EFFICACY TEST PROTOCOL

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1 TITLE: TEST OF PERSONAL INSECT REPELLENTS

2 PROTOCOL NUMBER:

EMD–003

3 SPONSOR:

EMD Chemicals, Inc.

3.1 Address:

7 Skyline Drive, Rona–Cosmetic Business Unit
Hawthorne, NY 10532 USA

4 PROTOCOL OBJECTIVE:

4.1 Type of Protocol:

This protocol will indicate the specific methods to be used and direct the conduct of the Study EMD–003. This protocol functions with the general permitted Carroll-Loye Protocol C-L–001, entitled "Protocol for Tests of

Personal Insect Repellents". That protocol presents the domain of and universal instructions for conducting tests of this class, as required by the California Environmental Protection Agency. That and this protocol were developed by Dr. Scott Carroll, Director of Research, Carroll-Loye Biological Research.

The study will be conducted in the laboratory with ticks.

5 STUDY OBJECTIVE:

5.1 Objective of Research

To test the repellent characteristics of the test materials. The design measures the barrier efficacy of the test formulation; biting is not assessed nor does it occur.

5.2 Rationale and Main Endpoint:

The main endpoint of this study will be the conclusion mosquito and tick repellent efficacy tests conducted in the field of an IR3535-based topical repellent.

5.3 Standards Applied:

U. S. EPA Good Laboratory Practice Regulations (40 CFR 160).
California State EPA study monitoring.

6 INVESTIGATIONAL AND TEST MATERIAL CONTROL:

6.1 Test Substance:

6.1.1 Description of the Test Substance

Formulations containing EMD's proprietary IR3535-based repellent will be tested. IR3535 is a US/EPA-registered repellent active ingredient, Ethylbutylacetylamino-propionate. It is the active ingredient in numerous registered commercial personal insect repellents marketed worldwide, including the US/EPA-registered Avon Bug Guard line. The three test formulations are Lotion WV29-01-9N (lot # M17345), Aerosol EUS26-16-9N (lot # M17346), and Spray EUS26-

15-9N (lot # M17279).). Details of the test formulations are in the Appendix.

6.1.2 Dosage Form:

Liquid applied to exposed skin.

6.1.3 Dose:

The substance will be applied to a defined test site on the subjects' lower arms and/or lower legs at a concentration of approximately 1.6 micrograms per cm^2 of skin surface area (to give 1.0 mg per 600 cm^2 of skin surface area.

6.2. Positive Control (Standard):

6.2.1 Description of the Control Substance

An industry-standard EPA-registered commercial arthropod repellent, such as Deep Woods OFF!™ (S. C. Johnson and Sons, Inc., Racine Wisconsin, USA) will be used as the positive control. It will be purchased over-the-counter prior to the test. Its market formulation is approximately 20% DEET.

6.2.2 Dosage Form:

Lotion applied to exposed skin.

6.2.3 Dose:

The standard will be applied to a defined test site on the subjects' lower arms and legs at a concentration of 1.0 mg per 600 cm^2, based on the manufacturer's test protocol.

6.3. Negative Controls:

6.3.1 Description of the Negative Controls

The negative control is untreated.

6.4 Test Arthropod Species:

Testing will be conducted against the western black-legged tick (*Ixodes scapularis pacificus*).

7 STUDY SCHEDULE:

7.1 Proposed Date of Initiation:

TBD, within one year of IRB approval.

7.2 Schedule of Events:

Test day	Date	Activities
-10—2	TBD	Begin subject recruitment. Introduce subjects to test plan and procedures; explain payment; review subject rights and consent forms; option to sign consent forms in order to participate; measure limb surface areas; calculate individual dosages.
1	TBD	Prepare individual dosages for application. Meet with subjects to review day plan and safety procedures. Travel to field site. Review safety and data collection procedures. Administer repellent, commence data collection. Monitor subject safety, comfort, comportment, compliance with data collection protocol.

7.3 Proposed Date of Completion:

Experimental Completion Date (Test Day 1): TBD.
Final Report Completion Date: TBD.

