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COVER PAGE

EFFICACY TEST PROTOCOL EMD-003

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TEST OF PERSONAL INSECT REPELLENTS

SYNOPSIS

The study pursuant to this insect repellent efficacy protocol is intended to provide data under the requirements of United Stated Environmental Protection Agency Guideline OPPTS 810.3700. This protocol, dated 8 September 2006, is a revision of EMD-003 as originally submitted to US/EPA on 27 April 2006. That protocol was dated 13 April 2006, (IRB approval date, 18 April 2006). Also on 27 April 2006, we submitted a related protocol, C-L-001, that provided certain information on subjects safety and recruitment. That information has been incorporated into the revision of EMD-003 presented here, and C-L-001 is no longer under consideration

This revision was made in response to the following principal sources:

1. June 27-30, 2006 EPA Human Studies Review Board Meeting Report (Proposed Final Draft v. 1) dated 28 August 2006.
2. Product Performance Protocol Review by EPA staff Kevin Sweeney, dated 6 June 2006.
3. Revised Draft OPPTS 810.3700. Product Performance of Skin-Applied Repellents of Insects and Other Arthropods, dated 9 & 12 June 2006.
4. Science and Ethics Review of Protocol for Human Study of Tick Repellent Performance by EPA staff John Carley and Clara Fuentes, Ph.D. (review of draft revised protocol EMD-003, dated 12 July 2006), dated 28 August 2006.
5. Review pursuant to Title 3, California Code of Regulations Section 6710, Department of Pesticide Regulation, Worker Health and Safety Branch, and the Office of Environmental Health Hazard Assessment, dated 13 June 2006.

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. The study will be conducted in the laboratory at the letterhead address with deer ticks.

Note that this protocol formerly functioned in tandem with the general Carroll-Loye Protocol C-L–001, entitled "Protocol for Tests of Personal Insect Repellents". That protocol presented the domain of

and universal instructions for conducting tests of this class, as formerly required by the California Environmental Protection Agency. Elements of Protocol C–L-001 have now been incorporated directly into this and C–L-001 no longer applies. Both that protocol and this protocol were developed by Dr. Scott Carroll, Director of Research, Carroll-Loye Biological Research.

5 STUDY OBJECTIVE, RATIONALE AND STANDARDS:

5.1 Objective of Research

To test the repellent characteristics of the test materials against *Ixodes scapularis* ticks. The design measures the barrier efficacy of the test formulation; biting is not assessed nor does it occur. Because this study tests repellents against a well-known disease vector (the deer tick, *Ixodes scapularis*, vector of the Lyme Disease pathogen), efficacy will be measured principally as Complete Protection Time. Complete Protection Time, or CPT, is defined herein as the time between application of Test Material and the First Confirmed Crossing (FCC). The FCC occurs when a questing tick placed adjacent to treated arm skin walks more than 3 cm into the treated area toward the elbow. A crossing will not be designated as an FCC unless it is followed by another crossing within 30 minutes. The work conducted pursuant to this protocol will be initiated by determining the amount of each of the repellents that subjects typically apply. Dosimetry will consist of a behavioral assay utilizing passive dosimetry.

5.2 Rationale and Main Endpoint:

This study will test the efficacy of new formulations of IR3535, created by the developer of IR3535, which are intended to increase cosmetic quality for better user acceptance. US/EPA requires new repellent formulations to be registered, and some registrants must present efficacy data as part of the registration review. The rationale for this study is to provide that efficacy data, which has not been previously collected. Compared to the insect repellent ‘DEET’ (N,N-diethyl-m-toluamide), there are few data examining the efficacy of IR3535 in different formulations. In addition, IR3535 has not been widely studied in the United States at end-product concentrations as high as those to be

tested here. Yet the excellent safety profile of IR3535 indicates that it is suitable for testing at higher concentrations than have typically been studied.

Stability of the end-products will be tested in a different study.

The main endpoint of this study will be the conclusion of a tick repellent efficacy study, conducted in the laboratory, of three IR3535-based topical repellent formulations, with the data set suitable for submission to US EPA for insect repellent registration purposes. The efficacy study will consist of a single laboratory trial, with 10 treated subjects testing each formulation, and serving as their own untreated controls.

Initial dosage determination ('dosimetry') will be conducted with a set of 12 subjects, many of whom will likely continue on to participate in efficacy testing. Dosimetry will be conducted at the letterhead address. When 12 subjects have completed dosimetry, those data will be used to determine dosing for all efficacy trials with the actives, including those against other arthropods (i.e., including the mosquito repellent efficacy test, which is described in Carroll-Loye Protocol EMD-004). The EMD-004 protocol and this EMD-003 protocol are independent in all other ways, except that individual subjects are not proscribed from participating in both studies.

5.3 Rationale for use of Human Subjects:

Human subjects are required because they represent the target system for the test materials, and sufficiently reliable models for repellency testing have not been developed. In addition, subjects will self-administer the test articles during dose determination. There are no accepted methods of modeling the complex relationship between spray delivery systems and target subjects. At least ten subjects are required in order to reduce variation around the population means we will describe. Data of this type are not available from other studies, and so it is advisable to test the comparatively large number of subjects proposed in case variance among them is high. The low toxicity of the test materials should mean that there is little incremental risk associated with increasing sample size. In addition, in

pre-test meetings, human subjects were deemed appropriate by the same US/*EPA* toxicologist who also evaluated risk for the sponsor's Federal registration of the active ingredient.

5.4 Balance of Risks and Benefits:

The study-associated risks are of two types: exposure to the test materials themselves, and possible exposure to arthropod-borne diseases. As described below, subject health and safety are unlikely to be impacted by any study-associated risks during or after the study.

The repellent active ingredient has a low acute and chronic risk profile, established both through experimentation and through long-term consumer use. The concentrations of the active ingredient in the product being tested match those of products currently EPA-registered and marketed in the US. Subjects with known allergic reactions to insect repellents and common cosmetics are excluded from participating. 'Repeat' exposures during dosimetry are all of very brief before the repellent is washed off, and total a much briefer duration of exposure than a typical single consumer application likely would. Risks associated with inhalation and ingestion would require gross intentional mishandling by subjects, a scenario that the study methods do not promote.

While no bites are expected from the implementation of this protocol, it is worth noting that the testing will be conducted with laboratory-reared ticks descended from field caught adults. They are reared on quarantined rodents screened to be pathogen-free for all tick-transmitted pathogens and hantavirus using appropriate culture, direct detection (PCR), and immunological screening assays.

In summary, the relatively benign quality of the repellents and the technical precautions we employ indicate that the chance that any subject will be at a health or safety risk is extremely small. If at anytime during the study a subject suffers a skin reaction or feels ill, he or she is instructed to inform the Study Director (i.e., the 'Principal Investigator'), or anyone else who is also working to direct the study. Such subjects will be immediately withdrawn from testing and medical management will be implemented (§9.5). At least one qualified researcher will remain with the other test subjects if other researchers depart with an injured or ill subject. Subjects are clearly and repeatedly

informed that they may remove themselves for any reason from the study at anytime, without penalty to their compensation.

Against the slight risks are balanced substantial and reasonably likely benefits. Arthropod-borne disease is of growing significance in the United States and around the world where U.S. citizens are active. Discomfort associated with nuisance biting restricts many work and pleasure activities. DEET-based repellents have been the only reliable personal protection for many decades. However, health, comfort and practical concerns about DEET have restricted its use below a level ideal for public and personal health issues. The majority of marketed DEET-alternatives is of relatively very low efficacy. This study tests a repellent of well-known high efficacy, consumer safety and acceptability. It is one of only two or three repellent actives that have ever been in a position to serve as a DEET-alternative of public health value. This study will give a good estimate of a minimum time of expected excellent protection, using standards, safety practices and design that are all conservative. Few studies have examined IR3535 at a concentration as high as that tested here. Hence its maximum potential efficacy, particularly as influenced by each specific application formulation, is poorly known. Because EPA-registration requires efficacy data, a test such as this one is the only path toward further product development and greater availability of superior IR3535 products to consumers in the United States.

