Carroll-Loye Biological Research

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COVER PAGE

EFFICACY TEST PROTOCOL EMD-004

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-004 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-004 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 August 28, 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 12 June 2006.
4. Science and Ethics Review of Protocol for Human Study of Mosquito Repellent Performance by EPA staff John Carley and Clara Fuentes, Ph.D. (review of draft revised protocol EMD-004, 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 16 May 2006.

EFFICACY TEST PROTOCOL EMD-004

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1 TITLE: TEST OF PERSONAL INSECT REPELLENTS

2 PROTOCOL NUMBER:

EMD–004

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–004. The study will be conducted in the laboratory at the letterhead address and at locales in nature with mosquitoes.

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 protocol, 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 mosquitoes, with efficacy measured as Complete Protection Time. Complete Protection Time, or CPT, is defined herein as the time between application of Test Material and the First Confirmed 'Lite with Intent to Bite.' A 'Lite with Intent to Bite', or 'LIBe', occurs when a mosquito alights on the treated test skin of a subject and extends its proboscis to the skin surface while ceasing locomotion. A 'First Confirmed LIBe' is that which is followed by another within 30 minutes. This 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 mosquito repellent efficacy study conducted in the field 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 two field trials, with 10 treated subjects in each trial testing each formulation, and two untreated subjects in each trial. Initial dosage determination ('dosimetry') will be conducted with a set of 12 subjects, some of whom may then go 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 tick repellent efficacy test, which is described in Carroll-Loye Protocol EMD-003). Protocol EMD-003, and this protocol, EMD-004, 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 three types: exposure to the test materials themselves, exposure to biting arthropods, and possible exposure to vectors of 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.

The risk of a skin reaction to a mosquito bite is reduced by excluding candidate subjects who are aware of having a history of such reaction. In addition, subjects will be trained to quickly remove any mosquitoes that attempt to bite them, before penetration or injection of saliva if possible. Moreover, a stopping rule instructs subjects to cover any treated skin immediately if more than one mosquito attempts to bite during any exposure period. Subjects will be exposing small areas of treated skin for only 4 minutes per hour. Other parts of the body will be protected with provided netting. Subjects will be teamed with a partner for joint observation and experienced technical personnel will be present at all times to assist.

The US Centers for Disease Control estimates that about 1-in-5 people who become infected with West Nile virus will develop West Nile fever. Subjects are instructed to be alert for any flu-like symptoms (unusual tiredness or unusually severe headaches, body aches, fever, or a rash on the trunk of the body) for up to two weeks after the test. About 1-in-150 infected people will develop more serious symptoms, which will be described to the subjects. Most people (about 4 out of 5) who are infected with West Nile virus will not develop any type of illness.

In addition, the techniques employed to minimize exposure to mosquitoes and mosquito bites render the possibility of contracting a disease carried by mosquitoes very low. Field tests are being conducted in an area where such viruses have not been detected by county and state health or vector/mosquito control agencies for at least a month, so the risk is probably low that any individual mosquito present carries a disease. Only experienced professionals (the Study Director and/or other qualified researchers) will expose untreated limbs to monitor biting pressure, at the same infrequent, brief intervals as treated subjects, and with multiple assistants to remove any mosquitoes that lite with intent to bite.

In summary, the combination of technical precautions and natural factors means that the chances that any subject will contract West Nile fever or another disease from a mosquito bite are probably extremely small. There is probably no more risk to subjects than they would experience when engaged in normal outdoor activities in a similar rural area at the same time of year. 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). Subjects may also request access to standard first aid materials (such as bandages, antiseptics, and mild topical and oral antihistamines) and request qualified first aid assistance at any time. Epi-Pens will also be on-site in case of Type 1 (anaphylactic) allergic reaction. 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. Insect-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 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 appended.

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 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 Control

The negative control is untreated for both dosimetry and repellency assays.

