COVER PAGES

EFFICACY TEST PROTOCOL LNX-001

EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin)-BASED PERSONAL INSECT REPELLENTS (20% CREAM and 20% SPRAY) WITH MOSQUITOES UNDER FIELD CONDITIONS

SYNOPSIS

KBR 3023 (Icaridin; Picaridin) is a new generation of synthetic repellent developed as an alternative to DEET. It was developed by molecular modeling techniques. From more than 800 substances, KBR 3023 showed the best performance regarding efficacy against a variety of arthropods (Boeckh, et al., 1996) and had the most desired attributes regarding safety, low skin penetration, compatibility with skin, and plastic materials. It was developed by Bayer and is now owned by Saltigo GmbH (Lanxess Group) and in the USA it is handled by Lanxess Corporation (previously a Division of Bayer Corporation).

Icaridin (US EPA Registration Name Picaridin), the current common name, was developed under the Code Name KBR 3023 and the registered trade name Bayrepel™ and was sold under the Brand name Autan. The chemical name for Icaridin is 1-PIPERIDINECARBOXYLIC ACID, 2- (HYDROXY-ETHYL), 1-METHYLPROPYLESTER. However, the INCI (International Nomenclature of Cosmetic Ingredients) name was given as HYDROXY METHYL ISOBUTYL PIPERIDINE CARB. The product was submitted to US EPA under the common name Picaridin. However, the common name, Picaridin, was rejected by ISO (International Organization for Standards) as it was not considered a pesticide. The common name Picaridin was also rejected by WHO/INN (World Health

Organization/International Non-proprietary Name) but the common name, Icaridin, was accepted by WHO/INN

The study pursuant to the following insect repellent efficacy protocol is intended to provide data under the Data-Call-In requirements (EPA Reg. No. 3126-LRN0) of United States Environmental Protection Agency Guideline OPPTS 810.3700. That protocol, dated 27 March 2007, was reviewed and approved by a private IRB, the Independent Investigational Review Board (IIRB), located in Plantation Florida, on 5 April 2007. The document in hand is that which IIRB reviewed, with the addition, on 10 April 2007, of the following elements for review by the United States Environmental Protection Agency, including its Human Studies Review Board. 1) These completed cover pages (pp. 1-2); 2) the approved, signed Informed Consent Form, which has been substituted for the submitted Informed Consent Form; 3) record of PI–IRB correspondence; 4) approved minutes of IRB protocol review meeting. The final Table of Contents on the next page reflects those changes and additions.

EFFICACY TEST PROTOCOL LNX-001

EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin) -BASED PERSONAL INSECT REPELLENTS (20 % CREAM and 20% SPRAY) WITH MOSQUITOES UNDER FIELD CONDITIONS

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EFFICACY TEST PROTOCOL LNX-001

1 TITLE: EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin)-BASED PERSONAL INSECT REPELLENTS (20% CREAM and 20% SPRAY) WITH MOSQUITOES UNDER FIELD CONDITIONS

2 PROTOCOL NUMBER:

LNX–001

3 SPONSOR:

LANXESS Corporation

3.1 Address:

111 RIDC Park West Drive
Pittsburgh, PA 15275-1112

4 PROTOCOL OBJECTIVE:

4.1 Type of Protocol:

This protocol will indicate the specific methods to be used, and direct the conduct of, Study LNX–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 objective of this study is to test the repellent efficacy characteristics of the test materials to mosquitoes. The active ingredient, KBR 3023 (aka Icaridin; Picaridin), is of known high broad spectrum efficacy, but has not been studied at very many concentrations in the United States.

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. The work conducted pursuant to this protocol will be initiated by determining the amount of each of the repellents that subjects typically apply. Dosimetry will consist of a behavioral assay.

5.2 Rationale and Main Endpoint:

Existing efficacy data on KBR 3023 has been developed over several years by Bayer/Lanxess sponsored studies, independent researchers, World Health Organization (WHO) and military research organizations of the United States and Australia. Twenty percent formulations were found to have efficacy of 8 hours against numerous species and under a variety of climatic condition in various parts of the world. However, LANXESS does not have efficacy data generated in the United States. As part of the registration review of the 20% formulations, US EPA has requested new, US-based efficacy data as condition of registration. The rationale for this study is to provide those efficacy data. The information will also be used in product labeling and for increased user acceptance.

This study will test the efficacy of two newly registered formulations of KBR 3023 that are intended to provide information on Complete Protection Time achieved by a single application of the product. KBR 3023-based repellents have been marketed around the world for a

decade, but the product is comparatively recent in the United States market (2005). The US Centers for Disease Control has acknowledged the existence of substantial consumer interest in new and effective insect repellent products, and that KBR 3023-based repellents are among the very few of sufficiently high efficacy to offer reliable personal protection against vectors of West Nile Virus (in April 2005). However, few 'Picaridin' products are currently marketed in the US. Thus there is substantial merit in the development and unconditional registration of new KBR 3023-based repellent products.

Information regarding the stability of the end-products is available from the sponsor in a separate study.

The main endpoint of this study will be the conclusion of a mosquito repellent efficacy field test of novel KBR 3023-based topical repellent formulations (KBR 3023 All-Family Insect Repellent Spray and KBR 3023 All-Family Insect Repellent Cream), with the data set suitable for submission to US/EPA to comply with the conditions of the registration. The efficacy study will consist of two field trials. In each trial, the Picaridin formulations will be tested with 10 subjects, with two untreated control subjects. Initial dosage determination ('dosimetry') will also be conducted with 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, the resulting 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 material, 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.

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 a history of consumer use. The concentration of the active ingredient in the product being tested is lower than that of other 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 brief duration before the product is washed off, and the likely total exposure time is much shorter than a typical single consumer application. Risks associated with inhalation and ingestion would only ensue from serious mishandling by subjects, a scenario that the study methods preclude. The summary toxicology profile of KBR 3023 is appended.

The risk of skin reactions 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 gloves, headnets and full body suits made of Tyvek, through which mosquitoes do not bite. Subjects will be teamed with a partner for joint observation and experienced technical personnel will be present at all times for assistance.

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), glandular swelling 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 trial, only two experienced, qualified subjects (qualification criteria described in §9.1) 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 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 a 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 any time, without penalty to their compensation.

Against these 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. Moreover, discomfort associated with nuisance biting

restricts many work and pleasure activities. Because EPA registration requires efficacy data, a test such as that proposed here is the only path toward further product development and greater availability of new KBR 3023 mosquito repellents 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 Materials

There are two test materials, a 20% KBR 3023 lotion ('cream') and a 20% KBR 3023 pump spray. Labels are appended.

6.1.2 Trade Name:

KBR 3023 All-Family Insect Repellent Cream
KBR 3023 All-Family Insect Repellent Spray

6.1.3 Dosage Form:

Lotion and Pump Spray applied to the skin.

6.1.4 Dose:

Determining dosage is part of the 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:

Laboratory-prepared by Saltigo GmbH, (Lanxess Group), Germany.

6.1.6 Test Material Storage During Study:

Prior to application, the test materials will be stored indoors, at room temperature and away from direct sunlight or direct sources of moisture or the conditions specified by the sponsor. 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 or 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 has been tested in animals for potential oral and dermal toxicity. The active ingredient (KBR 3023) has an extensive toxicity data file, has been previously registered by EPA, and has a positive safety record in consumer use.

A toxicology profile of KBR 3023 is provided in the appendices.

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 will be produced under applicable Good Laboratory Practice standards, 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 (19-24°C). The composition and content of active ingredient in the products used for the proposed efficacy studies will be confirmed by analytical methods prior to and following human subject efficacy testing. The sponsor believes that the formulations will be stable for the duration of the study, based on storage stability studies previously conducted. Additional one-year stability tests are in progress. The EPA has extensive experience with enforcing requirements for such tests based upon their history with similar products applied to humans and LANXESS 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 ensure 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 than two untreated control subjects is probably small in this case.

There are no controls by which the formulation matrices without the repellent active ingredient are 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:

None.

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 and from the protective clothing of all subjects during testing and identified in the laboratory using taxonomic keys and stereomicroscopy.

7 STUDY SCHEDULE:

7.1 Proposed Date of Initiation:

To be determine (TBD); within one year of IRB approval.

