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COVER PAGE

EFFICACY TEST PROTOCOL SCI-001
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TEST OF PERSONAL INSECT REPELLENTS

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Test Material Labels and MSDS are in a companion 'Supporting Documents' file: 'Carroll SCI-001 Support Docs.pdf'

EFFICACY TEST PROTOCOL SCI-001

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1 TITLE: TEST OF PERSONAL INSECT REPELLENTS

2 PROTOCOL NUMBER:

SCI–001

3 SPONSOR:

Scientific Coordination, Inc.

3.1 Address:

4629 Cherry Valley Drive
Rockville, MD 20853

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 SCI–001. The study will be conducted in the laboratory at the letterhead address and at locales in nature with mosquitoes.

5 STUDY OBJECTIVE, RATIONALE AND STANDARDS:

5.1 Objective of Research:

The objectives of this study are to test the mosquito repellent efficacy characteristics of the Test Materials, compare them to one another, and contrast the with a comparison article that is the US Military issue topical insect repellent. Note that efficacy will be 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.

5.2 Rationale and Main Endpoint:

This study will test the efficacy of new formulations of DEET (N, N-diethyl-m-toluamide) that 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 those efficacy data, which have not been previously collected. DEET has been used worldwide for decades, but continued consumer concerns about it attributes, including poor cosmetic quality, appear to have limited its use even in situations in which its public health value is clear. Thus there is potential public value from the development and registration of more acceptably, DEET-based repellents.

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 field test of three novel DEET-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. In each trial, each formulation, including the comparison article, will be tested by ten subjects, with two untreated control subjects. Initial dosage

determination ('dosimetry') will be also be conducted with a total of 10 subjects per formulation, some of whom may then go on to participate in efficacy testing. Dosimetry will be conducted at the letterhead address. When 10 subjects have completed dosimetry for each formulation, including the comparison article, those data will be used to determine dosing for the efficacy testing.

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. Ten subjects are required in order to reduce variation around the population means we will describe. The low toxicity of the test materials should mean that there is little incremental risk associated with increasing sample size.

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 are lower than those of many 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 brief before the repellent is washed off, and likely total a much shorter duration of exposure than would a typical single consumer application. 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 fabric. 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. In each trail, only two experienced, qualified subjects 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 created a niche for new formulations with better consumer acceptance. 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 DEET products to consumers in the United States. In addition, the US Military is seeking improved DEET formulations, and is limited in its consideration to EPA-registered products.

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 Materials

1. EPA Reg. # 82810-1- LipoDEET
   This is a 30% DEET formulation contained in cosmetic lipid spheres that inhibit evaporation to prolong duration of efficacy, improve feel, reduce plasticizing and reduce odor.
2. EPA Reg. # 50404-8- Coulston's Duranon Insect Repellent
   This is a controlled-release, low-odor formulation of 20% DEET.
3. EPA Reg. # 54287-8 - Associated Registrations - Insect Guard II
   This is a functionally synergistic formulation of 17.5% DEET synergized by N-octyl bicycloheptane dicarboximide (5%) and complemented by Di-n-propyl isocinchomeronate (2.5%) to repel flies.
4. EPA Reg. # 58007-1- 3M Ultrathon- 34.34% DEET.
   This polymer based lotion extends efficacy and reduces plasticizing caused by DEET. This is the comparison article and is the insect repellent used by the US Armed Forces.

Test Materials 1-3 are EPA registered insect repellents. Details of the test formulations are appended.

6.1.2 Trade Name:

TBD

6.1.3 Dosage Form:

Lotions applied to the 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:

TBA

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. 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 testing have all been tested in animals for potential oral and dermal toxicity. The DEET active ingredient has an extensive toxicity data file, has been re-registered by EPA, and has a positive safety record in consumer use for nearly 50 years.

MSDS documentation is appended.