5.5 Standards Applied:

U. S. EPA Good Laboratory Practice Regulations (40 CFR 160); 40 CFR 26 subparts K and L; FIFRA § 12(a)(2)(P); California State EPA Department of Pesticide Regulation study monitoring (California Code of Regulations Title 3, Section 6710).

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). They are "pending products" to be submitted to EPA for registration as insect repellents. Details of the test formulations are in the Appendix.

6.1.2 Trade Name:

TBD

6.1.3 Dosage Form:

Liquid applied to exposed skin.

6.1.4 Dose:

Determining dosage is a main objective of this study.
Dosage for repellency testing will be the mean of the individual subject means determined for each product in the dosimetry portion of this study. Dosage will be measured in weight and reported by weight and volume.

6.1.5 Manufacturing Site:

ACCRA PAC Inc., Elkhart Indiana USA.

6.1.6 Test Material Storage During Study:

Prior to application, test materials will be stored indoors, at room temperature and away from direct sunlight or direct

sources of moisture. Storage will be at Carroll-Loye Biological Research.

6.1.7 Test Material Safety:

EPA regulates use of inert ingredients (also termed "other" ingredients) by toxicology profiles in animal tests and by their inclusion in EPA lists of "approved" other ingredients. Ingredients on lists 4a or 4b are considered relatively safe for all uses. The ingredients in the proposed insect repellent formulations are mainly on lists 4a or 4b with a few ingredients on list 3 because of ocular irritation potential (e.g., alcohols). EPA normally regulates the presence of materials on list 3 by labeling to avoid contact with eyes and to prohibit application by children. The other ingredients in the test formulations are commonly used in marketed products for application to human skin as components of cosmetic and drug formulations.

The insect repellent products proposed for registration have all been tested in animals for potential for oral and dermal toxicity, dermal inhalation, ocular and dermal sensitization potential; studies on droplet size of spray and aerosol products showed that there was little if any potential for inhalation exposure. These studies will be submitted and reviewed by EPA as part of the registration process. The results of these tests showed a low order of toxicity characteristic of similar tests on the "neat" active ingredient cited by EPA in approvals of this product for application on humans. The IR3535 active ingredient has an extensive, positive safety record of in consumer use.

MSDS documentation is the same as that submitted with the previous version of this protocol.

6.1.8 Test Material Composition and Stability:

The Test Material formulations are typical of topical cosmetics and insect repellent products marketed to

consumers. They were produced under Good Manufacturing Practices (GMPs) with records available to EPA. Production of these insect repellents involves only simple mixing of the ingredients and does not involve chemical reactions that can be an issue with other pesticide products; ingredients are non-reactive as documented in storage stability studies that are required for submission to EPA as part of the registration process.

Test materials were produced in February 2006. They were couriered to Carroll-Loye Biological Research on 7 April 2006, with Chain-of-Custody documented. Since that time they have been stored at the Carroll-Loye Offices at in a closed cabinet at room temperature (20-24°C). The composition and content of active ingredients in the products used for the proposed efficacy studies will be confirmed by analytical methods prior to and following human subject efficacy testing. Storage stability testing is also being conducted. The EPA has extensive experience with enforcing requirements for such tests based upon their history with similar products applied to humans and EMD intends to provide any requested information as appropriate to safety and efficacy issues.

6.2. Negative Control:

6.2.1 Description of the Negative Controls

The negative control is untreated for both dosimetry and repellency assays. Each subject simultaneously serves as a treatment and control subject.

6.2.2 Rationale for Employing a Negative Control

The ‘negative control’ serves to insure that each tick employed in the study is attracted to the test subject before it is used in a repellency challenge. Ticks that fail to meet the questing criterion (§8.4.1) are not used against Test Materials. In this way the negative control serves as a pre-screening of the ticks, such that only actively questing ticks are then

exposed to the treatments. Based on this manipulation of a standard control design, the crossing rate on the negative control is judged to be 100%.

There is no control in which each formulation matrix without the repellent active is tested. There is no a priori basis for anticipating significant repellent activity in the matrices, and the study objective is to examine efficacy of the end products. The question of whether there is interaction between matrix and active is external to that objective. Accordingly, including additional subjects testing matrix-only formulations cannot be justified.

6.3 Test Arthropod Species:

Testing will be conducted against laboratory-reared *Ixodes scapularis* ticks. Ticks are descended from field caught adults. Methods employed for disease exclusion are described in §5.4. Tick rearing is at 23.5°C, >97% relative humidity and a 14L:10D light cycle. Laboratory nymphs are active in questing and feeding between approximately 2 weeks and one year post-eclosion (molt). Ticks will typically be between 6 and 12 weeks post-eclosion for testing.

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
-30– -2	TBD	Begin subject recruitment. Introduce subjects

		to test plan and procedures; explain compensation; review subject rights and consent forms; option to sign consent forms in order to participate; measure limb surface areas; determine individual dosage values.
1	TBD	Prepare individual dosages for application. Meet with subjects to review day plan and safety procedures. Review safety and data collection procedures. Administer repellent, commence repellency 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 two experimental groups, namely 1) a 'treated' group of subjects treated with the test products, of which there are three formulations, and 2) an untreated ('negative') control group.

8.2 Experimental Design:

The experiment will be treated as a partially randomized, experimenter and subject-blinded trial.

8.3 Randomization Procedures for Repellent Efficacy Testing:

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 their subject number and the treatment allocation table, which follows.

8.3.2 Treatment allocation table:

Materials will be distributed among subjects as tabulated below. (Alternatively, pending consultation with US/EPA, the Pump and Aerosol treatments, which have the same concentration of the active ingredient and which will be very similar to one-another after their carrying material evaporates may be tested together on alternate limbs of the same subjects. Doing so would reduce the absolute subject exposure by 10 individuals. In that case, repellents would be applied only to the upper half of each forearm, with the lower half serving as the untreated area for gauging questing avidity. Limbs would be exposed one at a time.)

Subject	Lotion	Pump	Aerosol	Untreated
1	Left limb			Right limb
2	Right limb			Left limb
3	Left limb			Right limb
4	Right limb			Left limb
5	Left limb			Right limb
6	Right limb			Left limb
7	Left limb			Right limb
8	Right limb			Left limb
9	Left limb			Right limb
10	Right limb			Left limb
11		Left limb		Right limb
12		Right limb		Left limb
13		Left limb		Right limb
14		Right limb		Left limb
15		Left limb		Right limb
16		Right limb		Left limb
17		Left limb		Right limb
18		Right limb		Left limb
19		Left limb		Right limb
20		Right limb		Left limb
21			Left limb	Right limb
22			Right limb	Left limb
23			Left limb	Right limb
24			Right limb	Left limb
25			Left limb	Right limb
26			Right limb	Left limb
27			Left limb	Right limb

28			Right limb	Left limb
29			Left limb	Right limb
30			Right limb	Left limb

8.4. Conditional Boundaries or Limits of Study

8.4.1. Ambient Host-seeking Pressure:

To be included in the test on a treated limb, each tick must first meet the crossing criterion on the untreated limb, following the procedure for the treated limb (§10.3.6), in the same test period.

8.4.2 Environmental Conditions:

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

8.5. Monitoring of Environmental Conditions During the Study

Records will be made of environmental conditions (temperature, relative humidity, wind speed, light intensity and precipitation (presence/absence and general rate/quality) at approximately one-hour intervals throughout the course of data collection.

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).
9.1.1.5 Language: Speak and read English

9.1.2 Exclusion criteria:

9.1.2.1 Known to be phobic of ticks.
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 Use of insect repellent within three days preceding the study.
9.1.2.6 Unwilling to refrain from use of perfumed products, alcoholic beverages or smoking after 9 PM the evening preceding the test and throughout 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.
9.1.2.8 Inability to deliver the test materials to own left and right limbs.
9.1.2.9 Student or employee of the Study Director.

9.1.3 Number of Subjects and Rationale for Sample Sizes:

Dosimetry: 12 subjects per treatment formulation (namely lotion, pump spray, aerosol). Repellent efficacy: 10 subjects per treatment formulation. Each subject is a replicate.

