LSU REPELLENT STUDY 4370

Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR3535 Against Mosquitos in Louisiana

COVER PAGE:

Document Contents:

Study Protocol: (Approved April 1, 2021)

ARCTEC REPELLENT TRIALS

Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR3535 against Mosquitos in Louisiana.

ARCTEC ref: 1215

LSU ethics reference: 4370

LSHTM ethics reference: 25116

ClinicalTrials.gov:

Document	Date	Reason for Change
Study Plan/Protocol	DD-MMM-YYYY	Signed by Study Director*
Amendments		

*Note: GLP compliant study plans do not have multiple versions, and instead rely on authorising signature of the Study Director. Changes after this signature are termed amendments and are numbered sequentially.

Trial Management Group:
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Expected start date: April 2021
Expected first experimental date: June 2021
Expected last experimental date: August 2021
Expected finish date: October 2021

Expected dates are subject to change, so for updates to these dates please contact the Study Director.

Good Laboratory Practice & Good Clinical Practice Compliance Statements

This study will adhere to the principles outlines in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) Guidelines. Good Laboratory Practices, as defined by 40 CFR part 160 will be followed throughout this study.

The Study Director has determined studies of this kind to be low-risk. Day-to-day monitoring will be carried out at the Test Facility by a member of the study team with delegated responsibility. A separate monitoring team will monitor the study before subject enrollment, during the study and on study completion.

Compliance Services International will perform all QA duties. The QA will conduct critical phase inspections at intervals adequate to ensure study integrity, and maintain written and signed records of each inspection. Records shall identify the study and include the date of the inspection, the phase inspected, the individual conducting the inspection, any findings or problems, actions recommended and taken to resolve negative findings, the scheduled date for re-inspection (if any), and the date(s) the findings are reported. All inspection findings will be reported to management and the Study Director. Any problems, amendments or deviations discovered shall be brought to the attention of the sponsor, Study Director and Test Facility Management immediately. The QA representative will review the final reports for accuracy and compliance with GLPs and the protocol. A signed QA statement will be included in the final report that lists the phase inspections that were conducted, their dates, and the dates the findings were reported to management and the Study Director.

Auditing activities may be sub-contracted to LSU EHS, in which case copies of the inspection reports will be provided to Kristen Healy in addition to the Study Director and Test Facility Management.

Signatures

This protocol has been read and approved by:

Kristen Healy	Date
Study Director	

Sponsor	Date
Job Title	

Study Synopsis

Sponsor:	Livful Inc.
Study Title:	Field Evaluation of two topically applied insect repellent products containing IR3535 against mosquitos in Louisiana.
Short Title:	Louisiana Mosquito Field Trial 1215
ARCTEC Ref:	1215
LSU IRB Approval Date:	
Study Director:	Kristen Healy
Test Facility Manager	Michael J. Stout
Quality Assurance:	Compliance Services International
Monitor:	Vanessa Chen-Hussey, ARCTEC, LSHTM, LG38, Keppel Street, London, WC1E 7HT
Objective:	To determine the efficacy and duration of protection of two topically applied insect repellent products at preventing landing by mosquitos. The study will follow the EPA Product Performance Test Guidelines[1]. It is intended to test the products against natural populations of mosquito species of public health importance within the genera *Aedes*, *Anopheles*, and *Culex*, and to replace data from one site previously tested in Florida with data from a site in Louisiana with adequate landing pressure from target mosquito species of public health relevance.
Study Design / Methodology:	A single-site field setting study using healthy volunteers to test two insect repellent product formulations (lotion, and wipe) against mosquitos. Subjects will have repellent applied to one lower limb at a standardised dose rate to account for skin area. They will then expose this area only in a field site where mosquitos are recorded landing at a rate of 5 mosquitos per 5 minute or higher. The exposure period will last five minutes and all mosquitos landing on the exposed skin will be collected using an aspirator. 5 minute exposure periods will be repeated every half hour for 14 hours for the lotion and 13 hours for the wipe, or until median CPT can be established by more than half of subjects reaching treatment failure.
Number of Subjects:	Based on power analysis, a sample size of 13 test subjects for each product in this study design would provide sufficient power (>0.90) to obtain a ratio of the lower limit of 95% CI of the estimated median CPT / estimated median CPT is ≥ 0.6, where the ratio of the lower limit of 95% CI of the estimated median

	CPT/estimated median CPT expresses the precision of estimated median CPT. Two additional subjects will serve as untreated controls for each field test to monitor the landing rate throughout the study. An additional 5 subjects will be enrolled as alternates to replace any test subjects who drop out before testing begins. In total, up to 20 people could be necessary for each test day. Assuming no subjects participate as test, untreated control, or alternate subjects more than once, total of 40 people could be necessary to complete all testing outlined in this protocol.
Intended Product Users:	The test product is designed for use by the general public in areas where mosquito biting is likely.
Test Product Name:	AKIVA 20 Wipe and AKIVA 20 Lotion
Test Product Description:	**TS name:** Akiva 20 Wipe **Lot/Batch:** XXX **Active ingredient:** IR3535 or ethyl butylacetylaminopropionate **CAS Number:** 52304-36-6 **Appearance:** Liquid, without aggregation **Color:** Visually white to off-white **Odor:** Characteristic **Active ingredient concentration:** 20% ±0.2% **50°C x 1 week: settling/creaming:** None observable **50°C x 1 week: coalescence:** None observable **50°C x 1 week: visual changes:** None observable **Use:** As per test protocols **Storage:** At 20-30°C in opaque container or away from direct light **Date manufactured:** XXX **Date of certification:** XXX **Certified by:** XXX **TS name:** Akiva 20 Lotion **Lot/Batch:** XXX **Active ingredient:** IR3535 or ethyl butylacetylaminopropionate **CAS Number:** 52304-36-6 **Appearance:** Liquid, without aggregation **Color:** Visually white to off-white **Odor:** Characteristic **Active ingredient concentration:** 20% ±0.2% **50°C x 1 week: settling/creaming:** None observable **50°C x 1 week: coalescence:** None observable **50°C x 1 week: visual changes:** None observable **Use:** As per test protocols **Storage:** At 20-30°C in opaque container or away from direct light **Date manufactured:** XXX **Date of certification:** XXX **Certified by:** XXX

Study Duration:	Subjects will undergo a screening evaluation, which includes the consent process, a mosquito attraction test, and a training session to detect mosquito landings and use an aspirator. Subjects recruited into the study will participate in up to two repellency tests, one lasting for up to 14 hours duration and the other for up to 13 hours duration. Field testing will not begin until two hours post-application. In total, the test substance lotion is to be tested for up to 14 hours and the wipe for up to 13 hours. Each subject will be followed up after each visit by email/in person/by phone within 72 hours of the visit and asked whether they experienced any unreported adverse events. The minimum duration of subject involvement would therefore be approximately up to 6 days (1-day consent, 1-day training and attractiveness test, 1-day field testing, up to 72 hours post-field testing monitoring). Total study duration (recruitment and subject involvement) is anticipated to be around 6 months.