8 STUDY DESIGN:

8.1 Treatment Groups:

There are three treatment groups, namely 1) a test product treatment group, in which there are three formulations , 2) a positive standard (DEET) group, and 3) an untreated negative control group.

8.2 Experimental Design:

The experiment will be treated as a partially randomized, experimenter and subject-blinded trial. However, control subjects will be chosen only from among individuals that are experienced in field biology or entomology.

8.3 Randomization Procedures:

8.3.1 Allocation of subjects to treatment groups:

Subjects will be assigned to the treatment groups on the basis of a randomly assigned subject number. Subjects will be assigned treatment based on subject number from the treatment allocation table, which follows. Treatments will be balanced between arms and legs.

8.3.2 Treatment allocation table:

Materials will be distributed among subjects as tabulated below. There will in addition be one positive control and one negative control. For blinding and logistic concerns, the actual distribution will differ inconsequentially from this.

Subject	Lotion	Pump	Aerosol	DEET	Untreated
1	Left limb				
2	Right limb				
3	Left limb				
4	Right limb				
5	Left limb				
6	Right limb				
7		Left limb			
8		Right limb			

9		Left limb			
10		Right limb			
11		Left limb			
12		Right limb			
13			Left limb		
14			Right limb		
15			Left limb		
16			Right limb		
17			Left limb		
18			Right limb		
19				Left limb	
20					Right limb

8.4. Conditional Boundaries or Limits of Study

8.4.1. Ambient Host-seeking Pressure:

To be included in the test, each tick must be active in locomotion and travel at least 8 cm in 3 min from the point of placement on the hand, in oriented fashion toward the body (elbow), on an untreated limb, in advance of being exposed to a treated, test limb.

8.4.2 Environmental Conditions:

Based on known behavior of *I. scapularis pacificus*, temperature should be between 20 and 25°C, humidity should be above 35%, and light should be indirect ambient.

9 STUDY PROCEDURES:

9.1 Test Subjects:

9.1.1 Inclusion criteria:

9.1.1.1 Age: At least 18 yrs
9.1.1.2 Sex: Male/female
9.1.1.3 Race: Any race
9.1.1.4 Written consent (see 9.4, below).

9.1.2 Exclusion criteria:

9.1.2.1 Known to be to be hypersensitive to mosquito bites.
9.1.2.2 Known to be to be sensitive to any of the test product ingredients.
9.1.2.3 Poor physical condition.
9.1.2.4 Unwilling to submit to brief query about personal condition.
9.1.2.5 Not able to write, and speak English at approximately the University of California college level.
9.1.2.6 Unwilling to refrain from use of alcoholic beverages or smoking during the test.
9.1.2.7 Known to be pregnant or lactating. Pregnancy will be self-checked by each female volunteer on the morning of the repellent test using an OTC test kit provided by the Study Director. Results of each such test will be immediately verified by direct inspection by a female technician trained to make that assessment. Only volunteers scored as nonpregnant will be allowed to participate.

9.1.3 Number of Subjects:

Approximately 6-10 per treatment group, and 1-2 per control group.

9.2 Blinding of Study:

9.2.1. Extent of the Blinding:

Subjects will be blinded to the treatments they receive.
Study Director will be blinded to the identity of all test substances until the conclusion of data evaluation.

9.2.2 Blinding Methods:

Data capture forms will be coded with respect to treatment, so that personnel recording data (subjects) will not be aware of the treatments that they have received.

9.3. Study Material Administration:

Study Materials will be administered to each subject by Carroll-Loye technicians. Test products will be applied volumetrically. Test sites are first cleansed with water and a fragrance free detergent soap, rinsed with an isopropanol/water solution, and then towel dried. Test products are applied to the test site from a syringe or micropipette; they are spread on the site as evenly as possible with two fingertips in a surgical glove, using a light rubbing motion.

9.4 Subject Consent:

Written subject consent (Carroll-Loye California EPA approved Participant Consent Form) is an inclusion criterion.