7.2 Schedule of Events:

Test day	Date	Activities
-30 to -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 dosing behavior and rates, mean dosing rates, and individual dosage values
1	TBD	Prepare individual dosages for application. Meet with subjects to review day plan and safety procedures. Administer repellent, or do so after travel to field site. Travel to field site. Review safety and data collection procedures. Commence repellency data collection. Monitor subject safety, comfort, comportment, and 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 of each test material, there are two experimental groups, namely 1) a 'treated' group of subjects treated with a single test product, and 2) an untreated ('negative') control group. The dosimetry study is an examination of dosing behavior for each test material. In that study, each subject will be treated, and will also serve as their own untreated control for the dosimeters.

8.2 Experimental Design:

The experiment will be partially randomized by subject. Because the treated condition will be evident to experimenters and subjects, and the test materials are readily distinguishable (opaque lotion versus clear, low viscosity liquid), neither group will be effectively blinded. The clear criteria on which failure is determined (definition of LIBe) will help to eliminate any influence of experimenter or subject bias. Also note that for subject safety, control subjects will be chosen only from among individuals that are experienced in entomological testing or biological science (see §8.3.1, below). 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 reconnaissance of the field sites prior to data collection.

8.3 Randomization Procedures for Repellent Efficacy Testing:

8.3.1 Allocation of subjects to treatment groups:

All subjects that are not untreated controls will be assigned to the treatment group. 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, or be a vector control professional, or

have participated in at least five 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 who will monitor ambient biting pressure with untreated limbs during the test are also shown.

Subject	Lotion	Pump Spray	Untreated
1	Left limb		
2	Left limb		
3	Left limb		
4	Left limb		
5	Left limb		
6	Right limb		
7	Right limb		
8	Right limb		
9	Right limb		
10	Right limb		
11		Left limb	
12		Left limb	
13		Left limb	
14		Left limb	
15		Left limb	
16		Right limb	
17		Right limb	
18		Right limb	
19		Right limb	
20		Right limb	
21			Left limb
22			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, all subjects:

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. Inclusion criteria specific to the two untreated subjects

9.1.2.1 To qualify for candidacy as a subject who exposes untreated skin, an individual must be regarded as competent to do so by the Principal Investigator, must have participated in at least five prior Carroll-Loye repellent efficacy trials, or have participated in at least three such trials and have at least two years of experience as a

college life sciences major, or be professionally employed in vector control services.

9.1.3 Exclusion criteria, all subjects:

9.1.3.1	Known to be hypersensitive to mosquito bites or exhibiting hypersensitivity during test
9.1.3.2	Known to be sensitive or showing sensitivity to any of the test product ingredients, after application.
9.1.3.3	Poor physical condition.
9.1.3.4	Unwilling to submit to brief query about personal condition.
9.1.3.5	Use of insect repellent within one day preceding the study.
9.1.3.6	Unwilling to refrain from use of perfumed products, alcoholic beverages or smoking after 9 PM the evening preceding the test and throughout the test.
9.1.3.7	Known to be pregnant or lactating. Pregnancy will be self-checked by each female volunteer on the morning of the repellent test using an OTC test kit provided by the Study Director. Results of each such test will be immediately verified by direct inspection by a female technician trained to make that assessment. Only volunteers scored as nonpregnant will be allowed to participate.
9.1.3.8	Unable to deliver the test materials to own left and right limbs.
9.1.3.9	Unable to see mosquitoes on skin or otherwise effectively monitor and remove mosquitoes that alight on skin.
9.1.3.10	Student or employee of the Study Director.
9.1.3.11	Does not regularly spend time in outdoor settings.
9.1.3.12	Withdraws from testing before receiving a confirming LIBe, when the total exposure duration is less than 90% of the mean of subjects who did not withdraw, and when not more than 2 of 10 subjects have so withdrawn.

If more that 2 of 10 subjects withdraw prematurely, those with the briefest participation will be replaced first. This exclusion factor is not automatically invoked if the Study Director ends exposures due to other factors, such as darkness; in such cases the data collected before termination may be sufficient to meet the study goals.

9.1.4 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. In the dosimetry portion of the study, 10 subjects will be engaged to apply each repellent. 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 considerations demand the opposite in the present study, particularly during the efficacy testing 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 $P<0.05$ (one-tailed test in which all treated values differ in the same direction from the untreated). 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, some or all of the subjects in one test normally do not participate in the other (due to 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 a normal probability density function that interval is ±1.96 standard error of the mean. In this study we anticipate that the distribution of Times to First Confirmed LIBe will be truncated towards the origin. Because available mean and variance data on efficacy (e.g., Carroll 2006 and other references listed in below) indicate that no individual values will be near zero, approximating the exponential model with the normal model is likely justifiable. 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. Alternatively, if tests indicate substantial deviation from a normal distribution, the deviation will be corrected prior to anlysis.

To consider an example, in a study of repellency employing eight subjects, Cilek et al. (2004) recorded a mean protection time of approximately 180 minutes, with a standard error of about 15 minutes. Had the *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, in the case of a highly efficacious repellent, adding subjects beyond six is likely to increase the precision of the means estimate only slowly. However, the regulatory justification for this study is predicated on an aversion to assuming that efficacy is closely predictable in new formulations. That conservatism is further justified on the basis of the individual and public health importance of avoiding inaccuracy in this study. That position, 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.

9.1.5 Individual subject influences on repellent performance and risks from participation, in relation to the choice of subjects

Carroll (2006) reviewed the factors that influence the performance of insect repellents and concluded that there is no *a priori* means of predicting an individual's attractiveness to a particular mosquito population, or likely impact on the data set of a repellency trial. Several studies have indicated that individuals differ in attractiveness to mosquitoes, but that individual attractiveness rankings shift substantially among mosquito taxa. Skin emanated volatiles influence attractiveness, and skin temperature and absorption properties may likewise influence repellent efficacy.

The search for general characters to predict repellent performance has led to studies of gender, age, race, hair color, complexion, weight, skin moisture, menses (females), hairiness, and sweat. Of these, only gender has been shown strong and significant effects on individual, in two studies with adequate sample sizes. One of these studies found females to be 25% less attractive to *Aedes* mosquitoes, while that other showed them to be significantly less well protected against *Anopheles* mosquitoes by deet– the opposite pattern. That difference is consistent with further findings that the type of repellent used also interacts complexly with individual subjects and mosquito species in determining efficacy. Nonetheless, because gender effects seem most plausible, we attempt to engage similar numbers of males and females.

For clarity, note that, for example, studies have shown that sweating increases attractiveness to at least one mosquito species. What is not clear is that individuals that sweat more than others, on average, tend to be more attractive to mosquitoes.

On the other hand, it *is* clear that conditions of use strongly influence repellent performance. We intentionally limit our testing to places and times where large number of mosquitoes are active. Further, we expose subject individuals as uniformly as possible to those mosquitoes, and have them engage in behaviors resembling common outdoor activities (walking, sitting, reaching) that may be attractive to mosquitoes. Subjects are monitored to prevent exposure of treated areas to external moisture or abrasion.

Analogous to the summation for repellency, there are few clear patterns permitting us to predict which individuals might be at relatively greater risk from participating in this study. Pregnant and lactating women are excluded on general medical principals, and persons over 55 are excluded due to slightly elevated health risks from West Nile Fever (above), even though the likelihood of contracting the causal agent during a repellent test is very low.

9.1.6 Choice of subjects and recruitment

9.1.6.1 Sampling Frame of Study Subjects

For reasons of practicality and control, we work with people from the community in which our business is located (Davis, CA). Davis is a university-dominated community, and so the population demography differs somewhat from non-university communities. Based on census data, the four major race/ethnicity grouping in the local population is approximately 70% White, 15% Asian, 8% Hispanic, and 2% African-American.

Initial contact is through word-of-mouth and telephone contact with subjects who have participated in similar previous Carroll-Loye repellent efficacy tests and have agreed or requested to be in our Volunteer Database. At present that database consists of 30 males and 28 females. Of the 58 total subjects, 44 (76%) identify themselves as Caucasian, 8 (14%) as Asian, 3.5 (6%) as Hispanic (white), and 2.5 (4%) as African American. These proportions match the city's racial distribution quite closely.