Inclusion Criteria:	• Able and willing to give fully informed consent;
	• Male or female;
	• Aged 18 to 55 years;
	• Consider themselves to be in good general health, and specifically:
	  ○ Not aware of having any cardiovascular or respiratory disorder (whether active or inactive)
	  ○ No previous anaphylaxis
	  ○ Not aware of having a compromised immune system
	• Non-smokers or willing to refrain for 24 hours prior to and during each test;
	• Willing to undergo a mosquito attraction test (putting an arm into a cage of mosquitos)
	• Able to speak and understand English
	• Able to stand outside for periods of at least 5 minutes at a time
	• Able to understand and comply with the study procedures, including:
	  ○ Willing to complete mosquito landing/aspirating training
	  ○ Able to withstand exposing the lower leg to mosquitos for periods of at least 5 minutes at a time
	  ○ Able to operate an aspirator

Exclusion Criteria:	• Participated in any other intervention study in the previous 3 months • Participated in a biting insect test as part of the current study in the previous 72 hours • Employees, managers, and spouses of employees of the LSU and of the study Sponsor (LivFul, Inc.) • Students of the Study Director or any other LSU faculty/researchers involved in the study

	• Individuals suspected or known to be sensitive or allergic to, or phobic of, mosquito bites • Women who are pregnant, nursing or intending to become pregnant during the course of the study • Individuals with localized skin disorders or problems affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes • Individuals with known or suspected allergy or sensitivity to the test product or any of its ingredients, or any insect repellent products • Individuals who are not attractive to mosquitos during mosquito attractiveness test • Individuals who have signs or symptoms related to COVID-19, have tested positive for COVID-19 within the last 15 days, or have had contact (within 6 feet for a total of 15 minutes or more) with someone who has tested positive for COVID-19 in the last 14 days.
Efficacy Endpoint(s):	Median Complete Protection Time for each repellent product tested against mosquitos for prevention of biting.
Safety Endpoint(s):	Adverse event data will be collected and summarized.
Statistical Methods:	Median Complete Protection Time will be calculated using the Kaplan Meier survival-function.
Test Facility:	Louisiana State University, Life Sciences building
Potential Field Sites:	Cameron Parish Mosquito Control, Calcaseau Parish Mosquito Control, Plaquemines Parish Mosquito Control, or Grande Isle Mosquito Control, Louisiana, United States.

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1. Introduction

Mosquitos, midges, sand flies and other biting insects are vectors of extremely important diseases such as malaria, yellow fever, filariasis and many viruses and also are of great nuisance value due to biting behavior. The use of repellent products can provide added personal protection from disease transmission and nuisance bites. New effective repellents would offer an additional option for protection against biting insects. The tests carried out will provide important information on the effectiveness of skin repellents, which will be used for label claims to accurately inform consumers and registration purposes.

The aim of this study is to provide longevity and efficacy data for two topically applied insect repellent products for prevention of mosquito biting. The products will be provided by LivFul Inc. The active ingredient of each product is IR3535 (ethyl butylacetylaminopropionate, also known as 3-[N-butyl-Nacetyl]-aminopropionic acid, ethyl ester), which has been used in repellent products in Europe for more than 30 years. It was registered with the EPA in 1999[2]. There have never been reports about serious side effects from the use of IR3535[3].

2. Objectives

To determine the efficacy and duration of protection of two topically applied insect repellent products at preventing landing by mosquitos. The study will follow the EPA Product Performance Test Guidelines[1]. It is intended to test the products against natural populations of mosquito species of public health importance within the genera *Aedes, Anopheles*, and *Culex*, and to replace data from one site previously tested in Florida with data from a site in Louisiana with adequate landing pressure from target mosquito species of public health relevance.

3. Trial Design

This is a single-center, single-site field study with all subjects testing at least one product at one site. The control for each test will be two untreated persons. Repellent product testing will take place in a field setting using 13 test subjects (with a minimum of six of either sex) for mosquitos. Each subject will test a single product at a time (see “Test Methodology” section 7). All testing of a single product at a single site will occur during one test period (i.e., one day). Two untreated subjects will also monitor the landing rate at each test site for each test substance. An additional 5 subjects will be recruited and enrolled as alternates for each test day and will need to be present at the start of each test day in the event they are needed to replace an enrolled subject.

3.1. Trial Endpoints

The primary endpoint is the first confirmed landing, which is used to estimate the median Complete Protection Time (CPT) of the repellent product. The CPT is defined as the time between application of the repellent product and the occurrence of the first confirmed landing. First confirmed landing is a landing followed by another landing within a 5-minute exposure period, or a landing in an exposure period immediately followed by an exposure period in which a landing occurred.

3.2. Risk and Benefit Assessment

This study will adhere to ICH GCP[4], the Declaration of Helsinki[5], and all applicable regulatory requirements including but not limited to the EPA's Human Studies regulation at 40 CFR 26, Subparts K-L. However, there remain some risks to subjects that result from involvement in this trial. These potential risks and benefits have been assessed, and for each risk identified, mitigating actions are described in **Table 1**.

The research provides no direct benefit to subjects. Indirect benefits to society will be additional products available to consumers to repel mosquitos, thereby reducing the potential for mosquito bites and transmission of vector-borne illnesses. The results of this study will inform the product labelling.

Table 1. Potential risks to subjects and mitigating actions.

Risk	Mitigating Actions
Mosquito bites	To prevent biting outside observation periods whilst in the field, all subjects will be asked to wear light, loose fitting clothing that fully covers their body. They will also be provided with and directed to wear a new disposable head net and gloves during the periods of exposure to biting insects, and will use a screened enclosure area in the intervals between exposures. The intention is for mosquitos to be captured before they have a chance to bite. To achieve this, all subjects will be trained to recognise mosquito landing behaviour, and to aspirate mosquitos.
Transmission of vector-borne pathogens through mosquito bites	Weekly mosquito sampling will take place during two months prior to test initiation. Sampling will be conducted using two trap types (CDC Light Trap and CDC Gravid Trap). Trapped mosquitos will be identified to genera (and species) and submitted for pathogen testing based on genera. In addition, the Study Director will have regular contact (at least one per week) with the local health department, which provides information on human cases reported in the region, as well as the number of positive pools of mosquitos in the region. All mosquitos that land during testing will be captured, identified and select genera will be submitted for pathogen testing. All *Culex* species captured will be tested for West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and Eastern equine encephalitis virus (EEEV). All *Aedes* species captured will be tested for Zika virus (ZIKV) and EEEV.
Irritation and itching caused by mosquito bites	The study sponsor will provide topical, over-the-counter anti-itch cream, which will be available at the conclusion of a test day upon

	request to anyone who is experiencing irritation and/or itching as a result of their participation in the study.
SARS-CoV-2 infection	The study will comply with all federal, state, and local restrictions and guidance related to COVID-19 in effect at the time of the study. The Study Director or delegated person will conduct phone screening for symptoms of COVID-19 1-2 days prior to the mosquito attractiveness assessment and training visit, and the test day visits. Appropriate PPE and handwashing supplies will be made available for all study personnel and subjects, and cleaning supplies will be available to disinfect surfaces after subject encounters. Staff will also be monitored for signs, symptoms and exposure to COVID-19 to reduce study subject exposure to sick individuals. Individuals over 55 will be excluded from the study as they are more vulnerable to the effects of SARS-CoV-2 infection.
Irritation of the eyes or respiratory tract from exposure to IR3535	During handling and application of the repellent, eye protection will be worn by the handler. Subjects will wear eye protection during application only. Application and handling will be carried out in a well ventilated area.
Allergy from exposure to IR3535	Volunteers will be excluded if they have a known allergy or sensitivity to any of the product ingredients or any insect repellent products, or any skin condition that may affect their reaction to the product.
Fatigue from long test day.	Subjects will be provided with breaks as needed between the test periods. Subjects will have an opportunity to eat meals and snacks, stretch and walk around, and will have opportunities to use the restroom as necessary. Where a subject enrolls to participate in more than one test day, at least 72 hours will lapse between each test day to allow subjects to rest and recover.
Risks associated with being outside in a hot humid climate e.g. sunburn, heat stroke.	Water and other drinks will be provided to prevent dehydration and snacks will be provided to maintain blood sugar levels if necessary. Subjects will be told at the consent meeting and reminded at the training to bring snacks, lunch/dinner, and entertainment to occupy their time during breaks between test periods. A cooler will be available to store meals and snacks brought by subjects.

