

### **PROJECT NUMBER:**

252-0060

### **STUDY TITLE**

EVALUATION OF THE EFFICACY OF LICEFREEE!<sup>™</sup> HEAD LICE TREATMENT SPRAY AGAINST BODY LOUSE OVA

> PROTOCOL NUMBER: N252060860078

IN-LIFE COMPLETION DATE: August 18, 2008

#### **STUDY COORDINATOR:**

Kristine Styer

### **PERFORMED FOR:**

Tec Laboratories, Inc. 7100 Tec Labs Way SW Albany, OR 97321

### **TESTING FACILITY:**

ICR, Inc. 1330 Dillon Heights Avenue Baltimore, MD 21228

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### **EXECUTIVE SUMMARY**

Lice Freee!®, formulation B2074D was evaluated for efficacy against eggs of the human body louse on corduroy cloth. Five replicates of approximately 30 louse eggs were placed on corduroy patches and sprayed with LiceFreee!® B2074D. Each patch holding the eggs was then covered with another treated patch and incubated for 10 days. After this incubation period, the numbers of hatched and unhatched eggs were counted and percent mortality was calculated. The results are shown below:

TREATMENT	% Mortality	
Control	14.4%	
LiceFreee! ® B2074D	100%	

LiceFreee! B2074D prevented all louse eggs tested from hatching. Lice Freee! formulation B2074D is highly effective as an ovicide as it demonstrated complete mortality against body louse ova.

Kristine Styer Study Coordinator Date

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### **OBJECTIVE:**

To determine the efficacy of a leave-in head lice treatment spray against body louse ova..

This is not a GLP (Good Laboratory Practices) study or protocol.

### MATERIALS AND METHODS:

The following sample was supplied by the sponsor:

1. LiceFreee!® Head Lice Treatment Spray formula B2074D.

The materials and methods were as stated in protocol # N252060860078.

An average of 3.22 grams of LiceFreee! B2074D was applied to the bottom treatment patches and 3.26 grams to the cover patches.

### **RESULTS:**

Five replicates of approximately 30 louse eggs were placed on corduroy patches and sprayed with LiceFreee!® B2074D. Each patch holding the eggs was then covered with another treated patch and incubated for 10 days. After this incubation period, the numbers of hatched and unhatched eggs were counted and percent mortality was calculated. The results are shown below in table 1:



TREATMENT	% Mortality
Control	14.4%
LiceFreee! <sup>®</sup> B2074D	100%

 Table 1. Percent mortality (percentage of unhatched eggs) of body louse ova treated with

 LiceFreee!® B2074D

LiceFreee! B2074D provided 100% mortality i.e., none of the eggs incubated between treated corduroy patches hatched. It is therefore an effective ovicide against body louse ova.

### CONCLUSIONS

LiceFreee! B2074D prevented all louse eggs tested from hatching. Lice Freee! B formulation B2074D is highly effective as an ovicide as it demonstrated complete mortality against body lice ova in this study.

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### **APPENDIX I: PROTOCOL**

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#### **PROTOCOL NUMBER:**

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### PROJECT NUMBER 252-0060

PROTOCOL TITLE N252060860078

EVALUATION OF THE EFFICACY OF LICEFREEE!<sup>TM</sup> HEAD LICE TREATMENT SPRAY AGAINST BODY LOUSE OVA

> PROTOCOL VERSION DATE: June 17, 2008

PROPOSED START DATE TBD

### PROPOSED COMPLETION DATE TBD

STUDY COORDINATOR Kristine A. Styer

#### SPONSOR

Tec Laboratories, Inc. 7100 Tec Labs Way SW Albany, OR 97321

### **TESTING FACILITY**

ICR, Inc. 1330 Dillon Heights Avenue Baltimore, MD 21228

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Tec Laboratories, Inc. Louse Ova Test ICR Project No 252-0060 In-Life Completion: August 18, 2008

### **OBJECTIVE**

To determine the efficacy of a leave-in head lice treatment spray against body louse ova..

This is not a GLP (Good Laboratory Practices) study or protocol.