Three-quarters of the subjects are age 20-40; the remainder is between 40 and 55. Educational levels are as follows: 7 PhD, 8 MS, 18 in graduate programs, 14 others with BS/BA, and 10 undergraduate students. Among those who are not students, there is one professor, 15 professional researchers, 5 professional artists, 3 teachers, 3 office workers, 2 business owners, 2 sales people, 1 massage therapist, and a few unspecified. The youth of the age distribution reflects the collegiate community. Education levels are very high for the same reason. Profession is heavily slanted toward life science researchers and students, reflecting the community as well as perhaps the nature of the studies and the people who are interested in participating in them. While many of the subjects with whom we have worked show a keen and enduring interest in participating, such interest is not likely predictive of anything atypical about the results stemming from their presence in a study.

Compared to the US population (all potential repellent users), our sampling frame tends to under-represent blacks and over-represent Asians. It is also young, and well educated. Based on review of the scientific literature regarding individual differences in repellent performance and attractiveness to mosquitoes, we conclude that those deviations from the ideal frame will not influence the representativeness of the results, or their generalizability to the greater population. Lastly, because our Volunteer Database cohort is comprised by individuals who regularly spend time in outdoor setting (and thereby may have relatively frequent encounters with biting arthropods), this group is probably appropriate for insect repellent users in general.

9.1.6.2. Initial Recruitment Process

In recent years our Volunteer Database has grown through people who initiate contact with Carroll-Loye Biological Research. Those individuals learn of our work from persons who have worked with us; we do not direct or actively encourage that process. In those initial contacts, the prospective subjects typically have prior knowledge of our work and its general purpose, and what their fellows have experience in prior studies. Each individual in the database has requested that we contact him or her in the event that test subjects are needed for insect repellent efficacy testing.

About half of our subjects are present or past University of California–Davis graduate and undergraduate students, and postdoctoral researchers, in life science programs. Students in the laboratory of the Principal Investigator who depend on him directly for employment or scholastically are not eligible to participate. 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, and have additional inclusion requirements.

9.1.6.3 Screening of Candidate Subjects

All such potential participants are screened or re-screened for suitability for each test in a private, one-on-one conversation with the Study Director. Some former subjects have been excluded from mosquito repellent testing, for example, because they passed the upper age limit, when the health risks of West Nile Fever are statistically more serious. 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 to female candidates 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. Candidates are then asked if they would like to retire from consideration at that point. If they wish to remain in consideration, it is 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., 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, or work directly with or otherwise regularly encounter mosquitoes in infested habitats, we regard their motivations and decisions to participate as being 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.

9.1.7 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.

9.1.8 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.

9.2 Blinding of Study:

None

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 one fingertip 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 35% 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 one day 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 '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 General Considerations

Dosimetry data collection may be conducted on subject arms, legs, or both. Which limbs are used depends on whether efficacy testing, which will follow dosimetry, is to be conducted with repellents sprayed on arms, legs or both. That determination will be made in advance of the dosimetry study, based on the behavior of the mosquitoes present at the chosen field study sites.

10.3 When Variable will be Assessed:

Dosage will be calculated on the basis of surface area of the 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.

10.4 Procedures for Assessing Variable:

10.4.1 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.

10.4.2 Familiarization with, and subject use, of the spray apparatus:

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

Subjects will practice application of test material to their own limbs under the procedure in the Training Materials appendix, which will be reviewed for the subjects by a researcher before practice commences. That material explains that goals of this behavioral part of the study, describes that partnership between the subject and the technician in dosimetry data collection, and details the procedures to be conducted in simple language that is intentionally scripted as somewhat redundant in order to emphasize the structure of the work.

10.4.3 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 limbs to 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 procedure appended under Training Materials.

10.4.4 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.

10.4.5 Equipment Used to Assess the Dosimetry Variables:

Passive dosimeters are 2.5 cm wide strips of Nexcare™ Co-Flex™ cohesive flexible bandage. They are applied to limbs in the manner of 'bracelets'.

Each test limb will be treated three times. Each subject will therefore use 12 bracelets per limb for dosimetry.

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 will be weighed before and after dispensing on a traceably calibrated Sartorius GC 2502 (measurement increment 0.001 g, 500 g capacity).

10.4.6 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 by careful dosing and application.

Subjects will have approximately one hour of training and practice 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, to observe the mosquito as it lands on the skin, and to watch to see if its needle-like mouthparts are placed against the skin. Once a mosquito lands on the skin, places its mouth against the skin and stops walking, subjects will immediately attempt to catch the mosquito in the plastic nozzle of the aspirator. They may practice as many times as they wish with additional mosquitoes, and the researcher will be certain that the use of the aspirator 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 2.5 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 to 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 a mechanical aspirator to remove any mosquitoes that land on treated skin and attempt to bite (LIBe) 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 about reporting when a mosquito that lands on skin treated with repellent.

Before a repellent is applied, subjects will be guided to wash the lower arms and/or legs with mild, fragrance-free soap, rinsing them with a spray of ethyl alcohol (35% in water), and then drying the limbs with a clean towel. A technician will then apply the test material to a forearm or lower leg of each subject, giving 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 the test material. Each subject will therefore be dosed at the same rate, even if their voluntary individual application rates might otherwise differ 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. For a one-minute period, members of a partner pair will watch their own exposed limbs and those of their partner for mosquitoes that land. A technician will advise subjects when the one-minute period begins and ends. During exposure subjects will immediately remove any LIBing mosquitoes from the

exposed skin with the aspirator. They may also use the plastic nozzle of the aspirator or a finger to interrupt any mosquito even more quickly. Partners will assist one another in removing mosquitoes as needed.

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, potentially for the first several hours, subjects do not experience close contact with mosquitoes. The probability of eventual direct contact, if any occurs before the cessation of exposure due to darkness or subject withdrawal, increases at a slow rate.

Stopping Rule: Subjects are instructed to immediately cover exposed skin with the protective clothing provided if more than one LIBe occurs in a one-minute exposure period. Similarly, if subjects 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, concurrent with treated subjects. Such negative control subjects are attended by two assistants who use mechanical aspirators, switched on throughout the exposure 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.

10.4.7 Forms for Retention of Source Data:

Dosimety data will be recorded on two data forms. 'Lite with intent to bite' (LIBe) data will be recorded on a field repellency data form. Data forms are appended.

10.5 Study Facility:

Dosimetry data collection will take place in the main laboratory 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. For efficacy testing, there will be 10 subjects treated with the 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 dosimeter set 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 or 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

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, number of pump actuations). We will use subject dose means for the test material to calculate the dosing grand mean (± SD). That mean, expressed as repellent weight per unit skin surface area, will be converted to volume and used to determine individual subject doses in the field repellency test. To accomplish that, the specific gravity of the test material will be used to convert the dosage weight data to volumes, prepared for each subject on the basis of their skin surface area.

Subject effects on dosing behavior will be examined with nonparametric tests for *n*- sample independent cases (Kruskal-Wallis tests). In multiple regression analysis, the average amount of test material intercepted by each subject's dosimeters, as well as dosing per unit skin surface area, will be examined in relation to the distance from nozzle to skin, the number of times the pump was actuated, and limb size. The relationship between dosing behavior and dosage will also be examined with Spearman-rank correlation tests.

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 material 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 repellency breakdown sufficient 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, and will be presented with standard deviation and 95% confidence interval information as well. Ambient LIBing pressure as measured by untreated subjects 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.

To examine the temporal pattern of failure further, we will employ Kaplan-Meier survival analyses by subject. Kaplan-Meier survival analysis permits us to account for censoring in the event that any subjects withdraw before failure. In addition, we will estimate the Kaplan-Meier median, and the time until 25% failure, for each test product, Variance

around median estimates is much more robust than it is around means estimates when censored data are used.