	To prevent sunburn, subjects will wear clothing that exposes minimal skin, including a hat. Chairs and a shaded, screened area will be provided for relief from the sun. Fans will be used for the comfort of subjects and staff.
Unintentional release of confidential information.	All efforts will be taken to maintain subjects' confidentiality. See the precautions in Section 9.4 Confidentiality.
Psychological stress relating to pregnancy testing.	Women will be given a private place to take a pregnancy test. A female member of the study team will verify a negative test result, and the Study Director will ensure confidentiality of any test result. The results of the test will not be recorded, discussed with, or released to anyone besides the subject. The confidentiality of the pregnancy testing will be discussed during the consent process.

3.3. Alternatives to Human Study Research

This study will use human subjects because no reliable models or surrogates have been found to adequately predict the efficacy of topically-applied insect repellents. As the objective of this study is to determine the efficacy of a repellent in protecting human beings against bites from mosquitos, it is necessary to complete this testing using human subjects. As human subjects are known to provide a complex combination of thermal, visual and olfactory cues that are attractive to mosquitos looking to bite and feed, an alternative model is not currently available that will test the repellent in a suitably realistic scenario. The repellent must repel the mosquito in the presence of the attractive host in order to be truly effective. The risks of this research and the steps taken to counteract the risks are described in section 3.2. Every effort will be made to protect the subjects in this study from all potential hazards. Products containing IR3535 have been registered by EPA, and the risk assessment has shown that this active ingredient presents little or no hazard when used as directed[6].

4. Subject Entry

4.1. Recruitment

Recruitment will not begin until EPA and the HSRB have reviewed the protocol and the associated informed consent document, these documents are revised to address comments from EPA and the HSRB, and the IRB has approved the final versions of these documents.

Subjects will be recruited from the local area (Baton Rouge, Louisiana), via advertising through traditional, digital and social media. Advertisements will be posted on notice boards and in local newspapers. Advertisements will be posted in digital and social media mediums, such as Facebook, Yahoo/Bing, Google and Craigslist (11.1 Appendix 1.Recruitment Advert). Email will not be employed for advertising.

The advertisement will comprise basic information about the project including the number of visits, how long they will take, basic information about the test and how to get more information (email and phone number for contact). Participants will be asked to provide contact information (both phone and email preferred).

Official recruitment for the study participants will begin on May 1st, 2021. This will consist of using LSU AgCenter promotions and social media assistance. Currently, the LSU AgCenter has roughly 50,000 followers on its Facebook page. Many of the followers consist of individuals living locally within the surrounding regions of Louisiana. The provides an excellent resource for reaching out to individuals around the state for recruitment. It also helps that many of these individuals are already aware of many of the research activities conducted at LSU. On Monday May 1st, we will have the AgCenter post a recruitment announcement on their Facebook page. Posts on the Facebook page will be left up until recruitment is completed.

In the event enough participants for the study are not recruited through the Facebook page. Assistance will be sought from the LSU AgCenter communications to develop a press release, which can be disseminated to news partners throughout the state. The study leader will maintain a database of potential participants including information on contact information and whether they meet the inclusion criteria.

Baton Rouge has a total population of 229,493 people (2010 U.S. census), of these 39.4% are White or Caucasian (includes White Hispanic), 54.5% are Black or African-American, 3.2% are Asian, 1.3% are multi-racial and the remaining 1.6% are other. By sex, 48.1% are male and 51.9% are female. Within the categories that can participate in the study (18-55 years) by age 21% are 18-29, 17%.9 are 30-39, and 14.8% are 40-49. The final report will specify the age and gender demographics of test subjects who participated in the study, taking into account the availability of test subjects on each test day.

Current repellent product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitos. To target users familiar with and that understand the product labels, we will be recruiting English speaking subjects. This research does not offer benefits to the subjects, so limiting recruitment to English speakers will not result in equity-of-access issues.

4.2. Consent Process

Individuals who express an interest in participating in response to the recruitment materials will be contacted by telephone or e-mail by the Study Director or appropriately trained and delegated study staff to inform them of the basic inclusion criteria and study procedures. At this stage, volunteers will determine their own eligibility and self-exclude if they are aware of being ineligible. If they believe they are eligible and are interested in enrolling in the study, they will be given an appointment to meet with the Study Director or appropriately trained and delegated study staff to learn more about the study and their potential role in it. They will be provided with the Participant Information Sheet (PIS) by email or post, and will be given time and instructed to read it thoroughly it before the consent meeting.

The screening and consent session will be held remotely (over the phone or video conference) to reduce physical contact. Before the start of the consent meeting, volunteers will be asked to present

a valid driver's licence, passport, or other valid identification to verify their age and name. They will be given the consent form (see 11.2 Appendix 2 Participant Information Sheet and Consent Form) and given time to read independently before starting any discussion. The staff member leading the discussion will briefly outline the study, its purpose, the volunteer's potential role in the study, the potential duration of testing, the identity and function of the repellent to be used, potential hazards associated with the study, and steps taken to mitigate these hazards, the inclusion/exclusion criteria, and the procedures for reporting adverse events. Test procedures including the aspirator training, product application and field landing catches will be described step-by-step. Female subjects will additionally be informed of the requirement for pregnancy testing on each test day, as well as the procedure for testing and how the subject's privacy will be respected. The subject will then be asked if they have any questions regarding the information presented. It will be made clear that the subject does not have to consent and does not need to give any reason for non-consent or withdrawal. While the trial briefing may take place as a group, all subjects will have a one-to-one session with an appropriate member of study staff during which they can ask questions and will sign the consent form.

If an individual still wishes to enroll in the study, he or she will be asked a number of questions to ensure they have fully understood the information given. These questions are given in the **Table 2** below. Only if the subject can demonstrate their comprehension of the study procedures, will they then be asked to sign the Informed Consent Form (ICF), which will be posted or emailed in to the Study Director. If the signed IFC is emailed, the subject will be asked to bring the original to their first in-persona session, whereupon they will be given a photocopy. The Test Facility will retain the original of the ICF.

Table 2. Questions posed to subjects to confirm understanding of study procedures and examples of acceptable responses.

Questions	Examples of acceptable responses
What part of your body will be treated?	Lower leg
What product will be used in this study?	IR3535, insect repellent
What will you be wearing during the exposure period?	Long pants, long-sleeved shirt, gloves, head net
How long will you be exposed to mosquitos for during the field test for each exposure?	5 minutes every 30 minutes
What are the potential discomforts or hazards from this study?	Exposure to mosquitos, being outside in hot weather,
Do you have the freedom to quit or withdraw from the study at any time?	Yes

If you quit or withdraw from the study, for how many hours will you be paid?	For the duration of my participation, or for 14 hours if I withdraw on a test day for a medical /emergency reason

4.3. Screening Procedure and Eligibility Criteria

Volunteers will be consented prior to any screening procedures being undertaken. Following consent, subjects will be checked for eligibility using a screening questionnaire. Identity and age will be verified using a driver's licence, passport, or other valid government-issued photo identification. Volunteers will be healthy individuals and chosen based on their insensitivity to the bites in order to limit any itchiness or discomfort. Volunteers who do not meet the criteria for eligibility will be excluded. Volunteers will be asked if they have a history of heat stroke; this is not an exclusion criterion, but a condition of which the Study Director will be informed.

Due to the nature of the study, a field study, it is important that all instructions are understood fully and quickly. Therefore, it is essential that the subjects understand English. If the study staff are concerned that the subject is not proficient enough in the English language they will not be permitted to enroll in the study. Current repellent product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitos. To target users familiar with and that understand the product labels, we will be recruiting English speaking subjects. This research does not offer benefits to the subjects, so limiting recruitment to English speakers will not result in equity-of-access issues.