6.2.2 Rationale for Employing a Negative Control

Repellent efficacy can only be measured in the presence of biting mosquitoes. In addition, the duration of repellency recorded is likely a function of the number of host-seeking mosquitoes active during the study. The US/EPA uses a standard minimum rate of mosquito attack on untreated subjects to insure that the repellents under study are sufficiently challenged to provide meaningful data. Traditionally the measure rate is termed the 'ambient biting pressure'. We adopt that value, but use LIBes ('Lites with Intent to Bite') rather than bites. A mean study LIBe rate of ≥ 1 LIBe per untreated (negative control) lower leg or lower arm per 1 minute is required.

We take several precautions to minimize the probability to untreated control subjects receive any bites (see §§ 5.4, 8.2, 8.3.1, 8.4.1, 10.3.6). Recognizing that individual subjects differ in their inherent attractiveness to mosquitoes, US/EPA science reviewers have recommended that we use two untreated control subjects for this study in order to improve the likelihood of sampling ambient biting pressure in a representative fashion, while still exposing a very small number of untreated subjects to risks from foraging mosquitoes. Having separate untreated subjects also avoids the problem of interaction between treated an untreated limbs that may obtain when subjects serve as their own simultaneous controls. In reviewing an earlier version of this protocol in May 2006, the California Department of Pesticide Regulation asked for use of a single negative control, but compromised at two such subjects based on the position of the US/EPA. The prospect of

receiving approval to use more untreated control subjects is probably small in this case.

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, the added risk of including additional subjects testing matrix-only formulations cannot be justified.

6.3 Test Arthropod Species:

Testing will be conducted with all or some of wild *Aedes vexans*, *Ochlerotatus melanimon, O. taeniorhynchus,* and *Culex pipiens* mosquitoes, and possibly other mosquito species that occur in the same habitats. Mosquito specimens will be collected from untreated control subjects during testing and identified in the laboratory using taxonomic keys and stereomicroscopy in the laboratory.

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. Travel to field site. 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. However, control subjects will be chosen only from among individuals that are experienced in field biology or entomology. Whether arms, legs or both are tested at a given site will depend on the species of mosquitoes present and their behavior. That decision will be made by the Study Director based on visits to the field sites prior to data collection.

8.3 Randomization Procedures for Repellent Efficacy Testing:

8.3.1 Allocation of subjects to treatment groups:

Subjects will be assigned to the treatment (but not negative control) groups on the basis of a randomly assigned subject number. Subjects will be assigned a treatment based on their subject number and the treatment allocation table, which follows. Treatments will be balanced between arms and legs if both limbs are used. Negative control subjects will be selected exclusively from among experienced personnel.

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 simultaneously on alternate limbs of the same subjects. Doing so would reduce the absolute subject exposure by 10 individuals. To preserve subject security from biting mosquitoes, limbs would be exposed one at a time.) Two personnel who will monitor ambient biting pressure with untreated limbs are also listed to clarify the design of the test.

Subject	Lotion	Pump	Aerosol	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		
21			Left limb	
22			Right limb	
23			Left limb	

24			Right limb	
25			Left limb	
26			Right limb	
27			Left limb	
28			Right limb	
29			Left limb	
30			Right limb	
31				Left limb
32				Right limb

8.4. Conditional Boundaries or Limits of Study

8.4.1. Ambient 'Lite with intent to bite' Pressure:

A mean study LIBe ('Lite with Intent to Bite') rate of ≥ 1 LIBe per untreated (negative control) lower leg or lower arm per 1 minute is required. No more than 10% '0' values for individual exposure periods are permitted. Ambient LIBe pressure is measured from continuous exposure during 1-minute exposure periods commencing once every 15 minutes, beginning at the onset of data collection. Negative control subjects are attended by two assistants who use mechanical aspirators to remove all mosquitoes that LIBe before biting commences.