12 STUDY LOCATION(S):

Field sites are in or adjacent to the Central Valley of California or southern California (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:

A separate, 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 Carroll-Loye Biological Research

14.2 Study Monitor:

Dr. G. K. (Ghona) Sangha

14.2.1 Address:

359 Country Club Drive #15
Simi Valley, CA 03065

14.2.2 Telephone:

Phone: 913-638-3968; Fax: 253-840-8047

14.3 Quality Assurance Unit:

Dr. William Donahue

14.3.1 Address:

Sierra Research Laboratories
5100 Parker Road
Modesto, CA 95357

14.3.2 Telephone:

209-521-6380

14.3.3 Training and experience of QAU:

CV on file with Carroll-Loye Biological Research

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 LITERATURE CITED AND SELECTED REFERENCES

Bernard, Donald R., Bernier, U. R., Posey, K. H. and Xue, Rui-De (2002). Repellency of IR 3535, KBR 3023, para-menthane-3,8,-diol, and DEET to Black Salt Marsh Mosquitoes (Diptera: Culicidea) in Everglades national Park). J. Med. Entomology 39(6): 895-899

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Cilek, J. E., Peterson J. L., and Hallmon, C. F. (2004) Comparative efficacy of IR3535 and DEET as repellents against adult *Aedes aegypti* and *Culex quinquefasciatus*. J. Amer. Mosq. Control Assoc. 20: 299-304

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Yap, H.H., Jahangir, K. and Zairi, J. (2000) Field Efficacy of Four insect Repellent Products against Vector Mosquitoes in a Tropical Environment. Journal of the Mosquito Control Association. 16(3): 241-244

16 PROTOCOL APPROVAL SIGNATURES:

27 March 2007

Scott P. Carroll, Ph.D.
Study Director

Date

27 March 2007

G. K. (Ghosa) Sangha, Ph.D.
Study Monitor

Date

INDEPENDENT
INVESTIGATIONAL
REVIEW BOARD INC.
Your Advocate for Clinical Research Participants

Kim Lerner
Chairman

Anita McSharry, R.N.
President

DATE: April 05, 2007

TO: Scott P. Carroll, PhD
Principal Investigator

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

SUBJECT: Approval Clinical Research Protocol dated: 3/27/2007
- Informed Consent Form (Ver. 4/3/2007)
- Site Questionnaire
- The Experimental Subject's Bill of Rights

PROTOCOL: EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin)-BASED PERSONAL INSECT REPELLENTS (20% CREAM and 20% SPARY) WITH MOSQUITOES UNDER FIELD CONDITIONS

The Independent Investigational Review Board, Inc. is an institutional review Committee structured in compliance with the regulations of the Food and Drug Administration contained in the Code of Federal Regulations (2ICFR 50 and 56) and is in compliance with the International Conference of Harmonization (ICH) Good Clinical Practice (GCP) guidelines for IRB/IECs.

At the meeting held on April 05, 2007, the Committee reviewed and unanimously approved the Research Protocol, the Investigator, Experimental Subject's Bill of Rights and Informed Consent Form for the above noted research study. The Site Questionnaire was reviewed and unanimously accepted.

The Informed Consent Form is unanimously approved as revised. The Committee recommended that changes be made to the Informed Consent Form. The approved Informed Consent Form is identified as Version 4/5/2007 and stamped, "Approved 4/5/2007". The Informed Consent Form contains all regulatory required consent elements.

The study has been approved for a 12 month period. At the end of this time, you are required to provide the Independent Investigational Review Board with a written progress report and completed Informed Consent Form for this research and obtain approval for continuing the research. Changes to the protocol or use of non-approved recruitment materials cannot be initiated without IIRB review and approval.

In the event of any serious adverse reactions, significant deviations from the protocol or problems in the research, written notice to the Independent Investigational Review Board is required. Please provide this reporting to the above-noted address so that appropriate follow-up will be initiated.

Thank you for your cooperation.

KL/AMS/yc:rr

INFORMED CONSENT AUTHORIZATION TO PARTICIPATE AS A RESEARCH STUDY SUBJECT

Title of Study: **(LNX-001)** EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin)-BASED PERSONAL INSECT REPELLENTS (20 % CREAM and 20% SPRAY) WITH MOSQUITOES UNDER FIELD CONDITIONS

Principal Investigator: Scott P. Carroll, Ph.D.
Carroll-Loye Biological Research
711 Oak Avenue
Davis, CA 95616
(530) 297-6080

Site of Investigation: _____
_____
_____

Sponsor: LANXESS Corporation

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 with improved formulations of the ingredient Picaridin, which is relatively new to the US market. More studies are needed to determine how well such new insect repellents work.

The purpose of the study is to test how well this new insect repellent, in lotion and pump spray formulations, works outdoors against mosquitoes. The new formulations have a higher concentration of Picaridin (20%) than do similar products already being sold, which have 5-15% Picaridin. The information gained from the study will assist in the development of these repellents for commercial marketing. During the study, we will first measure how much insect repellent subjects put on their own arms and legs in a visit to the study laboratory. On a later date, we will go to a field site to test the insect repellents against

APPROVED BY
Independent IRB
Signature 4/5/07 Date

Initials: _____
Date: _____

mosquitoes in nature. You may be asked to participate in one or in both parts of the study.

The sponsor, LANXESS, 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.

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.

Up to about 34 subjects will complete this 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, 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 (2-30 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.

<u>Visit 1 for Orientation and determining Dosage</u>

Within 30 days before the field study visit you will meet with a researcher to perform orientation 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 and you will sign this consent form. You will also 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

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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.

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.

The Principal Investigator may also ask if you would like to participate in a second field test of these products, using the same procedures as in the first test, on a later date. You may refuse to participate in additional testing without penalty to your compensation.

STUDY PROCEDURES

Study Design

The study will test one lotion and one pump spray. You will have an amount typical of what people commonly use applied to your forearms or lower legs.

Two experienced subjects will also participate to record the activity of mosquitoes by exposing their own arms or legs without repellent applied. Experienced subjects are pre-qualified by the Principal Investigator, and designated before the field test begins. Unless you have been qualified in advance as an experienced subject and agreed to expose untreated skin, 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 your 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/or lower leg. If you are participating in this part of the study, you will then practice using the product to decide how you best like to apply it 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

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application. Once you have a method you are satisfied with, you will wash your arms and/or lower legs with soap and water and dry them with a towel. The researcher will then ask you to apply an amount of the repellent product to your skin that you think gives complete and even coverage. We will use the amounts you and other subjects 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 aspirator. 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 aspirator 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 remove 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/or legs with mild, low fragrance soap, rinsing them with a spray of 35% ethyl alcohol, and then drying them with a clean towel. A technician will then apply insect repellent to your forearm or lower leg 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 coveralls and gloves to prevent bites on other parts of your arms and legs, plus a head net.

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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 repellent applied. You and a partner will watch your own exposed arms or legs and those of your partner for mosquitoes that land during a one-minute period. 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 during the one-minute period when asked by a technician who will record it on a data sheet. 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.

If you are one of the two untreated ("experienced") subjects, two technicians with aspirators will assist you in watching for and removing mosquitoes during each one-minute exposure, and in each exposure you should cover your limb with the protective fabric as soon as the first mosquito lands and attempts to bite, and keep it covered until the next exposure period, 15 minutes later.

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 a day prior to the start of the study
- 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 healthcare facility. You may also request access

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to standard first aid materials (such as bandages, antiseptics, and mild antihistamines) and request first aid assistance at any time. You may remove yourself for any reason from the study at anytime. At least one qualified researcher will remain with the other test subjects if other researchers depart with an injured or ill subject.

If you are treated with repellent, it will irritate the eyes on contact, and it is harmful if swallowed. You may obtain more information about the safety of the repellents by asking the Principal Investigator, and he will provide you more 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 or 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/or 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), swollen glands 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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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 may be hazardous. If you are a woman of childbearing potential, it is important that you do not participate in this study if you are, or if you think you may be pregnant, or are 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.

UNKNOWN / UNFORESEEABLE RISKS

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

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

TREATMENT ALTERNATIVE

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

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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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
There will be no costs to you from participating in this study.

PAYMENT
For participation in the study, each research study participant will receive a cash payment of $20 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, LANXESS Corporation, the U.S. Environmental Protection Agency (EPA), the California Department of Pesticide Regulation, and the Independent Investigational Review Board, Inc. (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 by 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.

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

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

Consent and signatures

I have read, in a language that I understand well, and understand the information which has been stated above. I have received satisfactory answers to all of the questions, which I have asked. I hereby voluntarily consent to take part in this study and to be a research study participant in this study. I 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	**Print** Carroll-Loye Biological Research Representative	**Sign** Carroll-Loye Biological Research Representative

Independent Investigational Review Board, Inc.
Approval: 4/5/07

APPROVED BY
Independent IRB

Signature 4/5/07 Date

Initials: _____
Date: _____

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment, or who is requested to consent on behalf of another, has the right to:

1. Be informed of the nature and purpose of the study.

2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be used.

3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.

4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.

5. Be given a disclosure of any appropriate alternative procedures, drugs, or devices that might be advantageous to the subject, and their relative risks and benefits.

6. Be informed of avenues of medical treatment, if any, available to the subject after the experiment if complications should arise.