The number of subjects is chosen as a compromise between several conflicting factors. In the absence of clear means of estimating the distribution of outcome values, it is difficult to predict an ideal sample size. From a strictly scientific standpoint an appropriate response under such circumstances is to increase size, but ethical and economic consideration demand the opposite in the present study, particularly during the repellency phase.

The US/EPA has historically required a minimum of six subjects. Given that test repellents are nearly certain to exhibit greater than zero efficacy, and that testing is conducted under adequate ambient tick questing pressure, it is nearly certain that no

untreated subjects will register fewer or later crossings than any treated subjects. As a result, from the standpoint of statistical **power**, as few as six treated and one untreated subject are sufficient to demonstrate a significant treatment effect at $P<0.05$. In the same vein, six is often regarded as a statistically sufficient sample for an observation set because the increment in the confidence of means estimate begins to drop off sharply at that point. Notably, under the historical guidelines, there seem to have been few problems with EPA registering repellents that commonly fail to meet their labeled performance specification.

The main scientific risk of using a very small sample is that the probability of over-representing subjects inherently unattractive to ticks is rather large, as is the risk of uncontrolled biasing from a single subject that generates unrepresentative values for undetected reasons. The fact that the study will not be replicated for registration purposes increases that risk substantially.

Note that in our interpretation of the draft EPA guidelines the response variable, Time to First Confirmed Crossing, is calculated as the average duration for all treated subjects. There is no consideration of variation. In any given study, increasing the number of treated subjects to 10 will nonetheless improve the probability of estimating the population mean accurately.

The 95% confidence interval computation is useful for assessing the certainty of a means estimate, and for normal probability density function that interval is ±1.96 standard error of the mean. The normal density function is part of the exponential family of density functions, and in this study we anticipate that the distribution of Times to First Confirmed Crossings will be truncated toward the origin. However, to the extent that they are transferable to ticks, the available mean and variance data on IR3535 performance against mosquitoes (Cilek et al. J. Amer. Mosq. Control Assoc. 20: 299-304, 2004) indicate that no individual values will be near zero. Using the rule of thumb that a distribution in which the mean is greater than three standard deviations above zero may be regarded as effectively normal, it is sensible to compute and report the normal 95% confidence interval in this study.

Employing eight subjects in a cage test, Cilek et al.the (2004) recorded a mean protection time against mosquitoes of approximately 180 minutes, with a standard error of about 15 minutes. Had their N been six, we can roughly predict that the 95% CI would be 148-212. At N=10, the estimate would be 155-205. At N= 20, the interval would be roughly 162-198. Evidently, adding the additional 10 subjects to reach an N of 20 shrinks the interval, in absolute terms, no more than did the addition of four subject to increase the sample size from 6 to 10.

To summarize, adding subjects beyond six increases the precision of the means estimate only slowly. However, the individual and public health importance of avoiding inaccuracy in this study, coupled with the fact that the data set will not be replicated, argues for a prudent approach. To reduce the risk of over-representing atypically unattractive subjects, as well the weight of the value obtained from any one subject, we regard 10 (rather than six) treated subjects as a better sample size for the repellency portion of the study. For dosimetry, in contrast to repellency, less general information is available, and the risk profile is more benign. Consequently, a slightly larger sample is prudent. In meetings with EPA toxicology staff in 2005, 12 was regarded as an acceptably sample size for estimating mean dosage for each to the repellent formulations. Accordingly, we propose to employ a total of 12 subjects for dosimetry.

9.1.4 Test Subject Recruitment:

[Note: This material is adopted from former protocol C-L-001]

9.1.4.1 Synopsis of Recruitment Process:

1) Source(s): Participants are recruited by verbal networking through our academic and personal communities of friends, neighbors and scientists in Davis, California. Individuals are recruited from the community specifically for each study. Studies are not conducted with individuals from particular employers or agencies.

2) Initial Contact Method: Initial contact is through word-of-mouth and telephone contact with individuals in our Volunteer Data Base.

3) Follow up Contact Method: Telephone interview, personal interview with the Study Director conducted at the Carroll-Loye Biological Research Offices.

9.1.4.2 Methods of Recruitment:

Our subjects are mainly University of California–Davis graduate and undergraduate students in life science programs with which the Study Director is associated. Students in his laboratory who depend on him directly for employment or scholastically are not eligible to participate. Other subjects are science, education and health care professionals, and mosquito and vector control professionals.

We contact subjects who participated in previous Carroll-Loye repellent efficacy tests by selecting them from our Volunteer Database. At that time interested individuals often ask if one or more of their lab mates or acquaintances may participate as well. All such potential participants are screened or re-screened for suitability for each test in a private, one-on-one conversation held at the office of the Study Director. The Exclusion Criteria (section 9.1.2) are exercised by asking each candidate to address them in the interview with the Study Director. It is explained that pregnancy will be assessed directly in on the test day. The PI encourages candidates to ask questions and ask for clarification at any time during the interview and in all activities that follow. To candidates that pass screening the Study Director describes the test purpose in plain language (in English), and the procedures and comportment to be followed are described in detail. Candidates are then asked if they would like to retire from consideration at that point. If they wish to remain in consideration, it is explained and emphasized that they may withdraw from the test at any time during the test without penalty to their compensation. Candidates are given copies of the State of California Department of Pesticide Regulation 'Experimental Subjects' Bill of Rights' to read as the Study Director reads it aloud. They are also given a copy of the IRB-approved consent form to read as the Study Director reads it aloud. The amount and form of compensation is described. They are again encouraged to ask any questions they have about the test, which may include understanding its purpose more fully, understanding

risks and discomforts more fully, and understanding treatment and compensation for injury more fully. While the majority of our subjects have worked with us on an occasional basis for a number of years, we encourage them to personally evaluate their interests and concerns about participation seriously each time. We ask them not to sign on immediately but to give the situation due consideration (normally at least one day, sometimes less for those who have participated in multiple prior studies). Because most of the volunteers are researchers and/or have advanced degrees in life sciences, we regard their motivations and decisions to participate as being unusually well considered and well informed. Accordingly, we normally accept their decisions to participate if they so choose following due consideration. Nonetheless, the Study Director retains the final right to refuse participation to any candidate.

9.1.5 Identification method and records retention:

Subjects will initially be identified by first and last name, and assigned a unique number for purposes of this study. Individual data will be entered into the computer for retention and analysis with reference to individual number, not name. Records relating individual names to individual numbers will be retained separately. The Study Director will retain records indefinitely. Subjects may obtain their own records from the Study Director.

9.1.6 Enrollment of alternate subjects and its relation to individual privacy:

We will enroll three more subjects than are required to meet our sample size. All subjects will be informed during the Consent process that on the day of testing, a small number of subjects may be designated as alternates and sent away after being compensated for coming to the test site. Alternate subjects may return later to replace subjects that initiate testing but withdraw before useful data are generated. They also serve as insurance against any enrolled subjects who fail to appear.

The possibility that any subject may be designated as an alternate will assist in protecting the privacy of any subject that must withdraw in or near the presence of other subjects at the start of

the test day (i.e., before treatment and testing begins), for reasons such as a positive pregnancy test result, or for any other personal circumstance to which possibly inappropriate attention might otherwise more readily be drawn. In the case of privacy concerns related to pregnancy detection, we regard this "indirect" approach as potentially as discrete and less likely to result in errors that would be the case if we were to employ, e.g., separate male and female Informed Consent Forms, with pregnancy only mentioned on the female form. The latter approach does not address loss of privacy among females, nor does it control the possibility of indiscrete revelation of pregnancy testing by females to males during the test or later, and it also creates the risk of a female subject using the wrong form. Separate forms would also assume that we may fairly treat individual subjects unequally on the basis of postulated gender-based differences in the information the merit receiving in to arrive at their informed consent decision. The soundness of making such an assumption enters ethically complex grounds requiring an intricacy of analysis and breadth of treatment beyond the scope appropriate to the privacy concerns of the present study.