Inclusion Criteria

Volunteers will be eligible to participate in the study if they meet all of the following criteria:

- Able and willing to give fully informed consent;
- Male or female;
- Aged 18 to 55 years;
- Consider themselves to be in good general health, and specifically:
    - Not aware of having any cardiovascular or respiratory disorder (whether active or inactive)
    - No previous anaphylaxis
    - Not aware of having a compromised immune system
- Non-smokers or willing to refrain for 24 hours prior to and during each test;
- Willing to undergo a mosquito attraction test (putting an arm into a cage of mosquitos)
- Able to speak and understand English
- Able to stand outside for periods of at least 5 minutes at a time
- Able to understand and comply with the study procedures, including:
    - Willing to complete mosquito landing/aspirating training
    - Able to withstand exposing the lower leg to mosquitos for periods of at least 5 minutes at a time
- Able to operate an aspirator

Exclusion Criteria

Volunteers will be excluded from the study if they meet any of the following criteria:

- Participated in any other intervention study in the previous 3 months
- Participated in a landing insect test as part of the current study in the previous 72 hours
- Employees, managers, and spouses of employees of the LSU and of the study Sponsor (LivFul, Inc.)
- Students of the Study Director or any other LSU faculty/researchers involved in the study
- Individuals suspected or known to be sensitive or allergic to, or phobic of, mosquito bites
- Women who are pregnant, nursing or intending to become pregnant during the course of the study
- Individuals with localized skin disorders or problems affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes
- Individuals with known or suspected allergy or sensitivity to the test product or any of its ingredients, or any insect repellent products
- Individuals who are not attractive to mosquitos during mosquito attractiveness test
- Individuals who have signs or symptoms related to COVID-19, have tested positive for COVID-19 within the last 15 days, or have had contact (within 6 feet for a total of 15 minutes or more) with someone who has tested positive for COVID-19 in the last 14 days should.

4.4. Withdrawal Criteria

Subjects will be told that they can withdraw at any time without giving a reason for withdrawing and without forfeiting benefits based on their participation prior to withdrawal.

Subjects will be told that they have the option to request their data not be used if they decide to withdraw from the study or if they are withdrawn. Subjects who withdraw from the study or are withdrawn by the Study Director will be notified at the time of the withdrawal that their data will be used unless they request otherwise. Data collected to the point of withdrawal will be used in the analysis of the study unless the subject requests otherwise, in which case it will be removed from the database

Subject's enrollment in the study may be ended at the discretion of the Study Director, where continued participation may affect the safety of the subject or where there is a development of any condition that might interfere with study participation. Withdrawal criteria include:

- The Study Director decides that continuing the study would be harmful to the subject.
- The subject is not following directions necessary for the study to proceed.
- Participation in the study has a bad effect on the subject.
- The study is cancelled.

If a subject withdraws during the test day, the Study Director or appropriately trained study staff member will be responsible for ensuring that they are safely returned to their own vehicle or other transport by the end of the test day. If the subject withdraws because they require medical treatment, the Study Director or appropriately trained study staff member will be responsible for ensuring that they are safely returned to their own vehicle, other transport or a medical facility as soon as possible.

At the end of the consenting and screening meeting, unscented soap will be posted to enrolled subjects or made available for collection from the Test Facility.

4.5. Subject Timeline

Participant involvement in the study is summarised in **Figure 1**.

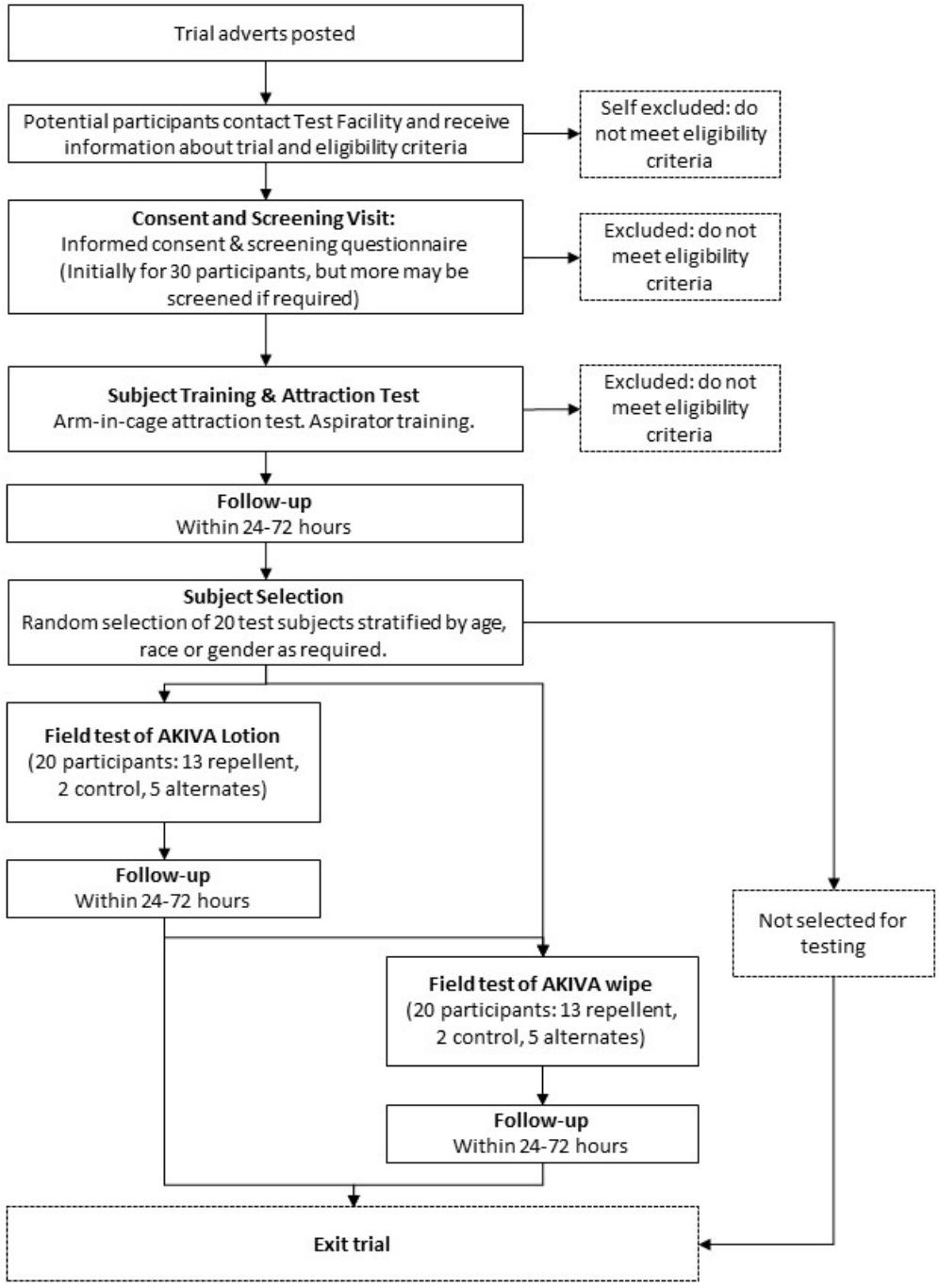


Figure 1. Flow diagram to show movement of subjects through the trial.