7. Be given an opportunity to ask any questions concerning the experiment or the procedures involved.

8. Be instructed that consent to participate in the study may be withdrawn at any time, and the subject may discontinue participation in the medical experiment without prejudice.

9. Be given a copy of a signed and dated written consent form when one is required.

10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

_____ _____

Signature of Subject Date

_____ _____

Signature of Witness Date

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_____ 4/5/07

Signature Date

Study LNX-001 **Dosimetry Data Form** **Date:**

Subject name: **Data recorder name:**

Subject number: **Data recorder signature:**

I. Practice Application				
Trial no.	Distance from skin (cm)	No.pumps for full coverage	Container before (g)	Container after (g)
A. Pump spray, left arm				
1				
B. Pump spray, right arm				
1				
A. Pump spray, left arm				
1				
D. Pump spray, right leg				
1				

II. Pump Sampling						
Trial no.	Distance from skin (cm)	No.pumps for full coverage	Container before (g)	Container after (g)	Dosimeter before (g)	Dosimeter after (g)
A. Pump spray, left arm						
1						
2						
3						
B. Pump spray, right arm						
1						
2						
3						
C. Pump spray, left leg						
1						
2						
3						
D. Pump spray, right leg						
1						
2						
3						

Dosimetry Data Form– Lotion

Study LNX-001

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: LNX-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	17	18	19	20	21	22	23	24	25	26	27	28	29	30

Data collected by:

Data collector's signature:

Carroll-Loye Biological Research **Study LNX-001**

Limb Measurement Form

Subject number: _____ **Date:** _____

Subject name: _____

		Circumference					
Limb	**Length**	**Lower (A)**	**Lower-mid (B)**	**Upper-mid (C)**	**Upper (D)**	**Mean circumference**	**Surface area**
Left forearm							
Right forearm							
Left lower leg							
Right lower leg							

Data collector name: _____

Data collector signature: _____

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Study LNX-001

Efficacy Dosage by Subject

Subject no.	Surname	Name	Sex	Limb	Length	Lower A	Lower mid B	Upper mid C	Upper D	Surface area (cm^2)	Dose rate gm per cm^2	Dose rate ml per cm^2	Total dose by limb ml
				L arm									
				R arm									
				L leg									
				R leg									

Surface area = [(A+B+C+D)/4] x Length
Dose rate in g is from dosimety analysis
Dose rate in ml = dose rate in g/specific gravity
Total dose by limb ml = Dose rate ml/cm2 x Surface area

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Test Reference: LNX-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. Learning 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 aspirator. 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 aspirator is correct.

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Test Reference: LNX-001

CLBR Training Manual

§1.c. Practicing and performing dosimetry with a Pump Spray delivery system

A. Goals of exercise

1. Determine your preferred practices for applying ('self-dosing') a pump spray repellent to your arms and/or legs.
2. Assist technicians in measuring the amount of repellent that you apply when using your practices

B. General information

1. A technician will measure the surface area of your forearms and/or lower legs. He or she will introduce you to the repellent and its dispenser
2. You will work in open air, practicing applying the repellent. A technician will help you keep track of your preferred technique.
3. Using small gauze "bracelets" around your limbs to collect samples of repellent you spray on, you will apply repellent with your preferred practices several times. The bracelets will be quickly removed and weighed. 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. Bracelet dosimeters with nonabsorbent backing
4. Temperature, humidity and wind speed measuring devices
5. Written copy of the procedures for subjects to read
6. 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.

Working with the pump spray and determining your preferred method of applying the repellents:

Your trainer/technician will help to introduced you to how the spray bottle works and how you will determine your preferred methods of applying them. You will read the written procedures that follow here together.

"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 may have had experience with applying pump 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 limb to light may aid in determining whether full coverage has been achieved. Spray as much as necessary to achieve full coverage.

---

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

You may work with the spray on your arms, legs, or both. The technician will inform you. Wash your limbs to be tested 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 a pump spray delivery system.

First, 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 10 °C (50 °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 limb designated by the technician. By successively moving the spray nozzle closer to and farther from the limb, 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.

Wash and dry the treated limb 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). 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, estimate its volume as '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. Discard your latex gloves, and wash both test limbs (arms or legs) with cleanser and dry them thoroughly with a towel.

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Next, repeat the application procedure and collect the same data for the other limb. In doing so, try to be consistent with your use of the spray apparatus. If you are clear and confident about the distance from the limb 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 on to the **Spray Sampling** exercise described in the next section."

Spray Sampling[2]

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 limbs. These dosimeters will intercept a portion of the spray applied to the limbs. 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 test limbs. The rings are about one inch (2.5 cm) wide. Each of these "gauze bracelets" will be centered on each of the four positions on the limb at which we initially

---

[2] **Equipment Used to Assess the Dosimetry Variable (technical detail):**

Passive dosimeters are 2.5 cm wide strips Nexcare™ Holdfast™ self-adhesive roll gauze.

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/or leg will be treated three times. Each subject will therefore have a total of twenty-four or 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).

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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 limb 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 limbs 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 limb 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 limb only. The technician will tell you to which limb to apply spray. You and the technician will collect the same data as previously.

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

Discard your gloves, and wash both limbs 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 limb, and three more for the second limb. If you have completed sampling on, e.g., both arms, the technician may then ask you to repeat the same measurement on both legs. Be sure to discard your gloves, and wash all limbs with cleanser and dry them thoroughly with a towel, including after the last application."

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Test Reference: LNX-001

CLBR Training Manual

§1c. 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

1. Study subjects
d. Dosimetry (lotion only)
i. practice
ii. performance
(v. 1, 1 December 2006)

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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 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 index finger, 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

[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, wash and dry the treated arm so that none of the repellent you have applied is visible on close inspection. You will continue until you have completed three successful applications. You may then repeat the entire procedure above, but with the lower leg."

KBR 3023 Insect Repellent Cream

Contains Bayrepel™. Long-lasting, effective protection from mosquitoes ticks, biting flies, and fleas. Not oily, greasy or sticky. It smells great, too. Repels insects for up to 8 hours.

ACTIVE INGREDIENT: Picaridin, 1-Methylpropyl-2-(2-hydroxyethyl)-1-piperidine carboxylate	20%
INERT INGREDIENTS**	80%
TOTAL	100.0%

**Other Ingredients: Purified water, glycerin, denatured alcohol, thickener, emollient, fragrance

KEEP OUT OF REACH OF CHILDREN

WARNING

STOP – Read This Entire Label Before Use

PRECAUTIONARY STATEMENTS

WARNING. HAZARDS TO HUMANS.

Causes substantial but temporary eye injury. Do not get in eyes. Wash thoroughly with soap and water after handling, returning indoors, and before eating, drinking, chewing gum, or using tobacco. Discontinue use and consult a doctor if irritation or rash occurs.

The information below describes the first aid procedures for incidents involving KBR 3023 Insect Repellent Cream:

FIRST AID

IF IN EYES:

- Hold eye open and rinse gently with water for 15-20 minutes.
- Remove contact lenses, if present, after the first five minutes, then continue rinsing.
- Call a poison control center or doctor for treatment advice.

IF SWALLOWED:

- Call a physician or poison control center immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to do so by a Poison Control Center or a doctor.
- Do not give anything to an unconscious person.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-410-3063 for emergency medical information.

The LANXESS Pittsburgh Emergency Response Telephone Number is 800-410-3063

PHYSICAL HAZARDS

Flammable. Do not use or store near heat sources, sparks or open flame. Do not smoke while applying,

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

For best results, read and follow all label directions.

Follow these guidelines when applying KBR 3023 Insect Repellent:

- Apply evenly to skin in a thin layer
- Excessive amounts or more frequent reapplication should be unnecessary. Do not apply more than 2 times a day.
- Repels insects and ticks for up to eight hours.
- Reapply every 8 hours. Do not exceed two applications per day.
- Do not spray directly on face.
- Avoid contact with lips, cuts, wounds, or irritated skin.
- Do not apply to excessively sunburned skin.
- Do not apply under clothing.
- Apply sparingly around ears.

STORAGE AND DISPOSAL

STORAGE: Store in a cool, dry place out of the reach of children. Keep away from heat, sparks and open flame.
DISPOSAL: Do not reuse empty container. Discard in trash.
IF EMPTY: Do not reuse this container. Place in trash or offer for recycling if available.
IF PARTLY FILLED: Call your local solid waste agency or 1-800-526-9377 for disposal instructions. Never place unused product down any indoor or outdoor drain.