9.2 Blinding of Study:

9.2.1. Extent of the Blinding:

The types of Test Materials and their identities will be evident to subjects as they apply them during the dosimetry portion of the study. During the repellency portion of the study, subjects will be blinded to the exact treatments they receive although some may note differences between the lotions and the clear liquids in the repellency potion of the study. The Study Director will be blinded to the identity of all test substances until the conclusion of data evaluation.

9.2.2 Blinding Methods:

The Test Materials, Dosing Administration and Data Capture forms will be coded by a researcher with respect to treatment, so that subjects and personnel recording data will not be aware of the treatments for which they are reporting. The Study Director

will access the codes to identify the Test Materials in the Study Report after completing the data analysis.

9.3. Study Material Administration:

Study Materials will be administered to each subject by Carroll-Loye technicians. Test products will be applied volumetrically to the skin surface from a tuberculin (1 ml) syringe, and spread on the site as evenly as possible with two fingertips in a surgical glove, using a light rubbing motion. Skin surfaces to be treated are first cleansed with water and a fragrance free detergent soap, rinsed with a 50% ethanol in water solution, and then towel dried.

9.4 Subject Consent:

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

9.5 Stop Rule and Medical Management

Specific adverse reactions in subjects to the test materials are not anticipated based on low acute and chronic toxicity profiles of the materials, the disease-free status of the ticks, and the training and oversight of subjects in handling ticks. Because the products are topical, subjects will self-monitor for allergic and irritant skin reactions, particularly redness, edema, itching or pain, and report any such reactions to the Study Director. Any subject showing adverse skin reactions will immediately stop further participation. The treated skin will be gently washed with clean water and mild soap to remove the test product, and the area will be gently dried with a clean towel.

On the day of testing, we will alert the nearest hospital of the scope of our activities in advance of commencing treatment and data collection. In unlikely event of a Type 1 allergic reaction (anaphylaxis), we will contact 9-1-1 by telephone and cooperate as instructed with emergency personnel. We will be prepared to instruct emergency personnel on how to reach our site via multiple routes. In addition, we will personally transport affected persons to

the nearest hospital if so advised by emergency personnel. There is sufficient redundancy in personnel that in such a case subjects remaining at the study site will still receive appropriate technical, scientific and safety guidance.

All subjects are asked to contact the Study Director and a physician of their own choice at any time should they develop a rash (a delayed hypersensitivity reaction) within 48 hours of the conclusion of the test day.

As part of Medical Management, the Study Director will record all benign and adverse health observations.

9.6 Subject training for research with ticks

Approximately one week to four days before repellent efficacy testing, subjects will be trained by technical personnel in handling and observing ticks. Subjects will learn how to manipulate ticks with fine paintbrushes, place them on their own forearms, observe and quantify tick movement on their arms, and dispose of used ticks. This training will be documented. This 'hands-on' experience will assist subjects in collecting data accurately and handling ticks safely during the repellent efficacy trial.

10 TEST VARIABLES AND THEIR MEASUREMENT:

10.1 Variables to be Measured:

Subject forearm surface area.
Subject self-dosing behaviors.
Weight of test materials delivered to the surrogate skin (gauze) dosimeters.
Number of tick Crossings on the treated surface of the skin.

10.2 When Variable will be Assessed:

Dosage will be calculated on the basis of surface area of the lower limb skin that is treated. Measurements to calculate that surface area will be made on each subject in advance of application of the test materials.

Self-dosing behavior (distance of spray nozzles from skin, number of pumps or sweeps of delivery apparatus) will be measured at least three days prior to Test Day 1.

Passive dosimeters (described in section 10.1.3) will be weighed before application of the test materials and again between one and five minutes after application of the test materials.

Subjects will record any Crossings as they occur. Data are recorded in three-minute exposures at 1- minute intervals. The time at which the application of a treatment is completed is recorded as t_0 ('time zero'). The first exposure begins 15 minutes after treatment.

10.3 Procedures for Assessing Variable:

10.3.1 Limb dimensions and surface area:

The term 'limb' refers to the forearm. The surface area of each limb is computed as the average of four evenly spaced circumferences (two peripheral, two central) of the forearm (elbow to wrist multiplied by the length of treatment area. The locale along the limb at which each circumference is taken will be recorded (for later use in the study, see section 10.3.3, below) as the distance in centimeters from the distal margin of the site of the most distal circumference site (i.e., at wrist or ankle).

10.3.2 Familiarization with, and subject use, of each spray apparatus:

Variable assessment will involve a two-step process, namely subject familiarization with the spray apparati, followed by dosage measurement.

Subjects will practice application of test materials to their own limbs under the following procedure (next paragraph), which will be reviewed for the subjects by a researcher before practice commences. The copies used during the study will be formatted for greater clarity and ease of use than is possible here.

"Read along on your copy of the procedure as the Researcher reads them to you. Ask questions of the Researcher as they occur to you or at any time thereafter. Be sure to get answers to any questions you feel should be answered before proceeding at any step of this work.

This is a study of your behavior in applying spray insect repellents. You will probably have had experience with applying spray products of some kind to your skin before. If you uncertain about how to use a spray dispenser be sure to ask the Researcher or one of the technicians. You will each have the opportunity to practice these procedures with the aid of a technician.

Insect repellents function to repel insects from biting the skin. Their effectiveness is influenced by the completeness of their application to the skin surface. Our goal is to determine your preferred method for achieving **full coverage**. At minimum, **full coverage** is defined as a continuous and complete layer of test material. Orienting the arm to light may aid in determining whether full coverage has been achieved. Spray as much as necessary to achieve full coverage.

In these instructions, the act of spraying a repellent on your arm will be termed 'spraying', 'application', or 'dispensing.'

If you are wearing a long-sleeved shirt roll the sleeves so as to expose the entire lower arm. Wash arms thoroughly with the provided cleanser and dry with a clean towel. Place new latex or vinyl gloves on each hand, choosing the size that fits you most snugly without being uncomfortably restricting or likely to tear when you put them on.

You will work with a technician who will assist you in measuring and recording your use of a repellent product in two delivery systems, a pump spray and an aerosol spray.

Work first with the pump spray, second with the aerosol spray. Because they are similar, the application instructions below

describe the procedures for each type of spray together in each paragraph.

Familiarize yourself with the spray mechanism. Any actuation (pushing down on the pump plunger) of the spray must take place out-of-doors. Work at a distance of no less than 6 feet (1.9 meters) from other subjects. Do not dispense the spray at or near your face or anyone else's. Minimize inhalation of airborne spray while working.

Testing will take place out-of-doors during daylight hours at an air temperature (shade) above 14 °C (57 F) and wind speed below 12 kph (7 mph), with no precipitation. The researcher or a technician will inform you when these conditions are not met and spraying of the repellents will cease until those conditions resume.

Dispense the spray on one forearm, using the opposite hand. By successively moving the spray nozzle closer to and farther from the arm, identify a distance between nozzle and skin that seems most appropriate for effective application to the skin. The technician will measure and record that distance to the nearest centimeter on the provided datasheet.

Have the technician wash and dry the treated arm so that none of the repellent you have applied is visible on close inspection.

Now, using the spray nozzle at or near the distance from the skin that you have just chosen to be effective for application, determine the minimum number of actuations (pumps of the pump spray) or longitudinal passes (aerosol) required to give full coverage of all surfaces of the forearm. For the pump spray, depress the plunger fully each time, and count them aloud beginning with "1, 2, 3 ...." etc. If you partially depress the plunger (rather than fully depress it) in order, e.g., to apply to a small skin area not covered be initial application, report that to the technician as a "half pump." Each partial depression should be so reported as it occurs. If on any given actuation material fails to be delivered, do not count that actuation. If a partial amount is delivered, consider it either 'whole', 'half' or 'none'

and report it as such. For 'none', simply resume counting at the next actuation that delivers material to the skin.

Report the count to the technician who will record it on the data sheet. The technician will also assist you in keeping track of whole versus half pumps.