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 are produced under Good Manufacturing Practices (GMPs) with records available to EPA. They will be couriered to Carroll-Loye Biological Research, with Chain-of-Custody documented. After that time they will be stored at the Carroll-Loye Offices 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 will also be conducted. The EPA has extensive experience with enforcing requirements for such tests based upon their history with similar products applied to humans and Scientific Coordination, Inc. 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 in the 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 that 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 and untreated limbs that may arise when subjects serve as their own simultaneous controls. In reviewing a similar protocol in May 2006, the California Department of Pesticide Regulation initially requested 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 Comparison Article:

6.3.1 Description of the Comparison Article:

The Comparison Article is 3M Ultrathon. This is a 34.34% DEET insect repellent polymerized to reduce the rate of

evaporation and thus extend duration of efficacy. It is the chief repellent used by the US Armed Forces.

6.3.2 Rationale for Employing a Comparison Article:

The US Armed forces have used Ultrathon as the principle insect repellent for deployed warfighters for almost two decades. Because of that, it is commonly regarded as the most effective insect repellent available. Yet for reasons similar to those experienced by other consumers, the military is actively seeking alternatives. These reasons include lack of cosmetic acceptance and problems with melting plastics. Because the DEET-based products to be tested under this protocol are expected to be superior to Ultrathon in these auxiliary performance categories, yet show excellent repellent efficacy, their repellency is most appropriately directly compared to Ultrathon. Indeed, the military is considering new DEET formulations for adoption, but will require efficacy comparisons in order to make further decisions.

6.4 Test Arthropod Species:

Testing will be conducted with all or some of wild *Aedes vexans*, *Aedes melanimon, Aedes taeniorhynchus, Culex tarsalis* 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:

For efficacy testing, there are three experimental groups, namely 1) a ‘treated’ group of subjects treated with the test products, of which there are three formulations, 2) comparison article group testing Ultrathon, and 3) an untreated (‘negative’) control group. The dosimetry study is an examination of dosing behavior. Hence, for dosimetry, all subjects are treated, and there is not an untreated 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. To be regarded as experienced personnel, a candidate subject must have an undergraduate (or higher) degree in life sciences, of be a vector control professional, or have participated in at least 5 Carroll-Loye repellent efficacy studies. In addition, that person must meet all of the other participation criteria listed in §§9.1.1.1 and 9.1.1.2.

8.3.2 Treatment allocation table:

Materials will be distributed among subjects as tabulated below. Two additional personnel will monitor ambient biting pressure with untreated limbs during in the test.

Subject	LipoDeet	Duranon	Bug Guard	Ultrathon
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
33				Left limb
34				Right limb
35				Left limb
36				Right limb
37				Left limb
38				Right limb
39				Left limb
40				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 the field trial.

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, including DEET, 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 one day 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:

In efficacy testing, we will use 10 subjects per treatment and 2 untreated control subjects per field trial. Each subject is a replicate. In the dosimetry portion of the study, 10 subjects will be engaged to apply each repellent, including the comparison article.

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 considerations 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 efficacy (e.g., Carroll, S., 2006, In Debboun et al. (eds.), *Insect Repellents*, CRC Press) 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. (J. Amer. Mosq. Control Assoc. 20: 299-304, 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 subjects 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.

9.1.4 Test Subject Recruitment:

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. Those who will serve as untreated control subjects are limited to experienced technical personnel, who are screened with the same exclusion criteria as are other subjects.
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 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. This freedom is especially re-emphasized in cases in which considerable effort or expense has been required to include a subject (e.g., air travel to a distant site), to discourage the

conception that that effort or expense creates any added obligation in the subject.

Candidates are given copies of the State of California Department of Pesticide Regulation 'Experimental Subjects' Bill of Rights' (Appendix 4) 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, a physician who has read the protocol and discussed the research with the Study Director will be on call. 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 one-half) 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 will be measured prior to Test Day 1.

In efficacy testing, 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'). The time between application of test materials and the initiation of exposure will be measured. 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.

10.3.4. Dosimetry

The amount of lotion applied to limbs will be quantified in a series of three applications for each repllent. 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. If you have sufficient lotion remaining, spread it evenly and thoroughly toward the

elbow. Do not spread it beyond 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 will instruct you to 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 to the arm. Then you will repeat the entire procedure above, but with a lower leg. You will complete this sequence of three arm applications, and three leg applications for each of the repellents being studied. For each repellent you will begin with a practice application to familiarize yourself with how it comes out of the tube, and how it covers and spreads on the skin."