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:	18-55 years
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 hypersensitive to mosquito bites or exhibiting hypersensitivity during test
9.1.2.2 Known to be sensitive or showing sensitivity to any of the test product ingredients after application.
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.
9.1.2.10 Do not regularly spend time in outdoor settings.

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 and 2 untreated control subjects. 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 biting pressure, it is nearly certain that no untreated subjects will register fewer or later LIBes than any treated subjects. As a result, from the standpoint of statistical **power**, 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 subset 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 mosquitoes is rather large. Note, however, that for US/EPA registration purposes, the test for mosquito repellency is conducted twice, once in each of two ecologically different habitats. In our experience, the subjects in one test normally do not participate in the other (due to large geographic distances between sites). In addition, two negative controls are used for a more robust baseline comparison. Those facts decrease the probability of such sampling error substantially.

However, further considerations indicate that a somewhat larger sample would be superior. Note that the draft EPA guidelines state that the response variable, ‘Time to First Confirmed Bite’ (or LIBe in this study) 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 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 LIBe will be truncated toward the origin. However, available mean and variance data on IR3535 performance (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. (2004) recorded a mean protection time 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 data collection is only 'replicated' once (in a different habitat at that), argues for a prudent approach. To reduce the risk of over-representing atypically attractive 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:

i) 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.
ii) Initial Contact Method: Initial contact is through word-of-mouth and telephone contact with individuals in our Volunteer Data Base.
iii) 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 Principal Investigator 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 Study Director 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 individual treatments until the conclusion of data evaluation.

9.2.2 Blinding Methods:

The Test Materials as well as the 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 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, as well as the research design to minimize exposures, and the training of subjects to aspirate landing mosquitoes before they probe or bite. Because the products are topical, technical personnel will monitor, and 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. The subject will be removed from further exposure to mosquitoes.

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 cellular or satellite 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.

The risk of mosquito-associated health risks is likewise regarded as very low due to the complementary precautions outlined herein. However, the Study Director will assess skin condition of affected subjects should any bites inadvertently occur during efficacy testing. In addition, subjects will be asked to make contact with Study Director at any time should they have health concerns relating to their participation in the efficacy testing.

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

9.6 Subject training for research with mosquitoes

Approximately one week to four days before repellent efficacy testing, subjects will be trained by technical personnel in handling mechanical

aspirators and observing mosquitoes in the laboratory. Subjects will be shown how to turn on and manipulate the aspirator to capture mosquitoes by a technician who first demonstrates the following procedure, which subjects then emulate: Two laboratory-reared, disease-free female mosquitoes are released in a cage. A small area (less than ½ of the forearm) is uncovered and exposed in the cage, with no insect repellent applied. Subjects will learn how to watch approach and land on the arm, how to detect a mosquito's intention to bite, and how to quickly remove LIBing mosquitoes with the aspirator. A technician will be present to instruct and guide throughout; mosquitoes will not be exposed to more than one subject before being destroyed. This training will be documented. This 'hands-on' experience will assist subjects in collecting data accurately and handling mosquitoes safely during the repellent efficacy trial.

10 TEST VARIABLES AND THEIR MEASUREMENT:

10.1 Variables to be Measured:

Subject forearm and lower leg surface area.
Subject self-dosing behaviors.
Weight of test materials delivered to the surrogate skin (gauze) dosimeters.
Number of mosquito lites with intent to bite (LIBes) on the treated surface.

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 'lites with intent to bite' (LIBes) as they occur. Data are recorded in one minute exposures at 15 minute intervals. 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. Subjects will practice removing mosquitoes exhibiting LIBes before the field test.

10.3 Procedures for Assessing Variable:

10.3.1 Limb dimensions and surface area:

The term 'limb' refers to the forearm and the lower leg. 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) or lower leg (back of knee to ankle) 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 are 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, you will return to the instructions above that you have just carried out and conduct the procedure for the lower legs, and then conduct Spray Sampling for the legs. Next 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. Then you will repeat the entire procedure above, but with the lower leg."