IN CASE OF EMERGENCY, CALL: CHEMTREC 800-424-9300
EPA REGISTRATION NUMBER: 39967-50
EPA ESTABLISHMENT NUMBER:

INTERNATIONAL 703-527-3887
Net Contents:
Lot No.:

LANXESS

LANXESS Corporation
111 RIDC Park West Drive ● Pittsburgh, PA 15275-1112

LABEL TEXT DATE: 12/19/06

KBR 3023 All-Family Insect Repellent Spray

Long-lasting, effective protection from mosquitoes, ticks, biting flies, gnats, chiggers and fleas. Use with confidence on the whole family. And your family will want to use it, too. Not oily, greasy or sticky. It smells great, too.

ACTIVE INGREDIENT: Picaridin, 1-Methylpropyl-2-(2-hydroxyethyl)-1-piperidine carboxylate	20%
INERT INGREDIENTS	80%
TOTAL	100.0%

KEEP OUT OF REACH OF CHILDREN

CAUTION

STOP – Read This Entire Label Before Use

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS

Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling, returning indoors, and before eating, drinking, chewing gum, or using tobacco.

The information below describes the first aid procedures for incidents involving KBR 3023 Insect Repellent Spray:

FIRST AID

IF IN EYES:

- Hold eye open and rinse gently with water for 15-20 minutes.
- Remove contact lenses, if present, after the first five minutes, then continue rinsing.
- Call a poison control center or doctor for treatment advice.

IF SWALLOWED:

- Call a physician or poison control center immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to do so by a Poison Control Center or a doctor.
- Do not give anything to an unconscious person.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-410-3063 for emergency medical information.

The LANXESS Pittsburgh Emergency Response Telephone Number is 800-410-3063

IN CASE OF EMERGENCY, CALL: CHEMTREC 800-424-9300

EPA REGISTRATION NUMBER: 39967-53
EPA ESTABLISHMENT NUMBER:

PHYSICAL HAZARDS

Flammable. Do not use or store near heat sources, sparks or open flame. Do not smoke while applying,

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

Follow these guidelines when applying KBR 3023 Insect Repellent:

- Hold 4 to 6 inches from skin while spraying, keeping nozzle pointed away from face. Slightly moisten skin with a slow sweeping motion.
- Excessive amounts or frequent reapplication is unnecessary.
- Apply on face by first spraying small amounts in palms of hands and spreading on face and neck.
- Do not apply to the hands of small children.
- Repels insects and ticks for up to eight hours.
- Reapply every 8 hours. Do not exceed two applications per day.
- Do not spray directly on face.
- Avoid contact with lips, cuts, wounds, or irritated skin.
- Do not apply to excessively sunburned skin.
- Do not apply under clothing.
- Apply sparingly around ears.

STORAGE AND DISPOSAL

Store in a cool, dry place out of the reach of children. Keep away from heat, sparks and open flame.

IF EMPTY: Do not reuse this container. Place in trash or offer for recycling if available.

IF PARTLY FILLED: Call your local solid waste agency or 1-800-526-9377 for disposal instructions. Never place unused product down any indoor or outdoor drain.

INTERNATIONAL 703-527-3887
Net Contents:
Lot No.:

LANXESS

LANXESS Corporation
111 RIDC Park West Drive ● Pittsburgh, PA 15275-1112

LABEL TEXT DATE: 12/19/06

TOXICOLOGY PROFILE OF KBR 3023 (page 1 of 2)

The toxicological profile of KBR 3023 is well characterized. All toxicology data were developed using the dermal route of exposure, the most relevant route based on the use pattern of the product (insect repellent for dermal application). The rationale of product development using the dermal route of exposure was considered at the suggestion of the USEPA and in agreement with USEPA and Bayer/Miles. All study protocols, scientific issues, methodology for dermal dosing for extended periods of time and rationale for dose selection were discussed with the EPA. Agreements regarding use of dermal route of exposure were also made with BGA (German authorities) and Health & Welfare Canada. A complete toxicology package required for the registration of an insecticide including acute and subchronic neurotoxicity and metabolism studies was conducted. Additionally, 14-day, 5-week and 14-week dietary feeding studies were conducted to assess any hazard associated with hand-to-mouth transfer from dermal use of KBR 3023. The highest dermal dose for long-term studies was 200mg/kg/day. Dermal absorption studies were conducted both in rats and human volunteers to assess the human risk on the absorbed dose analysis associated with the consumer use of the product.

KBR 3023 and its formulated products have low acute toxicity by oral, dermal or inhalation routes of exposure. They were not irritating to the skin nor sensitizers in the animal studies. A slight to moderate ocular irritation was observed in the animal studies.

KBR 3023 has no demonstrable neurological or developmental toxicity by dermal route of exposure. KBR 3023 shows no evidence of genotoxicity. Subchronic dermal dosing at 500 mg/kg/day produced no clinical pathology and only slight histopathology changes in the liver, and all changes were reversible after four weeks. Chronic dermal dosing in mice, rat and dogs produced no evidence of adverse toxicity changes and it was not oncogenic in mice or rats. In the oral toxicity studies (14-day, 5-weeks and 14-weeks), only kidney effects were seen in the male rats and were attributed to α_{2u} globulin accumulation. The toxicology profile by oral route of exposure did not reveal any new targets compared to the dermal route and. Cumulative effects were not evident in dermal or oral studies. The systemic NOAEL in the subchronic studies by oral route were similar (308mg/kg/day for oral/200mg/kg/day- the highest dose tested).

TOXICOLOGY PROFILE OF KBR 3023 (page 2 of 2)

The safety of KBR 3023 was further established by dermal absorption studies conducted in rats and in human volunteers. The dermal absorption study in human volunteers showed that KBR 3023 is poorly absorbed through the human skin. Only 1.66% of the material (AI) was absorbed compared to 19 – 60% for the rat. A conservative dermal penetration factor of 11.5 was used by the EPA for risk assessment. The excretion half-life in humans was 8.2 hours compared to 23.3 hours in the rat. The qualitative pattern of excretion is similar in humans and rats (primary urinary excretion) with similar metabolites. KBR 3023 has good skin feel and is odorless. No significant complaints have been reported over years of use.

In summary:

KBR 3023 has complete toxicology data supported by State-of-the–Art testing
KBR 3023 showed no foreseeable public health risks, including in children and is alternative to DEET
It has no end points of concern
Low acute toxicity
No irritant or sensitizing potential
No specific effects in rats or dogs in short-term and long-term studies
NOAEL = 200 mg/kg (dermal); NOAEL = 308 mg/kg (oral)
Not mutagenic
Not tumorigenic
No effects on reproduction
No neurotoxicity
No photo-sensitisation or irritation
It is poorly absorbed through the human skin
Does not bio-accumulate and is rapidly excreted

INDEPENDENT INVESTIGATIONAL REVIEW BOARD INC.

SITE QUESTIONNAIRE

Non-Local Review

Protocol # & Complete Study Title: **(LNX-001) EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin) - BASED PERSONAL INSECT REPELLENTS (20 % CREAM and 20% SPRAY) WITH MOSQUITOES UNDER FIELD CONDITIONS**

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

Sub Investigator(s): None

Please indicate the location where study activities will be performed (where patients will be seen excluding Diagnostics) If more than on location is being used you may attach additional pages.

Site Address: Carroll-Loye Biological Research
711 Oak Avenue
Davis, CA 951616 USA

PI's Mailing Address: _____
(If different) _____

If the study is being conducted at more than one location and information requested differs for each location, please provide separate information for each location.

Regulatory/Study Coordinator: Scott Carroll Phone: 530-297-6080 Fax Number: 530-297-6080

Office Phone: 530-297-6080 24 Hour Phone: 530-297-6080

Please complete the following: You may attach copies of relevant procedures.