When applying the aerosol, announce each onset of spraying with the word "START" and each cessation with the word "STOP". This will aid the technician who is counting your application time. Apply the aerosol in a series of full "sweeps" (passes) between the wrist and elbow. There may be more than one start and stop while working to achieve full coverage of the arm. Count each one-way sweep as one sweep, and count passes in a manner analogous to that used for pump spray (above). If you make a partial sweep that you judge to be closer to a "half sweep" than a "full sweep", call it out to the technician as a "half". Treat accidental under-applications in the same manner as for the pump spray (described above). Try not to let your awareness of the technician's timing to influence you dispensing behavior. If the technique of using mainly full sweeps seems awkward or unnatural to you, inform the technician immediately. Your preferred method should be demonstrated for the Researcher, who will determine how it may be quantified.

Repeat the application procedure and collect the same data for the other arm.

Discard your latex gloves, and wash both arms with cleanser and dry them thoroughly with a towel.

Put on new gloves, and repeat the application procedure twice more (both arms) with the pump spray. During these two repetitions the technician will again measure your preferred distance between the nozzle and the skin, and quantify the application as before. However, in these repetitions, if you are confident that you have learned and remembered your preferred distance, you and the technician can measure the distance you used *after* reporting the data on number spray pumps/number and duration of aerosol sweeps. This will avoid interrupting your application with additional arm washing by the technician.

Try to be consistent with your use of the spray apparatus. If you are clear and confident about the distance from the arm that works best, pay enough attention to keep the nozzle in that general range while maintaining a natural delivery as you would use the product under normal personal use. Keep the nozzle aimed at the skin surface, and avoid orienting the containers in any ways that you determine, as you proceed with the trial, to interfere with delivery of the repellent to the skin surface.

Now move onto the **Spray Sampling** exercise described in the next section for the spray pump. After completing that exercise, repeat all of the above with the aerosol."

10.3.3. Spray Sampling

Spray Sampling is the procedure by which the spray is subsampled with patch dosimeters. Dosimeters of known surface area will be placed on subject lower arms. These dosimeters will intercept a portion of the spray applied to the arm. Be weighing dosimetry patches before and after treatment, the mass of the intercepted material can be calculated. The spray delivery systems will also be weighed before and after each application.

Spray sampling will be conducted according to the following procedure.

"Please read along with the Study Director as he reads aloud the following description of the procedures you will employ in spray sampling. Please be sure to ask questions at any point.

This procedure is very similar to what you have just performed. The main difference is that for spray sampling, a technician will place four narrow rings of plastic-backed gauze around each of your forearms. The rings are about one-half-inch (1.5 cm) wide. Each of these "gauze bracelets" will be centered on each of the four positions on the arm at which we initially measured the circumference. These positions may be marked on the skin with small but visible dot using a temporary marker.

The function of the "gauze bracelets" is to capture some of the spray that would otherwise reach your arm as you apply the test products. It is important that you do not alter the way in which you apply the materials in any intentional or substantial way from what you have already determined is your best procedure. The technician will review your results from your previous applications with you to assist you in repeating your general procedure (distance of nozzle to skin, number of spray pumps or aerosol sweeps) as you apply the materials to one of your arms with the bracelets in place.

The gauze bracelets are narrow in order to minimize the extent to which your sensation of receiving the spray on the arm is changed. Do your best to proceed as if the sensation is not changed. In other words, attempt to avoid spraying additional material onto areas under the bracelets where the sensation of test material on the skin will be different or absent. Do not attempt to spray additional material directly onto a bracelet unless it is within an area that needs additional treatment. Again, attempt to repeat the procedure that you have already developed, and apply the materials "as if the bracelets were not there."

Put a new latex glove on each hand. Spray material onto one arm only. The technician will tell you to which arm to apply spray. You and the technician will collect the same data as previously.

After you have completed spraying, keep both arms from making contact with any surface. All bracelets will be removed by a technician and taken for weighing.

Discard your gloves, and wash both arms with cleanser and dry them thoroughly with a towel.

Repeat these procedures until you have made at total of three spray samples for the first arm, and three more for the second arm. Be sure to discard your gloves, and wash both arms with cleanser and dry them thoroughly with a towel, including after the last application."

10.3.4. Lotion sampling

The amount of lotion applied to limbs will be quantified in a series of three applications analogous to the Spray Sampling above. However, dosimeters are not required, nor are the extensive practice sessions. The amount applied is the weight difference in the dispensing tube before and after application.

The instructions are as follows:

"Put a new latex glove on each hand. You will apply lotion to one arm only. The technician will tell you to which arm to apply. You will begin with an amount that you suppose is about one half of what you will need to achieve thorough and uniform coverage. After spreading that around the lower part of your arm, you will apply more as needed to the area closer to your elbow. Begin by gently squeezing lotion from a tube with the cap open directly onto the horizontally-held surface of the opposite arm. Hand the tube to the technician. Using the tips of the index and middle fingers, spread the lotion as evenly as possible on all surfaces of the lower arm. Do not spread it onto the hand or beyond the marking on your wrist. If you have sufficient lotion left to spread it evenly and thoroughly toward the elbow, continue in the direction. Do note spread it beyond the elbow or past beyond the marking near the elbow. If you need more lotion to achieve thorough and even coverage, make sure you have wiped all repellent from your fingertips onto the skin and ask the technician to hand you the tube. Apply as much additional as you think you need, as before, but to complete the coverage. If you decide that you have applied more repellent that you would normally use to achieve thorough and even coverage, immediately have the technician wash and dry the treated arm so that none of the repellent you have applied is visible on close inspection, and begin again. Likewise, be careful to avoid dropping any lotion off of the arm, and if this happens, begin again as you would if you applied too much.

After you have completed an application successfully, the technician wash and dry the treated arm so that none of the repellent you have applied is visible on close inspection, and reweigh the tube. You will continue until you have completed three successful applications."

10.3.5 Equipment Used to Assess the Dosimetry Variable:

Passive dosimeters are 1.5 cm wide strips of 3M Brand Nexcare™ Holdfast™ self-adhesive roll gauze attached to the adhesive side of 1.5 cm wide strips of 3M Brand clear packaging tape. The tape will retard test materials from passing from the dosimeter to the subjects' skin. The tape strip length will match the circumference of a given region of a subject's arm. The gauze strip will be 1.5 cm longer than the tape, in order to permit a 1.5 cm overlap onto the self-adhesive gauze bracelet, thus securing the dosimeter in place.

On the non-adhesive side tape strip (the inner surface of the dosimetry bracelet) the following notations will be made before they are used.

a) Subject number
b) L (for left placement) or R (for right arm placement)
c) Position letter: a (wrist), b (next proximal), c (next proximal), d (elbow)
c) T (for treatment) or C (for control)
d) Replicate number (1, 2 or 3)

There will be eight bracelets per replicate. Each arm will be treated three times. Each subject will therefore have a total of twenty-four custom bracelets made and labeled in advance.

Bracelets will be weighed before and after treatment on a traceably calibrated Sartorius H51 balance (measurement increment 0.0001 g, 30 g capacity). Test material containers (pump spray and aerosol) will be weighed before and after dispensing on a traceably calibrated Sartorius GC 2502 (measurement increment 0.001 g, 500 g capacity).

10.3.6. Repellency:

Before the repellent is applied, subjects will be guided to wash the lower arms with mild, low fragrance soap, rinsing them with a spray of ethyl alcohol (mixed with an equal part of water), and then drying them with a clean towel. A technician will then apply insect repellents to one forearm to

give even, complete coverage of the skin on all sides of the arm. The treated area will extend a minimum of 10 cm along the forearm. The amount of repellent to be applied to any limb will be calculated in advance for each subject. The dosing rate will be the product of the subject's limb surface area multiplied by the grand mean (mean of all subject means) rate calculated in the dosimetry data analysis for that test material. Each subject will therefore be dosed at the same rate within a given repellent even if their individual application rates differed from the grand mean.

Three 'orientation' ink lines, approximately 5 cm long and perpendicular to the longitudinal axis of the forearm, are arrayed longitudinally on both ventral forearms of each subject, at 3 cm intervals. On the treated arm (or treated portion of the arm if both arms are treated (see §8.3.2) the first line is 3 cm distal to the treated area, the second line marks the threshold of the treated area, and the third line is 3 cm into the treated area. The untreated limb/limb portion has a spatially identical array of 3 lines for tick activity screening. The first line, used for placement, insures that ticks are not placed within the treated area and so can detect a gradient of repellent density to which to orient. The second line serves keep subjects aware of where the treated area begins and serves as a reorientation point for re-marking should either the first or the third line become obscured.