10 SPECIFICATION OF THE VARIABLES:

10.1 Variable to be Measured:

Number of ticks crossing or repelled from arm skin. A crossing is scored if a tick travels at least 2 cm in a vector toward the elbow from a line at the wrist that marks the beginning of the treated area within 3 minutes of entering the treated area. A repulsion is scored when a tick changes its orientation away from or parallel to the margin of the treated area upon approach, or does not cross more than 2 cm toward the elbow within 3 minute of entering the treated area. Ticks are observed one at a time by a subject. Every 15 minutes, each subject selects one or two new ticks from a pool of unused pre-screened, qualified ticks, and place it on the base of the palm two cm from the distal edge ('wrist') of the arm. Ticks are manipulated with the bristles of a fine artist's paint brush. Ticks are placed so that they face the elbow. Ticks may be oriented to locomote toward the margin of the treated area with the gentle action of the paintbrush. Subjects will record any crossings or repulsions as they occur. Repulsions are normally unambiguous reversals of direction. Subjects lift the tick off with the paintbrush after each assessment is complete. Any brushes that come into contact with a test material are discarded. Used ticks are retired from the study.

10.1.1 When Variable will be Assessed:

The variable is assessed in sequential trials beginning every 15 minutes after the treatment is applied. The time at which the application of a treatment is completed is recorded as t_0 ('time zero'). There may be a delay of no more than 10 minutes after treatment until exposure begins.

10.1.2 Forms for Retention of Source Data:

Data will be recorded on a data form.

11 DATA ANALYSIS:

11.1 Experimental Unit:

The individual subject will be the experimental unit.

11.2 Replicates per Treatment:

There will be a minimum of six subjects treated with the each test repellent, one serving as untreated controls, and one testing the standard.

11.3 Statistical Methodology

Percent repellency is calculated as the total number of challenges in which ticks did not cross the barrier divided by the total number of challenges made, with the quotient multiplied by 100. (Normally no ticks are repelled from the untreated controls.) Mean percent repellency is calculated for each subject; grand means and standard deviations of subject means are presented.

The hypothesis to be tested is that the treatment will significantly reduce the number of ticks crossing on treated versus untreated arms. The binary nature of the data ('0' = repelled, '1' = crossed) indicates the use of contingency analyses (e.g., Chi-square). Alpha is 0.05. Analyses may compare subsets of the data (e.g., first two hours) in addition to the entire data set.

12 STUDY LOCATION:

Carroll-Loye Arthropod Behavior Laboratory at the letterhead address.

13 PERSONNEL:

13.1 Investigator (Study Director):

13.1.1 Address

Dr. Scott Carroll
Carroll—Loye Biological Research
711 Oak Avenue
Davis, CA 95616

13.1.2 Telephone

530-297-6080
530-297-6081 (Facsimile)

13.1.3 Training and experience of investigator

CV on file with sponsor

13.2 Study Monitor:

Dan Giambattisto

13.2.1 Address

EMD Chemicals, Inc.

7 Skyline Drive
Rona–Cosmetic Business Unit
Hawthorne, NY 10532 USA

13.3 Quality Assurance Unit:

Dr. Jenella Loye

13.3.1 Address

Carroll—Loye Biological Research
711 Oak Avenue
Davis, CA 95616

13.3.2 Telephone

530-297-6080
530-297-6081 (Facsimile)

13.1.3 Training and experience of QAU

CV on file with sponsor

14 AMENDMENT/DEVIATIONS TO THE PROTOCOL:

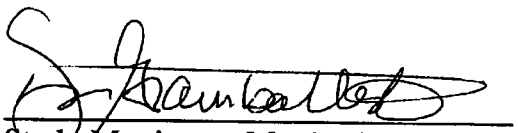
Protocol amendments or deviations will be reviewed by the Study Monitor and the Study Director. The amendments or deviations will be documented in the Study Director's final report. Documentation will include a description of the change, the reason for the change and the effect of the change on the experiment.

15 PROTOCOL APPROVAL SIGNATURES:

Note: Signing by appropriate persons of a "Study Initiation Protocol" that references the study described herein constitutes the completion of this Protocol Approval page (Section 14).