10.3.5 Equipment Used to Assess the Dosimetry Variable:

Test material containers 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 one-half 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. 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, Tyvek coveralls, latex, nitrile 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. 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 protective 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 10 treated subjects testing each of the three repellent formulations and the comparison article. For efficacy testing, there will be 10 subjects treated with each test material and two serving as untreated controls 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 arm in each trial will be calculated as the container weight before application minus the container weight after application.

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 weight changes.

We will statistically assess the strength of any individual subject differences in dosing with the test materials using Friedman two-way analysis of variance of 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 90 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:

Timothy H. Dickens, PhD.

14.2.1 Address:

Scientific Coordination, Inc.
4629 Cherry Valley Drive
Rockville, MD 20853

14.2.2 Telephone:

Phone: 301-570-4390
Fax: 301-570-5914

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 AMENDMENTS AND DEVIATIONS TO THE PROTOCOL:

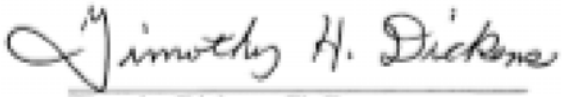
Protocol amendments or deviations will be reviewed by the Study Monitor and the Study Director. Any changes that may affect the health or safety of study participants must be approved the Study Director, the State of California Department of Pesticide Regulation, and the approving IRB. The amendments,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

14 December 2006

Scott P. Carroll, Ph.D.
Study Director

Date



Timothy Dickens, Ph.D.
President, Scientific Coordination, Inc.

14 December 2006

Appendix 1. Data recording forms.

Limb Measurement Form
Study EMD-003/004
Date:

Subject name: **Data recorder name:**
Subject number: **Data recorder signature:**

Limb Measurements	**Left arm**	**Right arm**	**Left leg**	**Right leg**
Length				
Lower (A)				
Lower-mid (B)				
Upper-mid (C)				
Upper (D)				

Lotion Data Form
Study EMD-003/004
Date:

Subject name:
Subject number:

Data recorder name:
Data recorder signature:

I. Practice Application		
A. Arm. Left or Right (circle 1)		
Trial number	Mass before (g)	Mass after (g)
1		

II. Lotion Sampling		
A. Arm. Left or Right (circle 1)		
Trial number	Mass before (g)	Mass after (g)
1		
2		
3		

B. Leg Left or Right (circle 1)		
Trial number	Mass before (g)	Mass after (g)
1		

B. Leg Left or Right (circle 1)		
Trial number	Mass before (g)	Mass after (g)
1		
2		
3		

Study: SCI-001

Date:

Site:

Application Time:

Time of first exposure: **LIBe recording code: 0=none, 1=1**

Incidence of LIBes at 15-minute intervals

Subject code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	71	18	19	20	21	22	23	24	25	26	27	28	29	30

Data collected by:

Data collector's signature:

Appendix 2. Subject training documents

711 Oak Avenue Davis, California 95616 Tel (530)297-6080 http://www.carroll-loye.com/

Test Reference: SCI-001

CLBR Training Manual

§1.c. Practicing and performing dosimetry with Lotion delivery systems

A. Goals of exercise

1. Determine your preferred practices for applying lotion repellents to your arms or arms and legs.
2. Assist technicians in measuring the amounts of such repellents that you apply when using your practices

B. General information

1. A technician will measure the surface area of your forearms and lower legs. He or she will introduce you to the repellents and their containers
2. You will work in the laboratory, practicing applying the repellents.
3. You will thoroughly wash your limbs with a gently skin cleaner between each application of repellent.

C. Materials and equipment needed

1. Test materials
2. Latex or vinyl gloves (various sizes)
3. Temperature and humidity measuring devices
4. Written copy of the procedures for subjects to read
5. Flexible metric rule

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D. Practicing the methods and performing the measurements

Measuring arms and legs[1]:
Limb is use to refer to your forearm and your lower leg. A technician will measure the distance around your limbs at four evenly spaced places on the forearm (elbow to wrist) and lower leg (back of knee to ankle), and also length of those limbs.

