (to keep fabric pulled tight over end).
- Enclose unfed 1st instar bedbugs, bed bug eggs, adults and debris inside jar (at least 7 day unfed)
- Shake jar over a clean surface(~30 sec.) and monitor for eggs and debris falling through the zippers

Zipper Feed through Method

- Expose zipper to body part for 15 minutes
- Monitor for any feeding

Species/Replicates:

Test Species	Stage/Age	# Reps	# per Rep	# per Product	# Test Products	Total # Specimens	# Test Arenas
Bed Bugs (susceptible)	Mixed	1	~ 500	N/A	2	~ 1000	2

Source of Test Specimens: Laboratory reared

Insect Exposure time: Fabric feeding - 15 minutes to body part
Shake through - 30 second "shake through" test
Zipper feeding - 15 minutes to a body part

Additional Test Details Preparer: Todd Smith

Preparer Signature: _____ **Date:** 11/05/10

Primary Researcher Signature: _____ **Date:** 11/11/10

Snell Scientifics LLC: Additional Test Details - Non GLP Tests **11/1/10**

Sponsor: **Bed Bud Supply/SafeRest**
Study: **Bed Bug Prevention of Mattress Covers 2010**

Observation Times: Shake through: 30 seconds
Feed through: 15 minutes each

Test Products:
SafeRest Premium Mattress Encasement (Snell Code: 110110-1-D-BBS)

1. Terry Cloth Fabric
2. Zipper Closure

Additional Test Details Preparer: Todd Smith

Preparer Signature: T. S **Date:** 11/05/10

Primary Researcher Signature: E **Date:** 11/11/10

Sponsor: Bed Bug Supply/SafeRest TM#: 314-2 Page 1 of 1

Study / Trial: Bed Bug Prevention Test System: Bed Bugs Sus. Strain

Asterisk	* =
Details:	** =

Arena Details: Fabric sealed on a pint jar containing Bed Bugs

Other Details: Zipper end sealed on a 6" PVC pipe with Bed Bugs

Rep #'s: A(fabric) Date(s): 11/05/10 Temp (F): 78 RH %: 49
Rep #'s: A(zipper) Date(s): 11/11/10 Temp (F): 76 RH %: 53

Fabric: SafeRest Premium Mattress Encasement

Fabric Feed Through Method: Terry Cloth Fabric

Rep	Exposure Time	Bed Bug Stage	Approx #	# Fed	# Escaped	# Blood Spots on Fabric
A	15min	mixed	~500	0	0	0
Initials					TS	
Date:					11/05/10	

Zipper Shake Through Method: Zipper

Rep	Exposure Time	Bed Bug Stage	Approx #	# Shaken Thru or (Yes/No)	# Escaped
A	30 sec	mixed	~500	0	0
Initials				TS	
Date:				11/11/10	