We will employ a 15-minute exposure interval as a good minimum in terms of both the temporal resolution of the data set and subject ability to remain focused. Every 15 minutes, each subject selects an unused tick and tests it for active questing behavior as described. To initiate a screening or a repellent challenge, a tick is placed on the ventral arm or proximal palm, in the most hair-free portion, at the first (most distal line). Ticks are manipulated with the bristles of a fine artist's paintbrush. 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. Forearms should be held from approximately 30° to vertically above the lab bench surface if that increases the propensity of ticks to travel toward the body.

A crossing is scored if a tick travels at least 3 cm in a vector toward the elbow into the treated area (i.e., at least as far as the third line) within 3 minutes of beginning to move up the arm from the first line. 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 3 cm toward the elbow within 3 minutes of entering the treated area.

Subgroups of approximately three subjects are led by a technician in the monitoring of time, ticks, and tick behavior. Time is monitored by referring to an electric chronometer with a highly visible display. The technician 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 immediately retired from the study by being transferred from the test arm to a container labeled "used".

Scientific Stopping Rule: Subjects are directed to cease tick exposures when a crossing is followed by another crossing within one-half hour, i.e., in either of the subsequent two exposure periods.

10.3.7 Forms for Retention of Source Data:

Dosimety data will be recorded by a technician on a data form for each test formulation. Repellency data will be recorded by technicians on a repellency data form. Data forms are appended. The recording technicians sign and date the data forms.

10.4 Study Facility:

Treatments and data collection will take place in the main building and on the terrace of Carroll-Loye Biological Research.

11 DATA ANALYSIS:

11.1 Experimental Unit:

The individual subject will be the experimental unit.

11.2 Replicates per Treatment:

For dosimetry, there will be 12 treated subjects, each serving as their own untreated control, testing each of the three repellent formulations. For repellency testing, there will be 10 subjects treated with each test repellent and serving as untreated controls.

11.3 Statistical Methodology:

Statistics will be computed with the software 'SAS JMP' Version 5.0.1.2 (SAS Institute, Cary NC).

11.3.1 Dosimetry:

Dosage will be calculated per square centimeter of skin. The amount of test material delivered to each dosimeter in each trial will be calculated as:

weight after application – weight before application

The **total captured** by all treated dosimeters per trial will be calculated by adding the mass changes in all four dosimeters together, and then subtracting or adding, respectively, any total gain of loss of weight in the paired control dosimeters.

The **proportion covered** of the total limb surface area by the dosimeters is:

$$\frac{\text{Surface area of a set of 4 dosimeters}}{\text{Surface area of the limb}}$$

The estimated **dosage per trial** is:

Total captured x 1/**proportion covered**

The specific gravity of each test material will be measured and used to convert the dosage weight data to volumes for preparing individual subject doses volumetrically for dispensing from the tuberculin syringes.

Subject means and standard deviations will be calculated for all measures of dosimeter weight changes as well as application behaviors (distance from nozzle to skin, duration of application, number of sweeps/pumps). Lotion, pump spray and aerosol statistics will be calculated separately and then compared with nonparametric tests for two- and three- sample independent cases (Wilcoxon match-pairs signed-rank and Kruskal-Wallis tests, respectively).

We will statistically assess the strength of any individual subject differences in application behavior and dosing in interaction with the three test materials using Friedman two-way analysis of variance subject dose means for each test material. We will use subject dose means for each test material to calculate dosing grand means (± SD) for each test material. Those means, expressed as repellent weight per unit skin surface area, will be used to determine individual subject doses in the field repellency test.

11.3.2. Repellency:

Because all subjects use different ticks, all ticks are used only once, and neither organism interacts directly with conspecifics at the level of the skin and the repellent during data collection, we will analyze data by subject as independent, replicated values. The hypothesis that the test materials will significantly reduce the number of ticks Crossing treated versus untreated skin is *not* the focus of this study. The focus is to compute, for each test material, a reasonable estimate of mean and standard deviation for the duration between application and sufficient repellency breakdown such that two ticks crossing on a subject within a half hour period. That pattern is here assessed at a resolution of 15 minutes.

Complete protection time (CPT) is measured as the length of time from initial application to the First Confirmed Crossing. A FCC is a Crossing followed by another Crossing within 30 minutes. For example, a Crossing at 120 minutes followed by another at 135 minutes is not confirmed, but a third Crossing

at 150 minutes would confirm that at 135 minutes, giving a CPT of 135 minutes.

CPT measured in this way will yield a single time value for each subject. Mean CPT will be calculated across all 10 subjects per treatment, and will be presented with standard deviation and 95% confidence interval information as well.

Because all subjects serve as untreated controls to verify tick questing sufficiency, Relative Protection (RP) may also be calculated. Its utility is limited to the time period from first exposure until the first subject testing a given repellent is withdrawn by invoking the Stopping Rule (after which continued calculation of RP would likely bias its value in favor of repellency). Within that limit, RP evaluation provides complementary information when considering CPT. Such complementary information is important because it gives a rate function for performance the is intuitively explicable, and also because CPT is not currently discussed in the draft EPA guidelines for tick testing. RP is calculated for each subject as a function of the total number of challenges in which ticks did not cross the barrier divided by the total number of challenges made. (Normally no ticks are repelled from the untreated controls.) Specifically, RP is the percentage prevented from crossing on the treated arm relative to the untreated arm, which is calculated as {[1 - (Mean comparator/Mean Untreated)]100} per unit time. Most simply, that time may be, e.g., per hour, with RP calculated for each hour as illustrated in the draft EPA guidelines of 12 June 2006. For each subject, cumulative RP may also be calculated for each time interval. This is the mean RP across all time intervals up to the selected point. In our design, Cumulative RP may be assessed at a maximum resolution of 15 minutes.

12 STUDY LOCATION:

Carroll-Loye Arthropod Behavior Laboratory at the letterhead address.

13 QUALITY ASSURANCE:

An independent, professional Quality Assurance Unit (QAU) will inspect several aspects of the study. The QAU will report to the Study Director. Protocol Review and Comments must take place before data collection commences. In-Life Inspection must include observing the measurement and recording of key variables by subjects and researchers. In addition, the Final Report will be audited for completeness and accuracy. A QAU Statement will address compliance and noncompliance or any omissions in auditing. Findings from the In-Life Inspection and the Final Report, as well as the QAU Statement will be transmitted to both the Study Director and to the Sponsor Monitor.

14 PERSONNEL:

14.1 Investigator (Study Director):

14.1.1 Address:

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

14.1.2 Telephone:

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

14.1.3 Training and experience of investigator:

CV on file with sponsor

14.2 Study Monitor:

Dan Giambattisto

14.2.1 Address:

EMD Chemicals, Inc.

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

14.3 Quality Assurance Unit:

Dr. Jenella Loye

14.3.1 Address:

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

14.3.2 Telephone:

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

14.1.3 Training and experience of QAU:

CV on file with sponsor

15 AMENDMENT/DEVIATIONS TO THE PROTOCOL:

Protocol amendments or deviations will be reviewed by the Study Monitor and the Study Director. Any changes that may affect the health or safety of study participants must be approved the Study Director, the State of California Department of Pesticide Regulation, and the approving IRB. The amendments, deviations as well as any adverse events 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 conduct and outcome of the study.

16 PROTOCOL APPROVAL SIGNATURES:

8 September 2006

Scott P. Carroll, Ph.D.
Study Director

Date

8 September 2006

Study Monitor or Monitor's Agent
Dan Giambattisto, EMD Chemicals, Inc.