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 and leg will be treated three times. Each subject will therefore have a total of forty-eight 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 and LIBes:

Repellency is assessed in the field. Preparatory training of the subjects to recognize and remove mosquitoes that lite with intent to bite contributes to subject safety. Subject safety is also enhanced by brief periods of exposure at intervals. as well as careful dosing and application.

Subjects will have approximately one hour of training and practicing observing foraging mosquitoes and catching them from their own arms in a laboratory cage, using an aspirator. A researcher will first demonstrate the procedure using his or her own arms, and will be present to instruct and guide each subject throughout the exercise. Subjects will be shown how to place both arms in a screen cage and to turn on the aspirator using the switch on the handle. One mosquito will be released in the cage. A small area (less than ½ of the forearm) will be uncovered, with no insect repellent applied. Subjects will be instructed to carefully watch the mosquito as it flies in the cage. The subject will be instructed to carefully observe the mosquito as it lands on the skin, and to watch to see if its needle-like mouths are placed against the skin. Once a mosquito lands on the skin, places it mouth against the skin and stops walking, subjects will immediately attempt to catch the mosquito in the plastic nozzle of the mosquito catcher. They may practice as many times as they wish with additional mosquitoes, and the researcher will be certain that the use of the mosquito catcher is correct. After several captures of single mosquitoes, a maximum of two mosquitoes will be placed in the cage. Two LIBing mosquitoes may be readily captured after little practice. Two represents the maximum number of mosquitoes that may LIBe on limb before the exposure stopping rule is reached (below), and so the exercise in the cage with two mosquitoes is highly appropriate.

The mosquitoes used for this training are *Aedes aegypti* reared in the laboratory and free from diseases. The source colony of *Aedes aegypti* was established from eggs collected in Northern Thailand in 2004. F_1 adults were tested by Vero cell (African green monkey kidney, *Cercopithecus aethiops*) plaque assay for possible transovarial infection of viruses. Typically, 20 females from subsequent generations are tested routinely, and no infection has been detected in the 2 years since this colony was established. A sample of 20 such females will be so tested before being employed in this study. Individual mosquitoes will not be used for more than one subject.

At the field site, the subjects and researchers will gather in an area without biting mosquitoes. Subjects are instructed not leave this area until guided by a researcher.

The technicians and other researchers who will assist subjects during the test will be introduced or reintroduced to the subjects. Subjects are instructed to call on them whenever they have questions. Each subject is given and must wear a head net, mesh jacket and pants, latex or vinyl gloves in their size, and is given an aspirator to suck any mosquitoes that land on treated skin and attempt to bite (LIBes*)* once formal exposures begin. A researcher will remind subjects about how to identify LIBes and when and how to operate the aspirator. Subjects will be further instructed about protecting themselves from mosquito bites during the test, and reporting on a mosquito that lands on skin treated with repellent.

Before the repellent is applied, subjects will be guided to wash the lower arms and/or legs 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 their forearms or lower legs to give even, complete coverage of the skin. 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 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.

Treated subjects will be partnered into groups of two. A researcher will then guide subjects into the area of the field site in which mosquitoes are active, approximately 15 minutes after the test materials are applied. Each member of a partner pair will watch their own exposed limbs and those of their partner for mosquitoes that land for one minute. A technician will advise subjects when the one-minute period begins and ends. Subjects will immediately remove any LIBing mosquitoes from the skin with repellent with the aspirator. They may also use the plastic

nozzle of the aspirator or a finger to interrupt any mosquito even more quickly.

At the end of the one-minute exposure period, subjects move away from the area with mosquito activity. Partners will assist one another in covering the treated skin with the sleeve of the net garments. Each subject will report the number of mosquitoes that attempted to bite their own treated skin during that one-minute period when asked by a technician who will record it on a data sheet. For perspective, note that in a typical test of a reasonably effective repellent, dozens of '0' LIBe values will be recorded for each '1' or '2'. In other words, during most exposure periods subjects do not experience close contact with mosquitoes.