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

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[1] **Limb dimensions and surface area (technical details):**
The term 'limb' refers to the forearm and [1]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 to place dosimeters) as the distance in centimeters from the distal margin of the site of the most distal circumference site (i.e., at wrist or ankle).

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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."

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Test Reference: SCI-001

CLBR Training Manual

§1.a. Observing mosquito landings and learning mechanical aspiration

A. Goals of exercise

1. Learn to determine when a mosquito on your arm is about to bite.
2. Learn to use a "mechanical aspirator" to remove such a mosquito before it bites. Catch at least 10 mosquitoes.

B. General information

1. A technician will show you how to watch mosquitoes that land on you to see if they are about to bite. He or she will then show you how to remove mosquitoes. quickly with a handheld mosquito catching device called a mechanical aspirator
2. You will work with you arms in a screen cage about 1 foot square, with up to two mosquitoes in the cage at one time.
3. You may be bitten by a mosquito while learning to use the aspirator. The mosquitoes were reared in the laboratory and are free from disease.

C. Materials and equipment needed

1. Mosquito cage with entrance stocking
2. Latex or vinyl gloves (various sizes)
3. "Ace" bandage
4. Approximately 12 mature unfed adult female *Aedes aegypti* mosquitoes
5. Mechanical aspirator with charged batteries and collection tube

D. Leaning the methods

Spend at least 15-30 minutes practicing observing and catching mosquitoes, working with one or two at a time. Aspirators resemble flashlights except that they have a small electric fan and suction tube rather than a light bulb. You will carry one with you during the field test of the repellent. Your trainer will first demonstrate the method of use and capture. The trainer will then cover your upper forearm with the bandage to protect that area from biting.

Put on gloves. Practice using the switch on the aspirator handle to turn it on, and insert the sucking tube into the cage through the elastic cloth. Then place your arm with the bandage into the cage. About half or your forearm will be uncovered, with no insect repellent. Carefully watch the mosquito as it flies in the cage. Once it lands on your skin, watch carefully to see if it stops walking and places its needle-like mouth against your skin. You may move your arms to get better views and access to the mosquito. 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. You may practice as many times as you wish, with one and then two mosquitoes, and the researcher will be certain that your use of the mosquito catcher is correct.

Appendix 3. IRB Approval Letter and Informed Consent Form

REVISION APPROVAL LETTER TO BE INSERTED HERE

The original protocol and ICF were approved by IIRB of Plantation, Florida on 7 November 2006. Following discussions of those documents with Mr. John Carley of the EPA OPR during the week of 11 December 2006, they have been subjected to minor revisions. Pending likely approval of the revisions by the IRB, we anticipate providing EPA with documentation of that approval in December 2006.

Appendix 4. California State Department of Pesticide Regulation Experimental Subject's Bill of Rights.

State of California Department of Pesticide Regulation

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

The rights below are the rights of every person who is asked to be in a research study. As an experimental subject I have the following rights:

1. To be told what the study is trying to find out.

2. To be told what will happen to me and whether any of the procedures pesticides or devices is different from what would be used in standard practice.

3. To be told about the frequent and/or important risks, side effects, or discomforts of the things that will happen to me for research purposes.

4. To be told if I can expect any benefit from participating, and, if so what that benefit might be.

5. To be told the other choices I have and how they may be better or worse than being in the study.

6. To be allowed to ask any questions concerning the study both before agreeing to be involved and during the course of the study.

7. To be told what sort of medical treatment is available if any complications arise.

8. To refuse to participate at all or to change my mind about participation after the study is started. This decision will not affect my right to receive the care I would receive if I were not in the study.

9. To receive a copy of the signed and dated consent form.

10. To be free of pressure when considering whether I wish to agree to be in the study.

If I have other questions I should ask the researcher. In addition, I may contact the Worker Health and Safety Branch, Department of Pesticide Regulation, which is concerned with protection of volunteers in research projects. I may reach them by calling (916) 445-4222 collect from 8:00 AM-5:00 PM., Monday to Friday or by writing to the Department of Pesticide Regulation, Worker Health and Safety Branch, 830 K. St., Sacramento, CA 95814-4268.