Date

Appendix 1. 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

Appendix 2. Sample data recording forms

TICKS ON ARMS 2006.xls

Study EMD-003						TEST OF REPELLENCY TO TICKS				Pg 1 of	
Date:							Subject Name:				
DATA: R = Repelled,											
C = Crossed treated area more than 2 cm							|----------|				
		R or C?				R or C?				R or C?	
TRIAL	TIME	Untreated	Treated	TRIAL	TIME	Untreated	Treated	TRIAL	TIME	Untreated	Treated
1				18				35			
2				19				36			
3				20				37			
4				21				38			
5				22				39			
6				23				40			
7				24				41			
8				25				42			
9				26				43			
10				27				44			
11				28				45			
12				29				46			
13				30				47			
14				31				48			
15				32				49			
16				33				50			
17				34				51			
OBSERVER SIGNATURE:											

Appendix. Data sheets (arm, rather than leg, examples are given).

Pump Spray Application

Subject name: Subject number

Date:

I. Quantification of application behavior

A. Left arm

Trial no.	Distance from skin	No. of pumps for full coverage	Mass before	Mass after
1		X	X	X
2				
3				
4				

B. Right arm

Trial no.	Distance from skin	No. of pumps for full coverage	Mass before	Mass after
1		X	X	X
2				
3				
4				

II. Spray sampling

A. Left arm

Trial no.	Distance from skin	No. of pumps for full coverage	Mass before	Mass after
1				
2				
3				

B. Right arm

Trial no.	Distance from skin	No. of pumps for full coverage	Mass before	Mass after
1				
2				
3				

Aerosol Application

Subject name: Subject number

Date:

I. Quantification of application behavior

A. Left arm

Trial no.	Distance from skin	No. of sweeps for full coverage	Seconds sprayed	Mass before	Mass after
1		X	X	X	X
2					
3					
4					

B. Right arm

Trial no.	Distance from skin	No. of sweeps for full coverage	Seconds sprayed	Mass before	Mass after
1		X	X	X	X
2					
3					
4					

II. Spray sampling

A. Left arm

Trial no.	Distance from skin	No. of sweeps for full coverage	Seconds sprayed	Mass before	Mass after
1					
2					
3					

B. Right arm

Trial no.	Distance from skin	No. of sweeps for full coverage	Seconds sprayed	Mass before	Mass after
1					
2					
3					

Lotion Application

Subject name: Subject number

Date:

A. Left arm

Trial no.	Mass before	Mass after
1		
2		
3		

B. Right arm

Trial no.	Mass before	Mass after
1		
2		
3		

Appendix 3. IRB Approval Letter and Informed Consent Form

INDEPENDENT
INVESTIGATIONAL
REVIEW BOARD INC.

Your Advocate for Clinical Research Participants

Kim Lerner
Chairman

Anita McSharry R.N.
President

DATE: September 12, 2006

TO: Scott P. Carroll, PhD
Principal Investigator

FROM: Kim Lerner, Chairman or
Anita McSharry, Vice-Chairman
Independent Investigational Review Board, Inc.

SUBJECT: Revised Protocol dated 9/8/2006
- Revised Informed Consent Form (Ver. 9/12/2006)

PROTOCOL: EMD-003

At the meeting held on September 12, 2006 the Independent Investigational Review Board, Inc. had an opportunity to review the Revised Protocol and the revised Informed Consent Form for the above noted research study. The revised Protocol includes numerous changes aimed at improving the science of the study to better reflect consumer habits, clarifying information provided to study subjects. Changes have been implemented in response to requests from the EPA Human Studies Review Board and California Department of Pesticide Regulation. In addition, the revised Protocol is independent of any generic procedural protocols.

The Revised Protocol is unanimously approved as submitted. The revised Informed Consent Form is unanimously approved as revised. The Informed Consent Form has been revised to accommodate the Revised Protocol. The approved revised Informed Consent Form is identified as Version 9/12/2006 and stamped, "Approved 9/12/2006". The Committee unanimously agreed that the risk/benefit ratio did not change. All current and future subjects must sign the revised consent form.

Thank you for your cooperation.

KL/AMS/RR/fc:

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INFORMED CONSENT AUTHORIZATION TO PARTICIPATE AS A RESEARCH STUDY SUBJECT

Title of Study: (EMD-003) Test of Personal Insect Repellents

Principal Investigator: Scott P. Carroll, Ph.D.

Site of Investigation: Carroll-Loye Biological Research
711 Oak Ave
Davis, CA 95161

Sponsor: EMD Chemicals, Inc.

Participant's Name: _____

You are being asked to participate in a research study. Your participation is voluntary. The information in this Informed Consent Form explains the study. You will receive a copy of this form, and you may take it home and think about it before making your decision. If you have any questions, or do not understand anything in this form, please ask the Principal Investigator to explain any words or information you do not clearly understand.

NATURE AND PURPOSE

Carroll-Loye Biological Research is conducting this research study in order to develop effective tick repellents. Many people are interested in having new and better tick repellents available to them. The tick repellents that we will study were developed from amino acids that are naturally occurring substances in animals. More studies are needed to determine how well such new tick repellents work.

The purpose of the study is to test how well new lotion, pump spray and aerosol insect repellent products work in the laboratory against ticks. These three products, which are similar to some already being sold, have been formulated to be more cosmetically acceptable to users. The repellent ingredient is a biochemical called 'IR3535'. The information gained from the study will assist in the development of these repellents for future commercial marketing. During the study we will first measure how much repellent you put on your arms in an initial visit to the study laboratory. On a later date, you will return to the laboratory to test repellents against ticks.

The sponsor EMD Chemicals, Inc has contracted Carroll-Loye Biological Research to conduct the study. Scott Carroll, Ph.D. of Carroll-Loye Biological Research is the Principal Investigator in charge of the study.

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SUBJECT SELECTION

You have been offered an opportunity to participate in this research study because you read and speak English, consider yourself to be in good physical condition and are at least 18 years of age. If you are a female of child-bearing potential, you cannot be pregnant or breastfeeding.

Approximately 30 volunteers will be enrolled in this laboratory research study, which is being conducted at only one site. A few more subjects will be enrolled than are needed in order to make up for anyone who is unexpectedly unable to participate once testing begins. If more subjects are present than are needed for any part of the test, you may be asked not to participate, but will instead be an 'alternate subject' who may be contacted to participate later if needed. If you are designated as an alternate subject, you will be compensated for your participation up to that point and for your inconvenience.

STUDY INTRODUCTION AND DURATION

Schedule of visits and time required to participate in the study

Activity	Visit 1 (1-21 days befc the test)	Visit 2
1. Orientation and Dosage visit	X	
2. Repellent Test visit		X
Total time	2-2.5 hours	8-14 hours

You will be given a training manual and will have a chance to review it and to read along with the instructions.

Visit 1 for Orientation and determining Dosage

Within 21 days before the second visit (in will test the repellents against ticks), you will go to the laboratory and meet with a researcher to perform introductory activities for the study. The researcher will also tell you more about what you will experience while participating and what is expected of you. You will work with a researcher to determine how much insect repellent you apply. Completing those measurements will take 1.5-2.0 hours.

You will also be shown how handle ticks on your skin with a small artist's paintbrush. This training and practice will take about ½ hour.

The total time for Visit 1 activities will be about 2.0-2.5 hours.

Visit 2 for the Tick Repellent Test

The study will also require a second visit to the same laboratory. This second visit will most likely require approximately 10 hours of your time. However, it may require as few as 6 hours or many as about 14 hours, depending on how long the repellents

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remain effective. Bathrooms are available, and meals, drinks and snacks will be provided.

STUDY PROCEDURES

Study Design

This study will test three different insect repellent products, namely a lotion, a pump spray and an aerosol spray. You will be randomly (by chance) assigned to receive one or two of the three products, so your chance of receiving any one of them is one-in-three or two-in-three. You will not have a choice as to which repellent product or products you receive. For each product assigned to you, you will have an amount typical of what people commonly use applied to one or both of your forearms. Neither you nor the Principal Investigator will know which of the study materials you are receiving; however, this information can be made available if medically necessary.

If you are a female, you will perform a pregnancy test using an Over the Counter (OTC) pregnancy kit in the morning prior to the start of each of the two study visits. The results of your test will be verified by a female technician that is qualified to make that determination. If you are pregnant, you will not be allowed to participate in the study. Information regarding your pregnancy test results will be kept in confidence.