Stopping Rule: Subjects are instructed to immediately cover exposed skin with the protective mesh provided if more than one LIBe occurs in a one-minute exposure period. Similarly, if subject receive a LIBe and recall receiving another in either of the two previous exposure periods, they are to ask their data recording technician to verify that recollection from the data record. If verified, the subject is instructed to immediately cover the limb as above.

Ambient LIBe pressure will be measured by experienced, untreated personnel from continuous exposure of a single limb during 1-minute periods commencing once every 15 minutes, beginning at the onset of data collection. Such negative control subjects are attended by two assistants who use mechanical aspirators switched on throughout the period to remove all mosquitoes that LIBe before biting commences. If mosquitoes are too abundant to permit ready aspiration, the controls may protect the exposed limb as soon as a LIBe occurs.

10.3.7 Forms for Retention of Source Data:

Dosimety data will be recorded on data form for each test material formulation. 'Lite with intent to bite' (LIBe) data will be recorded on a repellency data form. Data forms are appended.

10.4 Study Facility:

Dosimetry 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 two serving as untreated controls for repellency testing at each of two sites.

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:

Field tests are conducted with large populations of arthropods. This permits the analysis of the replicates (data by subject) as independent values. The hypothesis that the test materials will significantly reduce the number of mosquitoes LIBing on 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 mosquitoes LIBe on a subject within a half hour period. That pattern is here assessed at a resolution of 15 minutes. The untreated limbs serve to monitor whether the ambient biting pressure remains at or above the EPA standard.

Complete protection time (CPT) is measured as the length of time from initial application to the first confirmed LIBe. A confirmed LIBe is a LIBe followed by another LIBe within 30 minutes. For example, a LIBe at 120 minutes followed by another at 135 minutes is not confirmed, but a third LIBe 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. Ambient LIBing pressure as measured by the technical personnel serving as untreated controls will be presented tabulated by individual and exposure period. Mean LIBing pressure will be calculated as the number of LIBes received per untreated control subject and per period and span of exposure.

12 STUDY LOCATION(S):

Field sites are in or adjacent to the Central Valley of California, and the Florida Keys (depending on season). Test site information will be furnished to EPA once it is clear when testing will be permitted, since season influences the availability of test arthropods on both regional and local scales.

13 QUALITY ASSURANCE:

An independent, professional Quality Assurance Unit (QAU) will inspect 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:

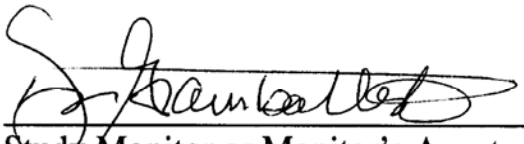
Protocol amendments or deviations will be reviewed by the Study Monitor and the Study Director. Any changes that may affect the health or safety of study participants must be approved the Study Director, the State of California Department of Pesticide Regulation, and the approving IRB. The amendments,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