Zipper feed Through Method: Zipper

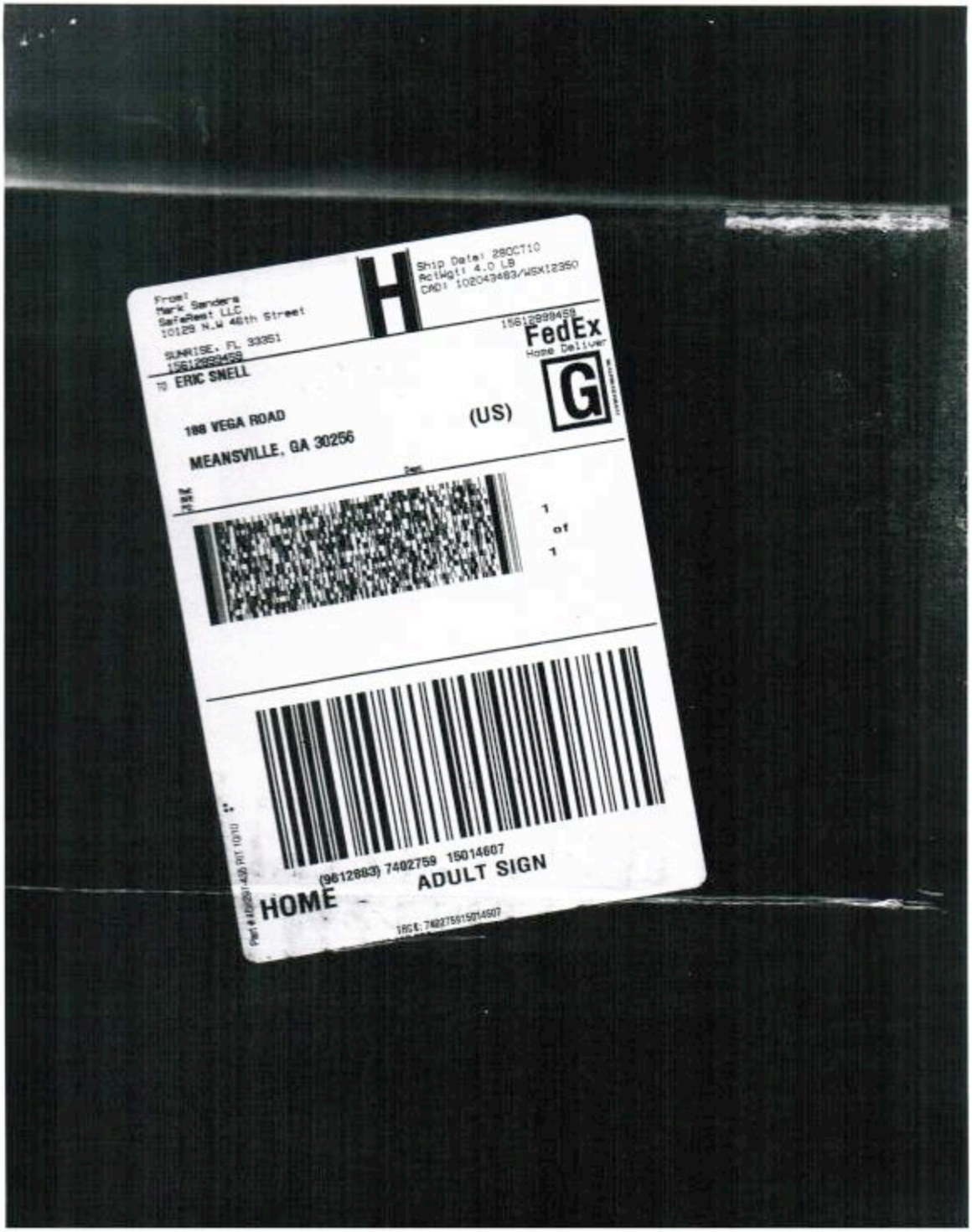
Rep	Exposure Time	Bed Bug Stage	Approx #	# Fed	# Escaped
A	15min	mixed	~500	0	0
Initials				TS	
Date:				11/11/10	

Researcher(s)
Name: Todd Smith Signature: T. S Date(s): 11/05/10 Role: Data Recorder
Name: Eric Snell Signature: E Date(s): 11/11/10 Role: Primary Res.

Appendix C: Test Substance Receiving Log

Snell Scientifics, LLC. Test Substance Receipt Log

Arrival Date	Test Substance Sponsor\Study	Substance Snell Sci. Code:	Test Substance (Trade name, % AI, formulation)	Lot/Batch #	EPA Reg. #	Amnt. Rec'd	Container Type	Shipper	Packaging Condition	Photo Taken (y/n)	MSDS Provided (y/n)	MSDS Down-loaded (y/n)	MSDS Logged (y/n)	Storage Location	Initials
11/1/2010	BB Supply BB Fabric 10	110110-1-D-BBS	SafeRest Premium Mattress Encasement	N / A	N / A	1 - 12 inch Twin Encasement	Clear Zippered Plastic Bag	Fed Ex	Good	Y	N	N	N	D	


Ship Date: 28OCT10
ActWgt: 4.0 LB
CAD: 102043483/WSX12350
From:
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FedEx
Home Delivery
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