Procedures

Visit 1

At the laboratory, a researcher will measure the length and circumference of your forearms. You will then practice using the products to decide how you best like to apply them and how much you would apply to your forearm or lower leg in order to have thorough and even coverage. The researcher will answer any questions you have about the application. Once you have a method you are satisfied with, you will wash your arms with soap and water and dry them with a towel. The researcher will then place three small "bracelets" made of medical gauze around your forearm. You will then spray that area, including the bracelets, with a repellent, and a technician will remove the gauze and weigh it to determine how much spray has clung to its surface. Similarly, we will ask you to apply an amount of the lotion repellent product to your skin that you think gives complete and even coverage. We will use the amounts you apply in this part of the study to determine how much repellent people normally apply.

You will also spend about 30 minutes practicing handling ticks in the laboratory in preparation for the repellent study. A researcher will show you how to catch the ticks, place them on your skin, take them off, and place them in a container. You will practice these tasks several times in order to familiarize yourself with how to handle the ticks carefully and successfully. You may ask the research for advice on how to do this at any time while you are practicing. The ticks used for this training are reared in the laboratory and free from diseases.

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Visit 2

This is the day of the actual repellent study. You will first be guided to wash your lower arms with mild, low fragrance soap, rinsing them with a spray of ethyl alcohol (mixed with an equal part of water), and then drying them with a clean towel. A technician will then apply repellents to one or two of your forearms to give even, complete coverage of the skin. The amount of repellent applied on an arm will be no more than about ¼ teaspoon.

During the test you and the Investigator will not know which repellent you are using. The study is done this way because knowing which repellent you are using can change the results of the study. If you start having any side effects from the repellent, the investigators can find out what you are taking in order to help you. Please ask the investigator if you have any questions at all about this kind of study.

You will be part of a group of about 6 treated subjects seated at a laboratory table, and a researcher will lead you in handling and keeping track of the ticks, of the time, and of your tick observations. Every 15 minutes, you will test a new tick on each arm and report the result to you lead researcher, who will record the data. That task will take between 5 and 10 minutes to complete. At times you may need to stand in order to so that the ticks may climb upward, which is their preference.

Every 15 minutes a project leader will announce the beginning of the next period for testing the treated skin. You will continue in this way until a tick crosses the repellent in two of three consecutive periods, as long as you are comfortable. There will time to eat comfortably and use the bathroom between test periods.

RESTRICTIONS

- You must not be a student or employee of the Principal Investigator
- You must not have a phobia of ticks
- You must not be sensitive to any of the test product ingredients
- You must not have used repellents within three days prior to the start of the study
- You must be able to apply spray and lotion repellents to your left and right arms
- You must not use perfumed products after 9 PM the night before and throughout the tests
- You must refrain from smoking or alcoholic beverages after 9 PM the night before and throughout the tests

RISK/DISCOMFORTS

If at anytime you feel ill, inform the Principal Investigator (or anyone else who is also assisting to direct the study) immediately, and you will be taken to receive medical attention at the nearest hospital. You may also request access to standard first aid materials (such as bandages, antiseptics, and mild antihistamines) and request first aid assistance at any time. You may remove yourself for any reason from the study at

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anytime. At least one qualified researcher will remain with the other test subjects if other researchers depart with an injured or ill subject.

The spray repellents contain alcohol and are flammable. The repellents may cause skin, lung and eye irritation. Excessive inhalation can cause lung irritation, headache and dizziness. Swallowing the products may cause temporary stomach distress. You may obtain more information about the safety of the repellents by asking the Principal Investigator, and he will provide you with the official "Material Safety Data Sheets" which give safety details similar to those found on commercial product labels.

Measures will be implemented to make sure that ticks are removed before they have an opportunity to bury in the skin.

PREGNANCY RISKS

The risks to the unborn are unknown and may be hazardous. If you are a woman of childbearing potential, it is important that you do not participate in this study if you are, or if you think you may be pregnant. 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.

UNKNOWN / UNFORESEEABLE RISKS

In addition to the risks and discomforts listed above, there may be some unknown or infrequent and unforeseeable risks associated with the use of this product, including allergic reaction or interaction with a medication. You will be informed in a timely manner both verbally and in writing of any new information, findings or changes to the way the research will be performed that might influence your willingness to continue participation in this study.

RESEARCH RELATED INJURIES

If you are injured as a result of being in this study, medical treatment will be available from a health care facility that is aware of the study. Carroll-Loye Biological Research will cover the costs of such medical treatment that are not covered by your own insurance or by a third party. If necessary, Carroll-Loye Biological Research will transport you to receive medical attention and pay costs associated with the reasonable and appropriate treatment for any injuries incurred as a result of participation in the study. For further information about this, the research test subject should call the office of Carroll-Loye Biological Research (530) 297-6080.

You DO NOT waive your legal rights by signing this form.

TREATMENT ALTERNATIVE

Since this study is not intended to provide any therapeutic or other health-related benefit, your alternative is to not participate in this study.

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BENEFITS
There are no immediate benefits to you from your participation other than compensation for your participation.

OFFER TO ANSWER ANY QUESTIONS ABOUT THIS STUDY
If you have any questions or problems during this study, or if you think that you may have experienced a research-related injury, you should contact Scott Carroll of Carroll-Loye Biological Research at (530) 297-6080 or (530) 902-8267.

If you have any questions regarding your rights as a research volunteer, please contact Kim Lerner, Chairman of the Independent Investigational Review Board, Inc. at toll free (877) 888-IIRB (4472) during regular working hours. The Independent Investigational Review Board is a committee established for the purpose of protecting the rights of volunteers in a research study.

COSTS AND REIMBURSEMENT
There will be no costs to you from participating in this study. For participation in the study, each research study participant will receive a cash payment of $15 per hour. Payment will be made at the end of each visit or whenever you withdraw from the study. If you are designated as an 'alternate subject', you will be paid for the hours you spent being trained, plus you will receive a payment of $50 dollars to compensate for being inconvenienced by the administration of the study.

CONFIDENTIALITY
Carroll-Loye Biological Research will retain records of this study indefinitely. You may access you own records by contacting the Principal Investigator. Representatives from the Sponsor, EMD Chemicals, Inc., the U.S. Environmental Protection Agency (EPA), the California Department of Pesticide Regulation, and the Independent Investigational Review Board, Inc. Review Board (an independent committee that reviewed the ethical aspects of this study to help protect the rights and welfare of study participants) may have access to all non-personal information collected in this study. Because of the need to release information to these parties, absolute confidentiality cannot be guaranteed. Any information or reports published as a result of this study would not identify you by name, or any other personal identification.

STATEMENTS OF UNDERSTANDING
Right to withdraw of removal from study
I understand that I am free to withdraw from this study at any time, and I agree to inform the Principal Investigator immediately if I intend to withdraw. It is understood that my decision to participate in this study or to withdraw from this study will not influence the availability of my future medical care and will involve no penalty or loss of benefits to which I am otherwise entitled. I may withdraw from this study at any time.

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I agree that the Principal Investigator in charge of the study can remove me from this study without my consent for any reason, including, but not limited to:

a. His/her judgment that any condition or circumstance may jeopardize my welfare or the integrity of the study.
b. My failure to follow the instructions of the investigator(s).
c. If the study is stopped by the sponsor and/or Principal Investigator participating in the study prior to completion.

Consent and Signatures

I have read, in a language that I understand well, and understand the information, which has been stated above. I have received satisfactory answers to all of the questions, which I have asked. I hereby voluntarily consent to take part in this study and to be a research study participant in this study. I authorize the use and disclosure of my medical information, and do **not** waive my legal rights by signing this Informed Consent Form. I shall receive a copy of the signed Informed Consent Authorization.

_____	_____	_____
Date/Time	**Print** Subject Name	**Sign** Subject Name
_____	Scott Carroll	_____
Date/Time	**Print** Carroll-Loye Biological Research Representative	**Sign** Carroll-Loye Biological Research Representative

Independent Investigational Review Board, Inc.
Approval: 4/18/06; Revised: 7/25/06; 9/12/06

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