C-L Field Record Mosq 8 hr

Test #		Note:		Test #		Note:		Test #		Note:		Test #		Note:	
Mosquitoes				Mosquitoes				Mosquitoes				Mosquitoes			
Name:				Name:				Name:				Name:			
Time appld:				Time appld:				Time appld:				Time appld:			
Time exposed:				Time exposed:				Time exposed:				Time exposed:			
LIMB:		Left Arm		LIMB:		Right Arm		LIMB:		Left Leg		LIMB:		Right Leg	
Min	Bites*	Min	Bites*	Min	Bites*	Min	Bites*	Min	Bites*	Min	Bites*	Min	Bites*	Min	Bites*
5		245		5		245		5		245		5		245	
10		250		10		250		10		250		10		250	
15		255		15		255		15		255		15		255	
20		260		20		260		20		260		20		260	
25		265		25		265		25		265		25		265	
30		270		30		270		30		270		30		270	
35		275		35		275		35		275		35		275	
40		280		40		280		40		280		40		280	
45		285		45		285		45		285		45		285	
50		290		50		290		50		290		50		290	
55		295		55		295		55		295		55		295	
60		300		60		300		60		300		60		300	
65		305		65		305		65		305		65		305	
70		310		70		310		70		310		70		310	
75		315		75		315		75		315		75		315	
80		320		80		320		80		320		80		320	
85		325		85		325		85		325		85		325	
90		330		90		330		90		330		90		330	
95		335		95		335		95		335		95		335	
100		340		100		340		100		340		100		340	
105		345		105		345		105		345		105		345	
110		350		110		350		110		350		110		350	
115		355		115		355		115		355		115		355	
120		360		120		360		120		360		120		360	
125		365		125		365		125		365		125		365	
130		370		130		370		130		370		130		370	
135		375		135		375		135		375		135		375	
140		380		140		380		140		380		140		380	
145		385		145		385		145		385		145		385	
150		390		150		390		150		390		150		390	
155		395		155		395		155		395		155		395	
160		400		160		400		160		400		160		400	
165		405		165		405		165		405		165		405	
170		410		170		410		170		410		170		410	
175		415		175		415		175		415		175		415	
180		420		180		420		180		420		180		420	
185		425		185		425		185		425		185		425	
190		430		190		430		190		430		190		430	
195		435		195		435		195		435		195		435	
200		440		200		440		200		440		200		440	
205		445		205		445		205		445		205		445	
210		450		210		450		210		450		210		450	
215		455		215		455		215		455		215		455	
220		460		220		460		220		460		220		460	
225		465		225		465		225		465		225		465	
230		470		230		470		230		470		230		470	
235		475		235		475		235		475		235		475	
240		480		240		480		240		480		240		480	
Subject signature:				Subject signature:				Subject signature:				Subject signature:			
*Measured as "ites with intent to bite"															

Page 1

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-004

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 including additional risk information. 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". It was noted that wording changes have been made to the "Risk" section of the consent form but 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:

phone 954-327-0778 • fax 954-327-5778 • 6738 West Sunrise Blvd., Suite 102 • Plantation, FL 33313 • info@iirb.com (email) • www.iirb.com

INFORMED CONSENT AUTHORIZATION TO PARTICIPATE AS A RESEARCH STUDY SUBJECT

Title of Study: (EMD-004) Test of Personal Insect Repellents

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

Site of Investigation: _____
_____
_____

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 mosquito repellents. Many people are interested in having new and better insect repellents available to them. The insect 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 insect repellents work.

The purpose of the study is to test how well new lotion, pump spray and aerosol insect repellent products work outdoors against mosquitoes. 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 insect repellent you put on your own arms and legs in a visit to the study laboratory, and train you to use a mechanical mosquito catcher. On a later date, we will go to a field site to test the insect repellents against mosquitoes in nature.

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.

APPROVED BY
Independent IRB
Signature
9/12/06
Date

Initials: _____
Date: _____

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 18-55 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 field research study. 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 before the field test)	Visit 2
1. Orientation and Dosage visit	X	
2. Field study visit		X
Total time	2-3 hours	8-14 hours

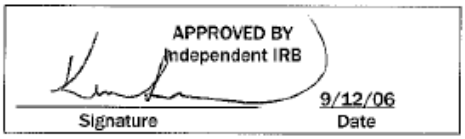
You will be given a training manual and will have a chance to review it and to read along with the instructions.

Visit 1 for Orientation and determining Dosage

Within 21 days before the field study visit you will go to the laboratory and meet with a researcher to perform introductory activities for the repellent 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 to use a handheld mosquito catching device called an aspirator. These devices resemble flashlights except that they have a small electric fan and suction tube rather than a light bulb. You will carry one of these devices with you during the field study. During this visit you will also practice removing mosquitoes from a small area of your arm with the aspirator. This training and practice will take about ½ to 1 hour.