### MATERIALS

TEST ARTICLE: The sponsor will supply the following sample for evaluation:

1. LiceFreee!® Head Lice Treatment Spray formula B2074D.

A Material Safety Data Sheet (MSDS) shall be provided for each test, control, and/or reference sample, which will include any hazardous information of the samples. The percentage of all active ingredients and any hazardous constituents must be included in all MSDS.

The sponsor is solely responsible for conducting the complete test sample, control sample, and any reference sample characterizations, and for retaining this documentation. If any of the test samples are currently available for consumer use and/or purchased in the marketplace, the sponsor should still conduct the same sample characterizations.

The stability of the test, control, and/or reference samples should be determined by the sponsor prior to the experimental start date. When relevant to the conduct of this study, the solubility of each test, control, and/or reference sample should be determined prior to the experimental start date.

The stability of test, control, and/or reference samples stored under the test site conditions should be determined by the sponsor prior to any studies.

All unused test samples will be returned to the sponsor within 30 days after the final report is sent to the sponsor. The sponsor will be responsible for all costs for the return of the samples, including

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any costs associated with hazardous materials shipping.

TEST ORGANISM:	Body lice eggs (ova).
TEST SUBSTRATE:	Cotton corduroy 2" X 2" patches.
TEST CONTAINERS:	Plastic petri dishes.
MISC:	Mettler® balance, forceps.

### **METHODS**

The study design consists of five replicates of 30 eggs. There will be an equal number of untreated controls.

### Spray application:

The weight of test sample delivered will be 5 sprays from the spray pump and container provided by the sponsor, from a distance of ca. 10 cm from the Petri dish containing eggs. The rate of discharge will be determined by weighing the spray container before and after discharge.

### Preparation:

An adequate number of adult female lice will be transferred to human hair cuttings, held in a petri dish, and incubated (31.7°C and 60% RH) for 24 hours. During this time, the lice will oviposit (lay eggs) on the hairs. The lice are then allowed to crawl off the egg-infested hairs by placing them on a rabbit's belly, leaving the hairs, and eggs attached to them, for testing. Thirty eggs per replicate and five replicates will be used.

#### Treatment:

The eggs/hairs will be placed on a 2 x 2-inch piece of corduroy fabric and placed in a Petri dish. The dish will be sprayed with 5 pumps from the sprayer from a height of ca. 10 cm as described above. The weight of formula will be determined for each strip of corduroy by weighing the spray unit before and after the application. After treatment, the eggs/hairs will be left on the treated 2" x 2" patch, covered with another treated patch, placed in an open clean Petri dish, and held in the incubator (31.7°C and 60% RH) for ca. 10 days, or until all hatching has ceased among the control eggs. The eggs will then be examined under a dissecting microscope and the numbers of hatched and unhatched eggs will be recorded. Hatched eggs are those from which the nymph has emerged completely with an empty, clear egg case with the operculum clearly open. Unhatched eggs are those that are opaque with the operculum closed or the nymph partly emerged. All Unhatched eggs will be counted as mortality.

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### DATA ANALYSIS

The counts of unhatched eggs will be converted to percentages. These percent mortalities will be corrected for any control mortality, using Abbott's formula.

### SCHEDULE OF EVENTS

DATE	PROCEDURE
Time Zero	Samples Received
Within 2 days	Test Conducted
At end of test	Telephonic or Electronic Report
Within 30 days of test	Report Written
After Report Submission	Samples Returned

### STATEMENT OF DEVIATION OR AMENDMENT

Any amendments to this protocol must be discussed with and approved by the Sponsor. All amendments to, and/or deviations from, this protocol will be documented in the final report.

Kristine A. Styer Study Coordinator ICR, Inc Date

Wendy S. Langley Date Regulatory Affairs Director



### RAW DATA COLLECTION SHEET #1 Weights Delivered of Licefreee Head Lice Treatment Spray

**Project No:** 

**Sponsor: Tec Labs** 

Date:

					Eggs								
Treatment Patches							Co	ver Patch	nes	+ 5			
	1	2	3	4	5	1	2	3	4	5			
wt initial, g													
wt final, g													
wt delivered													
avg. wt. delivered										5. III.			
# pumps													

Comments:

Recording Technician:



### RAW DATA SHEET #2 Egg Mortality Counts

**Project No:** 

Sponsor: Tec Labs

Date:

					Eggs					
Treated						Untr	eated Co	ntrol		
	1	2	3	4	5	1	2	3	4	5
No. Hatched										
No. Unhatched										
% Mortality										
avg. corrected % mortality										

Comments:

Recording Technician

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### **APPENDIX II: DATA ANALYSIS**

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Tec Laboratories, Inc. Louse Ova Test ICR Project No 252-0060 In-Life Completion: August 18, 2008

				Data F	Reduction	Table				
								1	124	
PROJE	CT #: 252-	0060	SPONS	OR:	Tec Labs			DATE:	08/2	7/08
Rep #	Total #	Hatched	Unhatched		Control Mortality	Treated Mortality	Abbott's correction	Ave	Asin	Mn Asin
				(	CONTRO	L				
1	32	26	6		18.8					
2	31	26	5		16.1					
3	30	27	3		10.0					
4	30	27	3		10.0					
5	29	24	5		17.2			14.4		
				LICE	FREEE! S	PRAY				
1	31	0	31		14.4	100.0	100.0		90.00	
2	30	0	30		14.4	100.0	100.0		90.00	
3	30	0	30		14.4	100.0	100.0		90.00	
4	30	0	30		14.4	100.0	100.0		90.00	
5	30	0	30		14.4	100.0	100.0	100.0	90.00	90.00

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### **APPENDIX III: RAW DATA SHEETS**

Tec Laboratories, Inc. Louse OvaTest ICR Project No. 252-0060

### **RAW DATA COLLECTION SHEET #1** Weights Delivered of Licefreee Head Lice Treatment Spray

Project No: 252-DD60 Sponsor: Tec Labs

Date: 7/11/08

					Eggs					
Treatment Patches						Cover Patches     1   2   3   4   5				
	1 2 3 4 5						2	3	4	5
wt initial, g	224.2	217.6	210.6	203.7	197.)	271.2	214.2	207.1	200.5	193.5
wt final, g	221.2	214.2	207.1	200.5	193.5	217.6	210.6	203.7	1971	189.9
wt delivered	30	3.4	3.5	3.2	3.6	3.6	3.6	3.4	3.4	3.6
avg. wt. delivered		3	3.340	<u>.</u>			3	.529	S	
# pumps	5	5	5	5	5	5	5	5	5	5

Comments: This is from the initial testing that was done. We did not proceed further b/c of 100% control mortality. Attaching this to show how much product we used. Recording Technician: priodice Atyur 7/11/08 (13)

Tec Laboratories, Inc. Louse OvaTest ICR Project No. 252-0060

### RAW DATA COLLECTION SHEET #1 Weights Delivered of Licefreee Head Lice Treatment Spray

Project No: 252-0060 Sponsor: Tec Labs

Date: 8 /5 /08

					Eggs					
Treatment Patches						Cover Patches				
	1 2 3 4 5						2	3	4	5
wt initial, g	187.41	180.31	173.12	146.98	160.89	183.92	176.69	170.08	163.87	158.05
wt final, g	183.92	176.69	170.08	163,207	158.05	190.31	173.12	166.98	160.89	154.99
wt delivered	3.49	3.62	3.04	3.11	2.84	3.61	3.57	3.1	2.98	3.06
avg. wt. delivered		3	.220	3		3	260	}		
# pumps	5	5	5	5	5	5	5	5	5	5

Comments:

Recording Technician: Knichine A Suyer 815108



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Tec Laboratories, Inc. Louse OvaTest ICR Project No. 252-0060

### RAW DATA SHEET #2 Egg Mortality Counts

Project No: 252-0060 Sponsor: Tec Labs

Date: 8/15/08 \$ 8/18/28

					Eggs					
-		Tre	ated				Untr	eated Co	ntrol	
	1 2 3 4					1	2	3	4	5
No. Hatched	D	0	0	0	D	26	26	27	27	24
No. Unhatched	31	30	30	3D	30	6	5	3	3	5
% Mortality	100%	100%	100%	100%	100%	18.75%	16.13%	10%	10%	17.24%
avg. corrected % mortality		10	00%							

Comments:

Recording Technician: Mistine A. Sayer 8/18/08