5. Test Products

5.1. AKIVA 20 Wipe

TS name: Akiva 20 Wipe
Lot/Batch: XXX
Active ingredient: IR3535 or ethyl butylacetylaminopropionate
CAS Number: 52304-36-6
Appearance: Liquid, without aggregation
Color: Visually white to off-white
Odor: Characteristic
Concentration: 20% ±0.2%
50°C x 1 week: settling/creaming: None observable
50°C x 1 week: coalescence: None observable
50°C x 1 week: visual changes: None observable
Use: As per test protocols
Storage: At 20-30°C in opaque container or away from direct light
Date manufactured: XXX
Date of certification: XXX
Certified by: XXX

5.2. AKIVA 20 Lotion

TS name: Akiva 20 Lotion
Lot/Batch: XXX
Active ingredient: IR3535 or ethyl butylacetylaminopropionate
CAS Number: 52304-36-6
Appearance: Liquid, without aggregation
Color: Visually white to off-white
Odor: Characteristic
Concentration: 20% ±0.2%
50°C x 1 week: settling/creaming: None observable
50°C x 1 week: coalescence: None observable
50°C x 1 week: visual changes: None observable
Use: As per test protocols
Storage: At 20-30°C in opaque container or away from direct light
Date manufactured: XXX
Date of certification: XXX
Certified by: XXX

5.3. Dose Rate

No dosimetry studies will be conducted to determine a consumer dose application rate. This (1) avoids exposure of human subjects who would be needed to perform the dosimetry phase of the study; (2) avoids the influence of outliers; (3) minimizes variability between studies; (4) reduces the time and cost of the study.

The test products will be applied at the standard dose rate based on dosimetry results from skin-applied insect repellent studies previously reviewed by the HSRB and EPA. AKIVA 20 lotion and AKIVA 20 Wipe will be applied at a rate of 1 gram of product per 600 cm^2 (1.67mg/cm^2).

5.4. Safety of IR3535

According to the EPA's risk assessment based on data submitted to EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classed as category III for acute dermal toxicity (LD50 > 3000 mg/kg in rats), category IV for acute oral and inhalation toxicity (LD50 > 5000 mg/kg in rats), and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats and for oral toxicity is 600 mg/kg/day in rabbits[7]. In its risk assessment, EPA used a 5% dermal absorption factor for IR3535 and based their calculations on an 11.8 kg child and 60 kg adult for a product at 7.5% active ingredient.

Since the initial registration, EPA has registered several products containing up to 20.07% IR3535. The products to be tested in the protocol may contain up to 20% IR3535 and Margins of Exposure (MOE) can be calculated as below. The calculation below is an example for calculating exposure estimates for a product containing 20% IR3535, the worst-case scenario of the two products proposed in the protocol.

For risk characterizations using 2680 cm^2 as the average area for the lower leg of an adult male (average lower leg area for a female is 2330 cm^2): (2680 cm^2 x 1000 mg formulation/600 cm^2) ÷ 60 kg = 74.4 mg formulation/kg (74.4 mg formulation/kg) (0.20 mg a.i./mg formulation) = 14.9 mg a.i./kg 14.9 mg a.i./kg x 0.05 dermal absorption factor = 0.75 mg a.i./kg.

Margins of Exposure (MOE) are calculated by dividing the oral NOEL of 600 mg/kg/day as below: 600 mg/kg ÷ 0.75 mg/kg = 800. To reach the EPA's level of concern (MOE ≤ 100), a person would need to make approximately 9 applications in a single day.

The amount of IR3535 applied in these studies does not exceed a level of toxicological concern to test subjects.

6. Test Methodology

6.1. Pregnancy Test

Under federal regulations, female subjects who are pregnant, nursing or lactating may not be enrolled in this study as regulations prohibit testing with pregnant, nursing, lactating women. To confirm that participating test subjects are not pregnant, at the beginning of any day when they will be exposed to mosquitos, female subjects will be required to perform an over-the-counter pregnancy test that will be supplied by LSU. Each female test subject will perform the test alone in a bathroom at the Test Facility. The test subject will keep the test and read their own result. After completion of the pregnancy test, a female employee associated with the study will ask, in a private setting, if the potential subject still wants to participate in the study. If they do, the negative test result will be verified by that employee. The results will be kept confidential, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director.

A positive test result will not be recorded on any documents. If the result is indeterminate, the test will be repeated. If two indeterminate results are obtained, then the subject will be excluded for that day. The potential for both false positive and false negative tests will be made clear to the subjects before and after taking the test.

Test subjects will not be required to disclose the results of the test, with the understanding that if they do not, they will not be allowed to participate in the test. Provisions will be made to allow the test subject to dispose of the test in a discrete manner (e.g. opaque plastic bags available in the restroom used for testing). However, the test subject may keep the test if she wishes to do so. This procedure will be repeated for each test day in which any female subject participates.

Women may be exempt from the pregnancy test if they are considered to not be of childbearing potential. This means either permanently sterile (through hysterectomy, bilateral salpingectomy or bilateral oophorectomy) or post-menopausal (no menses for 12 months without an alternative medical cause). Women will need to give signed confirmation of their eligibility for exemption, but will not be required to disclose the reason. The Study Director can choose to exclude women if they believe the statement of non-childbearing potential to be incorrect.

6.2. Mosquito Attraction Assessment

After completing the consent process, an in-person appointment will be scheduled for mosquito attractiveness tests and mosquito aspirating training. This will take approximately two hours. Within 48-72 hours prior to this test day, subjects will also be contacted to assess their health and potential exposures to COVID-19; those that do not meet the CDC's screening criteria will be excluded.

Due to COVID-19, all study staff and subjects in attendance at the mosquito attraction assessment and mosquito aspiration training will wear masks and where possible maintain 6 feet of social distancing. Masks may be removed during aspirator training periods to allow subjects to aspirate mosquitos. Staff and subjects will be required to have their temperature tested using an infrared thermometer.

For the attractiveness test, each subject's attractiveness to mosquitos will be verified before they proceed further in the study. They will roll up their sleeve and place one arm in a 45 x 45 x 45 cm cage of 78 *Aedes aegypti* or density equivalent to one mosquito per 1,160 cm^3. The study staff in attendance will watch to ensure the mosquitos do not have a chance to bite; they will be blown off or captured after they land on your arm. If the subject does not receive five landings in one minute they will be allowed to repeat the test up to two more times (each time with a fresh cage of mosquitos). If they still do not receive five landings in one minute they will be considered not sufficiently attractive to mosquitos and will not be allowed to continue with any other study procedures. Pathogen-free colony insects will be used that have been bred in colony for at least 10 years and have never had a blood meal. They will be tested prior to the study to confirm the absence of EEEV and ZIKV. After use in each test, the mosquitos will be destroyed and not reused.

6.3. Aspirator Training

After demonstrating sufficient attractiveness to mosquitos, subjects will also be trained to aspirate mosquitos. To prevent mosquitos from landing on parts of the body not involved in the aspirator training, subjects will be asked to dress as for a test day, with long sleeved shirts and pants and will be provided with a new set of gloves and a new, disposable head net. Subjects will be trained in a screened free-flight cage to identify mosquito landing behavior and to use aspirators to collect landing insects before they have time to probe or bite. Subjects will be shown how to aspirate a mosquito as it attempts to land on a partner's leg by study staff and then provided with their own aspirators to use. They will be paired up and asked to simulate the test day by aspirating mosquitos that land on

their partner's pant leg. Once the training is complete these aspirators will be placed in zip-lock bags labelled with subject numbers until the test days. Pathogen-free colony insects will be used that have been in colony for at least 10 years and have never had a blood meal. The mosquitos will be tested prior to the study to confirm the absence of ZIKV, EEEV, WNV, and SLEV (as appropriate by genera). Training will continue until the subjects are able to skilfully use the aspirator to remove a mosquito before it attempts to probe. If after one hour of training the subject is still unable to complete this activity they will not be permitted to continue with the study. After use in each test, the mosquitos will be destroyed and not reused.

6.4. Leg Measurement and Dose Calculation

In order to allow the test day to proceed as smoothly as possible, subject's leg measurements will be taken during the training visit. The vertical length of the leg from ankle to knee will be measured along with the circumference at the ankle, knee and two equally spaced intermediate points[1]. The surface area will be calculated by multiplying the average circumference by the length of the limb. Both left and right legs will be measured separately. Further calculations will be made of the dose required on either leg. All calculations will be checked by the Study Director, or a trained and delegated staff member, before the test day.