The total time for Visit 1 activities will be about 2-3 hours.



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Visit 2 for the Field Test against Mosquitoes

The study will also require one visit to the site of the field study. The field site visit will most likely require approximately 10 hours of your time. However, it may require as few as 8 hours (including travel time) and as many as about 14 hours, depending on how long the repellents remain effective. Bathrooms are available, and meals, drinks and snacks will be provided. There is a small chance that weather conditions will require that the test be canceled or rescheduled. The Principal Investigator will inform you in a timely manner if that happens.

STUDY PROCEDURES

Study Design

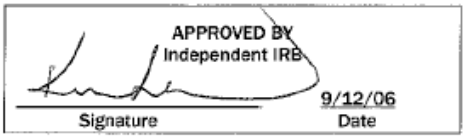
The study will test three different insect repellent products, namely a lotion, a pump spray and an aerosol spray. You will be randomly (by chance) assigned to receive one or two of the three products, so your chance of receiving any one of them is one-in-three or two-in-three. You will not have a choice 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 your forearms or lower legs. Experienced personnel will also be present to record the activity of mosquitoes by exposing their own arms or legs without repellent applied. However, you will not be asked to expose untreated skin and should avoid doing so.

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 forearm and lower leg. 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 and lower legs with soap and water and dry them with a towel. The researcher will then place three small "bracelets" made of medical gauze around your arm or leg. 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



Initials: _____
Date: _____

amounts you apply in this part of the study to determine how much repellent people normally apply.

You will also spend 15-30 minutes practicing catching mosquitoes in a laboratory cage, using an aspirator. You will be shown how to place both arms in a screen cage and turn on the aspirator using the switch on the handle. Two mosquitoes will be released in the cage. A small area (less than ½ of your forearm) will be uncovered, with no insect repellent applied. You will carefully watch the mosquitoes as they fly in the cage. Once they land on your skin, you will watch carefully to see if their needle-like mouths are placed against your skin. A researcher will be present to instruct and guide you. You may carefully move your arms to get better views and access to the mosquitoes. Once you observe a mosquito mouth touching your skin, you will immediately attempt to catch the mosquito in the plastic nozzle of the mosquito catcher. The researcher will first demonstrate the procedure to you using his or her own arms. You may practice as many times as you wish, and the researcher will be certain that your use of the mosquito catcher is correct. The mosquitoes used for this training are reared in the laboratory and free from diseases.

Visit 2

At the field site, the subjects and researchers will gather in an area without biting mosquitoes. You should not leave this area until instructed by a researcher.

You will be given an aspirator to suck any mosquitoes that land on your treated skin and attempt to bite you once the test begins. A researcher will show you again how to operate it. You will also be introduced to the technicians and other researchers who will assist you during the test. You will be instructed to call on them whenever you have questions about using the aspirator, protecting yourself from a mosquito, or reporting on a mosquito that lands on skin treated with repellent.

Before the repellent is applied, a technician will guide you in washing the lower arms and legs 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 your forearms or lower legs to give even, complete coverage of the skin. The amount of repellent applied on any one arm or leg will be no more than about ¼ teaspoon. You will also be given protective material to prevent bites on other parts of your arms and legs, plus a head net.

During the field 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

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Independent IRB

Signature 9/12/06 Date

Initials: _____
Date: _____

help you. Please ask the investigator if you have any questions at all about this kind of study.