1. Is this study federally funded requiring review under HSS standards? X No ☐ Yes

2. How will Study Participants be recruited?
   - ☐ Principal Investigator's Clinical Practice
   - ☐ Referrals from other clinical Practices
   - X Data base of potential Volunteers
   - ☐ Advertising in the community* *(*advertisements Must be approved by the IIRB)*
   - X Other *(please specify):* Word of mouth via Volunteers in data base

3. Will you recruit volunteers from vulnerable study populations? X No ☐ Yes *(please specify below)*
   - ☐ Persons kept in detention
   - ☐ Nursing Home Resident/Elderly
   - ☐ Patients in emergency situations
   - ☐ Persons of limited capacity
   - ☐ Minors
   - ☐ Pregnant women
   - ☐ Illiterate
   - ☐ Members of the Armed Forces
   - ☐ Patients with incurable disease
   - ☐ Unemployed/on Public Assistance
   - ☐ Homeless
   - ☐ Employees (Site or Sponsor, etc)
   - ☐ Disabled
   - ☐ Other: _____

   If yes, describe procedures to be followed (if applicable): 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.

4. Do the subjects that you intend to enroll in this study come from any type of ethnic background or cultural environment that might have an impact on their ability to understand that participation in the

study is voluntary and refusal to participate or discontinuing their participation will not have any adverse impact on the care that they will receive? No

5. Indicate the approximate demographics of your site's anticipated subject population:
___5__% African American __65__% Caucasian __15_% Hispanics __15__% Asian __<1__% Other

6. Will you be enrolling only subjects who speak English in this study? X Yes ☐ No
If No, Is a "local dialect" or translation needed? Translation needed: ☐ Spanish ☐ Other _____

7. Who will discuss the research study with the volunteer and obtain informed consent (signed informed consent)? *(Check all that apply)*

X Principal Investigator ☐ Sub Investigator ☐ Study Coordinator
Explain consenting procedures: 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 can 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 Principal Investigator (PI). The Exclusion Criteria (section 9.1.2) are exercised by asking each candidate to address them in the interview with the PI. The PI encourages candidates to ask questions and ask for clarification at any time during the interview and in all activities that follow. To candidates that pass screening the PI 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. They are also given a copy of the IRB-approved consent form to read as the PI 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 PI retains the final right to refuse participation to any candidate.

8. Describe the setting(s) where the study will be conducted (ie, private office, clinic, hospital environment) and if the Investigator is required to seek any type of administrative or Corporate approval in order to implement the study:
Private Laboratory owned by Principal Investigator.

*If being done in a Hospital or Outpatient Surgery Center, please provide a copy of that facility's License/accreditation and/or Hospital IRB Waiver Form.

9. Distance between the nearest hospital and research site: 1.8 miles from Laboratory, within 25 miles of field sites.

10. Describe the on-site emergency equipment available for the subjects: First aid kit, skin washing soap and mild dermal detergent, eye wash.

11. How long has the PI been conducting clinical research? ___17_____years ____1_____months

12. Within the past 3 years has the FDA/OHRP audited your site/Principal Investigator? X No ☐ Yes*
**If yes, please provide a copy* of all 483's *and any applicable correspondence.*

13. Has the FDA/OHRP or any State Medical Board ever sanctioned the Principal Investigator? XNo ☐Yes*
**If yes, please provide a summary of the action and applicable correspondence.*

14. Are subject files adequately stored and protected to ensure subject confidentiality, i.e. HIPAA, HIV, etc.? ☐ No* X Yes
**If no, please explain:* _____

15. Does the Principal Investigator, Sub Investigator(s) or any immediate family member have a conflict of interest with the study sponsor, sponsor representatives or other study related entities? X No ☐Yes*
**If yes, please provide explanation:*
_____
_____

Subject Compensation:

Will subject be paid for participation in this study? ☐ No X Yes*
**If yes, please specify the total amount, the amount for each visit and the timing of payment (i.e. at each visit, at the last visit, within 2 weeks of the last visit) in the draft Informed Consent Form.*

Site Specific Informed Consent Form Information

Is there any additional wording needed in the Informed Consent Form? X No X Yes*
**If yes, please specify the section and additional wording below.*
Already present in attached draft form.
_____
_____

Investigator Acknowledgment

On behalf of all of the investigators listed on page1, I agree that the responses provided on the Site Questionnaire are true and accurate and I agree to notify the Independent Investigational Review Board, Inc. of any changes in the research activities and to report any unanticipated problems involving risk to the research subjects. In addition, I agree not to make any changes in the research without IRB approval. I confirm that study personnel are familiar with the study and that either an Investigator or a study coordinator acting as my designee will orally explain the Informed Consent Form to all prospective subjects before obtaining their signed informed consent. Furthermore, by signing this form I confirm that I agree to conduct the study in accordance with the requirements of the protocol, for which I am seeking approval.

Scott P. Carroll
Print name of individual completing Site Questionnaire

Signature of individual completing Site Questionnaire — Date: 27 March 2007

Scott P. Carroll
Print Name of Principal Investigator

Signature Principal Investigator — Date: 27 March 2007

Please contact the Independent IRB, if you have any questions regarding this questionnaire 954.327.0778

INDEPENDENT INVESTIGATIONAL REVIEW BOARD INC.

Study Specific Instructions

Protocol Title: (LNX-001) **EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin) - BASED PERSONAL INSECT REPELLENTS (20 % CREAM and 20% SPRAY) WITH MOSQUITOES UNDER FIELD CONDITIONS**

Sponsor: Lanxess Corporation
Contact Info:

Contact/Title	Phone/Fax	email
Scott P. Carroll, Ph.D/Study Director	(530) 297-6080/6080	spcarroll@ucdavis.edu
Dr. G. K. Sangha/Sponsor Monitor	(913) 638-3968	sangha8@adelphia.net

CRO:
Contact Info:

Contact/Title	Phone/Fax	email

PROGRESS REPORT NOTIFICATION PROCEDURES: (To whom do we send the notice, etc.) Study Director

SPANISH LANGUAGE REQUIRMENTS: (If it is determined that a Spanish language ICF is necessary).

___ Use translations Services though IIRB (Americo Gomez)

___ We will provide our own Spanish Translations

Mailing Instructions: address for Sites do NOT need to be listed – just identify as "sites" (so that we have on file who get copies and who gets originals!)

Originals to: Scott P. Carroll — Sent by: FedEX **X** UPS - USPS
Address: Carroll-Loye Biological Research, 711 Oak Avenue, Davis, CA 95616 — Account #: 177-484-318
Copies to: — Sent by: FedEX – UPS - USPS
Address: — Account #:

Notes: (include if routine correspondence get copies sent to CRO/Sponsor, sent US Mail, etc.)

Progress Report Information:
To Study Director
Scott P. Carroll, Carroll-Loye Biological Research, 711 Oak Avenue, Davis, CA 95616

Billing Instructions:
To Study Director: Scott P. Carroll

Billing Address: Scott P. Carroll, Carroll-Loye Biological Research, 711 Oak Avenue, Davis, CA 95616

INDEPENDENT INVESTIGATIONAL REVIEW BOARD INC.

Membership Roster

Page 1 of 2 approved 1/02/07

Chairman, Kim Lerner, B.S.

Ms. Lerner is co-founder of the Independent Investigational Review Board and has acted as Chairman for the past 16 years. She has transformed her experience as Director of the IRB at a large teaching hospital into directing a large and diverse independent IRB. Her experience serving as the Director of a Hospital Quality Assurance Program provides the foundation for implementation of the Independent IRB's continuous quality improvement and regulatory compliance programs

Vice Chairman, Anita Mc Sharry, RN (scientific)

Ms Mc Sharry is co-founder of the Independent Investigational Review Board and has acted as President for the past 16 years. She has extensive knowledge of principles of medical research, regulatory compliance and clinical safety. Previous experience included development of Research Study budgets, liaison between the Pharmaceutical Sponsor and the Principal Investigator at the University of Miami, Department of Clinical Pharmacology.

David D. Wells, MD (physician/scientific)

Dr. Wells graduated from the University of Havana, is English-Spanish bilingual and brings this international experience to the IRB. He has served as the Emergency Medicine Department Chairman at a local hospital, has health care Clinic experience working with financially disadvantaged patients and is presently volunteering as a Family Practice Physician serving migrant farm workers. This hands-on experience enables him to clearly assess overall research risks and benefits and understand vulnerable population issues.

Rabbi Akiva D. Mann, M.A. (non-scientific)

Rabbi Mann is presently the Spiritual Leader of the Hallandale Jewish Center and is the Director of the Institute of Jewish Knowledge and Learning. He has served on Mayorial Commissions addressing the issues of Medical Ethics and Geriatric Care, as well as having served on Hospital Ethics Committees.