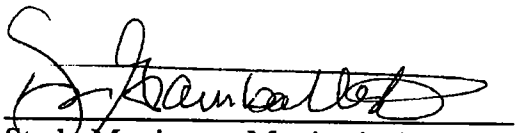
Scott P. Carroll, Ph.D.
Study Director

23 February 2006
Date



Study Monitor or Monitor's Agent
Dan Giambattisto

13 April 2006
Date



Study Monitor or Monitor's Agent

13 April 2006
Date

Appendix. Test repellent formulations.

Insect Repellent Spray with IR3535®
(EUS26-15)

Ingredients	INCI	[%]	CAS No.	EPA Inert List
Phase A				
IR3535®	Ethyl Butylacetylamino-propionate	20.00	52304-36-6	**Active Ingredient**
Carbowax 400 /Union Carbide	Polyethylene glycol 400	5.00	25322-68-3	4B
Arlamol E	PEG-15 Stearyl Ether	1.00	25231-21-4	4B
Phase B				
Ethanol SD 40B	Denatured Alcohol	35.00	61116-08-3	4B
Carbowax 1450 /Union Carbide	Polyethylene glycol 1500	4.00	25322-68-3	4B
PVP/VA Copolymer E-735 /ISP	PVP/VA copolymer	2.00	25086-89-9 64-17-5	
Polysorbate 20 / Uniquema	Tween 20	1.50	9005-64-5	4B
Water, demineralized	Aqua (Water)	31.50	7732-18-5	4A

Insect Repellent Aerosol with IR3535®
(EUS26-16)

Ingredients	INCI	[%]	CAS No.	EPA Inert List
Phase A				
IR3535®	Ethyl Butylacetylamino-propionate	20.00	52304-36-6	**Active Ingredient**
Phase B				
Ethanol SD 40B	Denatured Alcohol	21.67	61116-08-3	4B
Propylene glycol / Union carbide	Propylene glycol	4.34	57-55-6	
PVP/VA Copolymer E-735 /ISP	PVP/VA copolymer	1.73	25086-89-9 64-17-5	
Water, demineralized	Aqua (Water)	17.26	7732-18-5	4A
Phase C				
A31, Isobutane /Aeropres	Isobutane	35.00	75-28-5	

Insect Repellent Lotion with IR3535®
(WV29-01)

Ingredient	INCI	(%)
PHASE A		
Water, demineralized	AQUA (WATER)	ad 100
1,3-Butanediol (Merck KGaA)	BUTYLENE GLYCOL	4.00
Titriplex® III (Merck KGaA)	DISODIUM EDTA	0.10
PHASE B1		
Rhodicare-S (Rhodia GmbH)	XANTHAN GUM	0.20
Carbopol ETD 2050 (Noveon)	CARBOMER	0.30
PHASE B2		
Triethanolamine (Merck KGaA)	TRIETHANOLAMINE	0.20
PHASE C		
Arlacel 165 VP (Uniquema)	GLYCERYL STEARATE, PEG-100 STEARATE	3.50
Dow Corning 200 (100cs) (Dow Corning)	DIMETHICONE	0.50
Isopropyl palmitate (Cognis)	ISOPROPYL PALMITATE	4.00
Lanette 16 (Cognis)	CETYL ALCOHOL	1.00
Crodamol STS (Croda)	PPG-3 BENZYL ETHER MYRISTATE	2.00
IR3535®	**ETHYL BUTYLACETYLAMINOPROPIONATE**	**10.00**
Stearic acid (Merck KGaA)	STEARIC ACID	2.00
PHASE D		
Seibel 305 (Seppic)	LAURETH-7, POLYACRYLAMIDE, C13-14 ISOPARAFFIN	1.00
PHASE E		
Triethanolamine (Merck KGaA)	TRIETHANOLAMINE	0.10
PHASE F		
Paragon II/McIntyre	PROPYLENE GLYCOL, DMDM HYDANTOIN, METHYLPARABEN, PROPYLPARABEN	1.00