The Principal Investigator or one of his technicians will guide you into the area of the field site in which mosquitoes are active approximately 15 minutes after you have had the test repellents applied. You and a partner will watch your own exposed arms or legs and those of your partner for mosquitoes that land for one minute. A technician will let you know when the one-minute period begins and ends. If any mosquitoes land and attempt to bite the skin with repellent, you will remove them immediately with the mosquito catcher. If at any time you have difficulties using the mosquito catcher you should push the mosquito from your skin with the plastic nozzle of the catcher. You may also use your finger to brush any mosquito aside. If you brush a mosquito aside watch carefully because it may quickly return to your skin. You will report the number of mosquitoes that attempted to bite your own treated skin on a data sheet during the one-minute period when asked by a technician who will record it on a data sheet. At the end of the one-minute period you should immediately cover the skin with the protective mesh or clothing provided. Every 15 minutes a project leader will announce the beginning of the next one-minute period for testing the treated skin and watching for mosquitoes that might attempt to bite it. If more than one mosquito attempts to bite you on your treated skin in one of the one-minute periods, or if one mosquito attempts to bite in two of three consecutive exposure periods (that is, 15 or 30 minutes apart), you should cover the skin and not expose it again.

RESTRICTIONS

- You must not be a student or employee of the Principal Investigator
- You must not be hypersensitive (allergic) to mosquito bites
- You must not be sensitive to any of the test product ingredients
- You must regularly spend time in outdoor settings
- You must not have used repellents within 3 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
- You must wear specified protective clothing during mosquito testing

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

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yourself for any reason from the study at 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.

In addition, even if you have not had a serious skin reaction to a mosquito bite previously, it is possible that such a reaction could occur if you receive any bites during this study. Swelling, redness and itching near the site of the bite are all symptoms of an allergic reaction to a mosquito bite. You should inform the Principal Investigator of one of his technicians if you are having such a reaction. There will be a first aid kit at the field site with treatments to reduce allergic symptoms from bites. Inform the Principal investigator if you are allergic to any nonprescription medicines. At least one technician with current first aid training will be present during the field test.

In addition, there is a slight possibility that you will contract a disease carried by mosquitoes if you are bitten, such as West Nile virus or equine encephalitis. This test is being conducted in an area in which such viruses have not been detected by state health or mosquito control agencies for at least a month, so the risk is probably low that any individual mosquito that might bite you carries a disease. In addition, since you are wearing repellent and other protective measures, and are carefully watching for mosquitoes that land and try to bite, you are probably at no more risk than you would experience when engaged in normal outdoor activities in a similar rural area at the same time of year.

The US Centers for Disease Control estimates that about 1-in-5 people who become infected with West Nile Virus will develop West Nile fever. For up to two weeks after the test, be alert for any flu-like symptoms (unusual tiredness or unusually severe headaches, body aches, fever, or a rash on the trunk of the body). About 1-in-150 infected people will develop more serious symptoms including neck stiffness, stupor, disorientation and possibly coma and paralysis.

Most people (about 4 out of 5) who are infected with West Nile virus will not develop any type of illness. Since you will work to quickly remove mosquitoes before they have an opportunity to bite, and few of the mosquitoes present are likely to carry the virus, your chances of getting West Nile fever or another disease from a mosquito bite are probably extremely small.

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Date: _____

If you experience any of the symptoms described above in the month following the field test you should contact a medical practitioner and inform the Principal Investigator.

PREGNANCY RISKS

The risks to the unborn are unknown and 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 <u>DO NOT</u> 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.

BENEFITS

There are no immediate benefits to you from your participation. However, by serving as a participant you may assist in making new insect repellent products available to consumers

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Signature Date

Initials: _____
Date: _____

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 Study Director. 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 will not identify you by name, or any other personal identification.

STATEMENTS OF UNDERSTANDING

Right to withdraw or 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 compensation to which I am otherwise entitled. I may withdraw from this study at any time.

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:

APPROVED BY
Independent IRB

Signature

9/12/06
Date

Initials: _____
Date: _____

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 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
Date/Time	Scott Carroll **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

APPROVED BY
Independent IRB
Signature
9/12/06
Date

Initials: _____
Date: _____