Edward Wiederhorn (non-scientific)

Mr. Wiederhorn is the community representative to the IIRB and is a member of the American Association of Retired Persons (AARP). Mr. Wiederhorn has longstanding experience as a Civic Activist and has been a member of Fraternal and Charitable Organizations, at present he is actively involved in support of the City of Hope.

Shari Somerstein, B.S., R. Ph. (scientific)

Ms. Somerstein has served as a member of the Independent IRB for more than 6 years and has extensive experience in the interpretation and assessment of clinical research findings and study design. She brings extensive clinical pharmacy experience in a Hospital setting and in the community. She has broad administrative experience in IRB activities including, protocol review, assessment of adverse drug experiences and informed consent form development.

George J. Garbarino (non-scientific)

Mr. Garbarino has been an advocate for Labor Union members and brings a wide range of experience in the area of worker's rights and contract negotiations. He is the Business Manager of the Tile, Marble and Stone Workers Local 121 and is associated with the School Board of Broward County.

INDEPENDENT INVESTIGATIONAL REVIEW BOARD INC.

Membership Roster

Page 2 of 2 approved

Glenn K. Moran, DO, FACOFP (Alternate for: physican/scientific)
Dr. Moran is Board Certified in Family Practice and is presently in private practice. He maintains privileges at local hospitals and is active in the areas of Medical Quality Assurance and Peer Review and other community organizations. He is an Assistant Clinical Professor at Nova Southeastern College of Osteopathic Medicine and is familiar with current medical research requirements.

Marcos Rejtman, DO, (Alternate for: physican/scientific)
Dr. Rejtman is Board Certified in Family Practice, Geriatric Medicine and Hospice &Palliative Medicine and is presently the Medical and Team Director for VITAS Innovative Health Care (Hospice) and provides in hospital patient management for a multi-specialty group. He also has recent experience in Emergency Department Medicine. He maintains privileges at local hospitals and is active in the areas of Medical Quality Assurance and Peer Review and other community organizations. He is English-Spanish bilingual.

Maria L. Rodriguez, MS, CCRC,RHIT (Alternate for: non-scientific)
Ms Rodriguez brings to the Board extensive experience in the clinical research field and knowledge of regulatory requirements, policy and procedure development, and the implementation of the quality assurance function. Her educational background focuses on education and training in the clinical research field. She is English-Spanish bilingual.

Robert Lettman, Esq. (Alternate for: non-scientific)
Mr. Lettman is a practicing Attorney in South Florida with extensive experience in civil litigation and serves as a resource in the consideration of the legal aspects of the informed consent process. Having been in the community for 30 years he brings insights and knowledge of the needs of the community.

MEMBERSHIP CHANGES FROM PREVIOUS ROSTER (Dated: 01/03/06)
Resignation
Elsie P. Remy, MSN, ARNP-c (Alternate for: scientific)

Replaces Roster dated 1/03/06

CITI Course in The Protection of Human Research Subjects

Tuesday, May 31, 2005

CITI Course Completion Record
for Scott Carroll

To whom it may concern:

On *5/31/2005*, *Scott Carroll* (username=scottpcarroll; Employee Number=) completed all *CITI Program* requirements for the *Basic CITI* Course in The Protection of Human Research Subjects.

Learner Institution: *University of California, Davis*

Learner Group: *Group 1.*

Learner Group Description: *This course is suitable for **Students, Investigtors and staff** conducting BIOMEDICAL RESEARCH with human subjects. The VA module must be completed if you plan to work with subjects at a VA facillity.*

Contact Information:

Gender: Male
Department: Entomology
Which course do you plan to take?: Biomedical Investigator Course Only
Role in human subjects research: Principal Investigator
Mailing Address:
711 Oak Avenue
Davis
CA
95616
United States
Email: spcarroll@ucdavis.edu
Office Phone: 530 297 6080
Home Phone:

The Required Modules for *Group 1.* are:	**Date completed**
Introduction	05/31/05
University of California, Davis	05/31/05
Additional optional modules completed:	**Date completed**
History and Ethical Principles - SBR	05/31/05
History and Ethical Principles	05/31/05
Basic Institutional Review Board (IRB) Regulations and Review Process	05/31/05
Informed Consent	05/31/05

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

RECORD OF ADDITIONAL CORRESPONDENCE WITH IRB

To: Robert_Roogow
From: Scott P Carroll <spcarroll@ucdavis.edu>
Subject: Submission of new protocol LNX-001
X-Attachments: :André Rublev:840769:Carroll-Loye LNX-001.pdf:

Dear Robert,

The protocol and consent form of this protocol are again very similar to others you have considered recently. Only the sponsor is different, and the test materials are formulations of the insect repellent active ingredient Picaridin, or KBR 3023, which is relatively new to the US.

I have attached the Protocol and ICF for your consideration, along with the set-up form and site questionnaire. I am finalizing the protocol now.

The protocol document has the following notable features. There is a two-page cover sheet intended for the US EPA functionaries to whom the protocol will be submitted after your review and possible approval. In addition, the ICF, set-up form and site questionnaire also appear as appendices in the protocol document.

Please let me know if you have any questions.

I will be traveling the rest of the week and can best be reached on my cell at 530-902-8267.

Thanks very much,
Scott
--
Scott P. Carroll, Ph.D.
Carroll–Loye Biological Research
711 Oak Avenue
Davis, CA 95616

Tel (530) 297-6080
Fax (530) 297-6080
email spcarroll@ucdavis.edu
http://www.carroll-loye.com/

From: "Robert Roogow" <rroogow@iirb.com>
To: <Carley.John@epamail.epa.gov>
Cc: "Scott P Carroll" <spcarroll@ucdavis.edu>
Subject: Protocol LNX-001
Date: Tue, 10 Apr 2007 15:22:50 -0400
Thread-Index: Acd7paDdh9U2fiSkQE+UME27QpcyYQ==
X-UCD-Spam-Score: 0.002 () BAYES_50,HTML_MESSAGE
X-Scanned-By: MIMEDefang 2.57 on 128.120.32.35
Status:

Dear Mr. Carley,

Please find attached, the meeting minutes for the LNX-001 protocol on behalf of Scott Carroll. There have been no changes to our Board Policies & Procedures. Please let me know if you should have any questions or need anything further.

Regards,
Robert Roogow, MS, RAC
Director of Operations
Independent Investigational Review Board, Inc.
Ph: 954-327-0778
Fax: 954-327-5778
rroogow@iirb.com

Thursday, April 05, 2007
MINUTES

ATTENDANCE:

PRESENT	ABSENT
David Wells, MD	Anita McSharry, RN
Shari Somerstein, RPh	Rabbi Akiva Mann
Edward Wiederhorn	
George Garbarino	
Kim Lerner	

I. CALL TO ORDER

The meeting was called to order at 9:30 AM, by Chairman, Kim Lerner. The meeting was held at 6738 West Sunrise Blvd., Suite 102, Plantation, FL 33313.

II. APPROVAL OF THE 4/3/2007 MINUTES

The minutes of the meeting held 4/3/2007 were reviewed and unanimously approved as reviewed.

III. REVIEW PROTOCOLS

C (Protocol LNX-001) EFFICACY TEST OF KBR 3023 (Picaridin; Icaridin)-BASED PERSONAL INSECT REPELLENTS (20% CREAM and 20 % SPARY) WITH MOSQUITOES UNDER FIELD CONDITIONS
Principal Investigator: Scott P. Carroll, PhD

- Approval Clinical Research Protocol dated: 3/27/2007
- Informed Consent Form (Ver. 4/5/2007)
- Site Questionnaire
- The Experimental Subject's Bill of Rights

Motion was made, seconded and the Committee unanimously approved the Research Protocol, the Investigator(s), Informed Consent Form, The California Experimental Subject's Bill of Rights for the above noted research study. The Site Questionnaire was reviewed and unanimously accepted.

The Informed Consent Form is unanimously approved as revised. The Committee recommended that changes be made to the Informed Consent Form. The approved Informed Consent Form is identified as Version 4/5/2007 and stamped, "Approved 4/5/2007". The Informed Consent Form contains all regulatory required consent elements. The California Experimental Subject's Bill of Rights is stamped "Approved 4/5/2007".

The Committee evaluated that the risks to the subjects were minimized and that a reasonable risk/benefit ratio is established. Based on the duration of the study and the risks to the subjects, the approval is granted for a 12 month period, with a progress report required prior to continued approval. See Approval letter for Investigator's responsibilities and file for supporting documents.