The following formula will be used to determine the amount to be applied:

[Area of the Limb/600 cm2] * 1 gram = weight (amount) of product to apply

Individual doses will be converted from weight to volume (mg to ml) using the specific gravity of the test substance.

Subjects will be shown how the test substance will be applied to their leg on test visits. They will also be informed that they will wear gloves to protect their hands, and head nets to protect their head, face and neck, and will be required to wear a face mask. They will also be provided with an unscented soap that they can use to wash during this 24 hour period prior to their test visit.

6.5. Subject Selection

To ensure there is a sufficiently large pool of subjects to choose from, double the expected number of subjects required for the study will be screened ((13 test subjects + 2 control subjects + 5 alternates) * 2 test days * 2 = 80). At least 80 informed and consenting volunteers will be enrolled to take part in the study. This pool of eligible subjects will be checked to be ensure it is representative of the age, gender, and ethnicity of the general population (see **Table 3**).

Table 3. Criteria for determination of bias in pool of eligible subjects and decision to continue recruitment.

Measure		Expected characteristics based on U.S. population[8]	Range permitted for trial selection to proceed	Number of subjects at which stratified selection should be considered	Number of subjects at which recruitment of specific groups should be considered
Age	18-25 years	20%	10-30%	>24	<8
	26-34 years	26%	16-36%	>29	<13
	35-55 years	55%	45-65%	>52	<36
Gender	Percentage female	51%	41-61%	>49	<33
Ethnicity	White	60%	50-70%	>56	<40
	Hispanic	18%	8-28%	>22	<6
	Black/African American	13%	3-23%	>18	<2
	Other	9%	0-19%	>15	Not applicable

If the recruited pool of 80 subjects falls outside the range given in **Table 3**, then the Study Director will make a documented decision as to whether to continue recruitment of target groups, and whether stratified selection of test subjects will be required.

There will be at least six females and six males testing on each test day. Subjects selected for testing will be contacted 48-72 hours prior to the test day to confirm availability and to remind them of compliance requirements. They will also be asked about potential exposures to COVID-19; those that do not meet the CDC's screening criteria will be excluded. Finally, subjects will be reminded of items to bring, by providing them with a checklist of suggested items.

6.6. Field Site Selection

The field site for human landing catches will be selected based on preliminary trapping data that demonstrates suitability for the study. The site will be monitored for at least two months prior to testing by the study team. Three trap types will be used: CDC Gravid Traps, CDC Light Traps baited with carbon dioxide, and BGS trap with counter. These will be used to collect mosquitos from the site. Initially, one CDC Gravid Trap (designed to collect *Culex* mosquitos) and one CDC Light Trap (intended to catch a range of host-seeking and used to assess the diversity of mosquitoes in an area) will be placed at each of five locations across the test site. On addition to mosquito diversity, these traps provide information on relative abundance.

Closer to the test dates, as the location of testing is decided, traps will be placed at each of three locations. A BGS trap with counter will be used at each site as a way of assessing the temporal distribution of the different mosquitoes, as it records in real time as mosquitoes enter into a trap. This will help to determine if there are mosquitoes active during the typical duration period of a study.

The trap catches will be placed on dry ice and the cold chain preserved while they are transferred to the Test Facility. The mosquitos will be identified using relevant mosquito guides (Mosquitos of the Southeastern United States by Nathan D. Burkett-Cadena, and the Louisiana Mosquito Control Association training manual). Mosquitos will then be sorted by species and all vials of mosquitoes will be stored at -80°C, for any potential virus testing. Starting 1 month prior to the study, samples of mosquitoes will be submitted for virus testing. Mosquitoes will be tested in pools of up to 50 specimens for pathogens at the Louisiana Animal Disease Diagnostic Laboratory (LADDL) using PCR. *Culex* will be tested for WNV, SLEV and EEEV. *Aedes* mosquitos will be tested for EEEV and ZIKV. *Aedes* will be tested for chikungunya and/or dengue if there is an identified threat in Louisiana. This testing will be conducted specifically for this study and is in addition to routine surveillance conducted in the parish.

The field site will be selected in an area with an active vector-borne illness monitoring programmes. The Study Director routinely receives emails from the local health department and will receive emails at least weekly in the two months prior to the test initiation to confirm the absence of reported vector-borne disease cases in humans. The field site will only be used if no vector-borne diseases have been detected within 25 miles of the proposed site in the month preceding test initiation. The Study Director will coordinate with the local health department and mosquito control districts to confirm the absence of reported mosquito-borne disease cases in humans within 25 miles of planned test site a week before each test day is conducted. The sites will also not be known Zika virus transmission areas. Current areas under monitoring include Cameron Parish Mosquito Control, Plaquemines Parish Mosquito Control, Calcaseau Parish Mosquito Control, or Grande Isle Mosquito Control.

The monitoring trap catches should demonstrate the presence of three genera of mosquitos: *Aedes*, *Anopheles* and *Culex*. Trapping data prior to testing should show presence of mosquito species of public health importance within each of these genera. There is no minimum density required for any species or genus. The catches should also demonstrate that there could be mosquito host-seeking throughout the collection period. The Study Director will make the final decision on the suitability of the site, bearing in mind the requirement for 5 landings per 5 minutes per control person. The decision

will be made utilizing pre-existing data on population densities as well as monitoring prior to test days. The state health department has provided mosquito control districts with BGS counters, which attach to mosquito traps and provide population density information in real time. To determine that there is a suitable landing pressure at the test site, five CDC Gravid Traps and five CDC Light Traps at the test site will be fitted with BGS counters and set to cover at least 12 hours. Catch rates will be monitored to determine the most suitable 12-hour window for testing.

6.7. Field Test

The Study Director will make every effort to check the weather conditions ahead of time and only schedule the field study in good weather.

Compliance Check

Within 48-72 hours prior to the test day, subjects will be contacted by telephone to assess their health and potential exposures to COVID-19; those that do not meet the CDC's screening criteria will be excluded. Subjects will be told what time to arrive at the Test Facility. They will be advised not to use scented soaps, shampoo, deodorant, perfumes, or cosmetics for the 24 hours immediately preceding the study, and to wash only with hot water and the unscented soap provided at the attractiveness test meeting. Additionally, subjects will be asked not to drink alcohol, smoke or chew tobacco, or engage in vigorous exercise for the 24 hours prior to each test. Female subjects will be reminded that a pregnancy test will be conducted prior to any treatment. Subjects will be asked to wear light, loose fitting clothing that fully covers their body.

Subject Arrival

Subjects will be asked to arrive at the Test Facility at a specific time, 3-4 hours prior to the start of testing. This will allow time for registration, randomization and treatment before travel to the test site. The subjects will have a briefing and a compliance check. Subjects will be reminded that they are free to withdraw at any time and that they will be compensated for their participation up to the time of their withdrawal. They will also be reminded that they have the option to request that their data not be used.

Test subjects will be reminded not to engage in activities that could promote sweating and abrade the treated leg (e.g., rolling down pants, crossing legs), and to be careful when using the restroom to protect the treated leg.

Due to COVID-19, all study staff and subjects in attendance on test days will wear masks and where possible maintain 6 feet of social distancing. Masks may be removed during exposure periods to allow subjects to aspirate mosquitos. Staff and subjects will be required to have their temperature tested using an infrared thermometer. Subjects who fail to abide by social distancing and mask wearing provisions will be asked to leave and may be replaced.

A study staff member will orally verify each subject's compliance with the pre-test conditions on each test day prior to performing any treatment with a test substance. They will additionally check that eligibility criteria have not changed for example any new health conditions that may affect participation.

Randomization for Assigning Test Subjects and Alternates

The first seven males and first seven females to arrive at the Test Facility will be selected as test subjects, plus the eighth male or female. The remaining subjects will be alternates.

The 15 selected test subjects will be randomized to either control or repellent treatment on the day of testing. To achieve this the RANDTREAT command will be used to generate a random allocation of six or seven treatments and a single control to the list of male and female participants (**Figure 2**). It will also be used to assign the treatment to the right or left leg (**Table 4**). The do-file for this will be pre-prepared, and run at the start of the test day when the participant list is finalised. On the test day, the Study Director will refer to this list, and assign the subjects to control or repellent treatment accordingly. For example, in **Table 4**, the third subject will be the control subject.

Figure 2. Do File Example for randomisation in STATA

```
generate PPT_ID = .

[Manually write in 7/8 ID numbers for males/females]

splitsample, generate(TREATMENT) split(1,6) rseed (0)

[if 8 numbers, split (1,7)]

splitsample, generate(LEG) split(3,4) rseed (1)

[if 8 numbers split (4,4)]
```

Table 4. Example STATA randomised dataset, where TREATMENT = 1 is control and 2 is repellent, and LEG = 1 is right leg and 2 is left leg.

	PPT_ID	TREATMENT	LEG
1	121501	2	1
2	121502	2	1
3	121503	1	2
4	121504	2	1
5	121505	2	2
6	121506	2	2
7	121507	2	2

Pregnancy Test

Female subjects will be asked to conduct a pregnancy test prior to any treatment, as described above. All efforts will be taken to maintain the confidentiality of the pregnancy test results. The results will not be disclosed to anyone other than the test participant, the verifying employee, and/or Study Director.

Repellent Application

The lower leg to be treated (or left untreated for control subjects) will be washed with unscented soap and water, rinsed with isopropyl alcohol (70% in water) and dried with paper towels.

The dose required for each subject will have already been calculated, checked and recorded in their case report form following the participant's leg measurements being taken. This dose will be measured and applied evenly over the lower leg from the ankle to the knee using a single gloved finger to ensure uniform coverage. The AKIVA 20 Lotion dose will be measured directly by weight, but the AKIVA 20 wipe will be wrung out first and a measured weight of the liquid applied.

- For the lotion, a spatula will be labelled with the participant ID and placed on the balance, and the balance tared. The required weight of the test substance will then be added to the spatula. Then the product will be applied to the subject using a gloved finger directly from the spatula. The spatula will be reweighed until it contains less than 0.05 g product.
- For the wipe, a beaker will be placed on the balance and the weight recorded. One to two wipes will be squeezed into the beaker and the weight recorded. The beaker will be labelled with the participant ID and the excess product will be removed from the beaker using a spatula. Then the product will be applied to the subject using a gloved finger directly from the beaker. The beaker will be reweighed until it contains less than 0.05 g product. The excess product that is removed will be weighed and recorded as "disposed" in the product accountability log.

Due to the number of test subjects requiring repellent application, more than one trained technician may apply the test substances to subject's legs. Technicians will ensure consistency as best as possible. The time of application will be recorded for each subject on their case report form.

Transport to and from the Field Site

Subjects will be transported to the field site by the study staff and will be returned to the Test Facility at the end of the testing day. Subjects may also drive in their own vehicles if they wish. In order to maintain the integrity of the treatment, subjects will again be reminded to avoid abrading, rubbing, touching or wetting the treated area. Multiple vehicles will be used to ensure that subjects can remain at an appropriate social distance as far as possible.

If a subject experiences product failure before the end of the full test day they will remain with the group until the next vehicle is available to return them to the Test Facility.

Randomization for Human Landing Catches

Nine collection stations ('A'-'I') will be set up at the field site, with a minimum distance of 3 meters between each station. Subjects will be paired and assigned to the collection stations according to a randomization procedure.

Control and repellent treated individuals will not be paired together to avoid diversion of landing to control subjects. Additionally, difference in attractiveness can result in diversion of landing from one control to another. Therefore, the two controls will not be paired together, but with study staff. Study staff will perform counts, but will not expose their own legs. Unpaired test subjects will also be assigned a staff member in the same manner.

To achieve the randomization, first, the initials of three staff members will be listed in a column in a STATA dataset (**Table 5**, column B), alongside a column in which random numbers are generated (column C). The two lists will be 'sorted' according to the random number list, from smallest to largest. The first staff member will make a pair with the female control subject, the second with the male control subject, and the third with a treated subject (column A).

To determine the pairings, another dataset will be prepared in which the third staff member and the participant IDs of the 13 treated subjects are listed in a column (**Table 5**, column E), alongside a column in which a random number will be generated (column F). The two lists will be 'sorted' according to the random number list, from smallest to largest. The first two individuals in the list will make test pair 1, the second two individuals will make test pair 2, etc. (column D). The eight pair will be the male control pairing and the ninth pair will be the female control pairing. The pairs will not change throughout the test day. Test pairings will be assigned to test stations through a Latin square design (see Appendix 3).

A record of which subjects were paired together and where they were positioned throughout testing will be maintained to facilitate contact tracing, if necessary.

Table 5. Example randomization of subject pairings.

A	B	C	D	E	F
Pairing	Staff member initials	Random number	Test pair	Participant ID/ staff initials	Random number
Female control	TJ	0.1329	1	215021	0.0518
Male control	CH	0.2691	1	LC	0.0691
Treated	LC	0.5786	2	215034	0.1139
			2	215022	0.3075
			3	215064	0.5146
			3	215007	0.5797
			4	215013	0.6217
			4	215018	0.6599
			5	215024	0.6745
			5	215044	0.7217
			6	215049	0.7448
			6	215077	0.8894
			7	215003	0.9544
			7	215050	0.9646

Human Landing Catches

Human landing collections will start 2 hours after product application. Other products registered with the EPA that contain 20% IR3535, have been shown to give a similar level of protection to 10% DEET[9]. In addition, these test products were previously tested in field sites in Florida, and the EPA allowed data from those studies to support a claim of 3 hours complete protection. Therefore, it is highly unlikely the test products will fail within this time delay. The 2-hour time delay will allow repellent application to be carried out at the test facility where measuring equipment can be kept calibrated and in good condition, before travelling to the field site to start collections.

A minimum of three consecutive exposure periods should occur before subjects experience a first confirmed landing. If a first confirmed landing occurs within the first three exposure periods, the CPT will be counted as 0 hours. Subjects experiencing a first confirmed landing at two hours post application or within the first three consecutive exposure periods should not be replaced.

The mosquito landing pressure will be determined before the treated volunteers expose their legs. Control subjects will expose their lower leg for 5 minutes or until 5 landings occur, whichever is sooner. As soon as 5 landings occur, regardless of whether 5 minutes have elapsed, the control subject can cover the lower leg to minimize the potential for mosquito bites. Landing pressure will be assessed for each 30 minute interval. The time of the landings and when the threshold number of landings occur will be recorded. If the landing pressure is insufficient, test subjects will carry out their exposures in that time period unless the total number of exposure periods with low landing pressure exceeds three consecutive periods or four non-consecutive periods with inadequate landings on controls, and the test should be stopped.

The treated lower limb of the test subjects will be exposed for 5 consecutive minutes, during each 30 minute interval for the trial period. Under supervision of a trained member of staff or another subject, the number and timing of each landing during each exposure period for each subject will be recorded.

All insects landing on the treated and control subjects' exposed lower legs will be collected by aspiration (before they have chance to probe or bite) and labelled with the subject number, treatment status, date and time of collection. Mosquitos will be identified to genus or species as appropriate. Then samples will be pooled by subject and date and tested for pathogen prevalence as appropriate. Mosquitos landing elsewhere on the body will not be collected.

Human landing collections for each subject will continue until treatment failure. Treatment failure is defined as one mosquito landing followed by a confirmatory landing either in the same 5 minute exposure period, or in the following exposure period. If treatment failure is reached in more than half of subjects, all other subjects will also stop testing and the test will be concluded. If treatment failure is not reached in more than half of subjects, the test will continue until 14 hours post application for the lotion, or 13 hours for the wipe. A median CPT can be established when there is time to treatment failure data for half the subjects, so at this point there is no need to risk exposure of subjects who have not reached treatment failure.

Between exposure periods all subjects will have access to restroom facilities, and a shaded, screened location with adequate seating. In addition, subjects will have access to cold drinks (water, soft drinks, etc.) and snacks to keep them hydrated and to maintain their blood sugar. Subjects may also consume their own food or drinks they bring; read, or engage in other leisure activities. Test subjects will be reminded not to engage in activities that could abrade the treated leg and to be careful when using the restroom to protect the treated leg.

Early Stopping Criteria

The Study Director can decide to stop the test day early if they believe this is in the best interests of the trial subjects, study data or both. Data from uncompleted test days will be included in the final

report, but will not be included in final calculations unless this can be justified. The reason for stopping the test day must be recorded, and the sponsor informed within 24 hours.

The most likely reason for stopping the test day is anticipated to be insufficient landing pressure on controls. In this case the efficacy of the repellent cannot be demonstrated, and subjects would be exposed to landing mosquitos unnecessarily. Low landing pressure is defined as fewer than 5 landings on either of the two controls in a 5 minute exposure period. If there are 4 non-consecutive exposure periods with low-landing pressure the test day should be stopped. If there are 3 consecutive exposure periods with low-landing pressure the test day should be stopped.

Mosquitos are unlikely to fly in bad weather, and it is unpleasant for the subjects to be exposed to, so exposure periods will be skipped if wind speed exceeds 16 kph/10 mph, or if it is raining. If there are 4 non-consecutive exposure periods missed due to bad weather the test day should be stopped. If 3 consecutive periods are missed due to bad weather then the test day should be stopped.

If a single landing on a test subject during an exposure period is followed by a missed exposure period (due bad weather) then the first landing will be treated as a confirmed landing. If a confirmed landing occurs during an exposure preceded by a period of low landings or by a missed exposure period due to bad weather, then CPT will be recorded as the earliest time point in that preceding period.

Subjects will be instructed to wash their treated limb as soon as possible after their participation ends and will be provided soap, water, and paper towels at the field site or at the Test Facility where they will be returning to at the end of the testing day.

6.8. Environmental Monitoring

Weather conditions (temperature, relative humidity, cloud cover, precipitation, light intensity, and wind speed) will be recorded at the start and end of the study day. Temperature and wind speed will additionally be recorded for each half hour test period.

6.9. Follow-up

Subjects will be followed up within 24-72 hours after any exposure to mosquitos or test product to assess any possible adverse events. Subjects will also be asked about COVID-19 symptoms and diagnoses. Three attempts will be made to contact the subject. Initial contact will be by email, second and third contacts will be by phone and email. If it is still not possible to contact the subject, then a protocol deviation will be submitted to the IRB.

Alternate contact information will be gathered for each subject for someone who can reach the study participant if contact tracing is necessary. If any subject reports COVID-19, a contact tracing protocol will be followed so that staff and other subjects others potentially exposed to SARS-CoV-2 can be contacted.

In the event that a mosquito captured during testing is found to carry an arbovirus, the Study Director will contact the subjects testing that day. Contact will be made via telephone.

7. Statistics and Data Analysis

7.1. Sample Size Calculation

EPA statisticians used a Weibull distribution to simulate the sample size required to estimate the median CPT to a given degree of precision. A power analysis of these outputs resulted in a sample size of 13 subjects being chosen. This number allows for over 90% power at a range of expected median CPTs from 2-8 hours (Appendix 4: Sample size calculation).

This sample size is appropriate to achieve at least 90% power that the ratio of 95% LCL mCPT/mCPT ≥ 0.6), given that the P5MR (i.e., CPT5th percentile/mCPT) is assumed to be equal or greater than 0.5. The assumption for the variation of CPT data distribution is characterized by the value of P5MR, associated with the selected sample size and the result of power. The P5MR value characterizes the spread of the CPT data distribution. For two products with same mCPT, the product with lower value of P5MR has a wider range of CPT data.

To maintain gender balance among the volunteers, at least 6 volunteers of each sex will be recruited to test each product. An additional 2 subjects will be required to act as untreated controls. Five alternate subjects will be invited to take part in each study day. Subjects will be able to take part in one or both test days. Therefore, a minimum of 20, and a maximum of 40 subjects will be required to complete the trial.

7.2. Statistical Methods

The endpoint for Complete Protection Time will be time to treatment failure for each subject test. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the time at which the first confirmed landing occurred. First confirmed landing is a landing that is followed by another landing within a 5-minute exposure period, or a landing in an exposure period immediately followed by an exposure period in which a landing occurred. The times to treatment failure will be analyzed using Kaplan-Meier Survival functions, and from these the median Complete Protection Time and 95% confidence intervals (CI) will be calculated. The 95% CI of the estimated mCPT will be calculated with the log-log transformation applied to survival function and Kaplan-Meier survival curves will be presented in the study report.

A first confirmed landing occurring within the first 3 consecutive exposure periods will be considered as 0 for statistical purposes.

Participants that withdraw before the start of testing will be replaced with alternates. Participants that withdraw during the test day will have their data included in the statistical analysis as right censored data. Right-censored data are those data that, despite monitoring of the outcome event, the event does not occur within the study duration or because the participant has withdrawn. Withdrawn subjects whose data are right censored should not be replaced. If 3 or more participants on an individual test day withdraw before they record treatment failure, the Study Director will make the decision as to whether to continue testing and include these right-censored values in the analysis, or end the test day, and attempt a repeat at a later date. In this case the data will be reported, but not included in statistical analysis. The decision, and reasons to continue or discontinue testing, will be recorded.

Adverse events will be tabulated and included in the study report. Adverse events occurring after the end of subject participation but before the end of the study will be listed separately.

8. Safety Reporting and Data Monitoring

8.1. Medical Monitor

The medical monitor will be familiar with the protocol prior to the start of the study. They will be available via email and telephone for the study team to consult in cases where inclusion and exclusion criteria maybe unclear. In the case of adverse events, they will be asked to review the information provided and determine the severity and relatedness of the event. Quality assurance will review the use of the medical monitor, and if appropriate may communicate directly with them.

The medical monitor will be Lake Line Direct, a 24 - Hour Nurse Advice and Health Information service available on 225-765-5253 or 1-877-765-5253 (toll free).

8.2. Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study subject
Serious Adverse Event (SAE)	A serious event is any untoward medical occurrence that: Results in death Is life-threatening Requires inpatient hospitalisation or prolongation of existing hospitalisation Results in persistent or significant disability/incapacity Consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the subject or require an intervention to prevent one of the above consequences.

The medical monitor will decide whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

8.3. Adverse Events

Volunteers will be monitored throughout the duration of the tests by investigational staff for any adverse events. A certified first aider (American Red Cross Adult First Aid/CPR/AED certified, or

equivalent) will be present during the training and test days should any subject require emergency first aid on-site. If any adverse events related to mosquito bites or the repellent product are apparent at any time during the trial, testing will stop immediately and details of how to access treatment, and how treatment will be paid for will be offered. Volunteers can only participate in a test a minimum of 72 hours after the screening bite test and subjects with known allergies to any of the product ingredients will not be eligible to take part.

Within 72 hours after laboratory testing an email will be sent to subjects asking them to report any adverse events that might have occurred since the end of testing. Adverse events that occur >72 hours after the end of participation in the trial will be passively monitored. An adverse event which is ongoing at the time of subject withdrawal or completion will be followed up until it resolves or until 30 days after the subject terminates from the study, whichever comes first.

8.4. Compensation Due to Adverse Events

If a subject is injured or becomes ill as a direct results of his or her participation in this study, the Sponsor will pay for all reasonable and necessary medical expenses required to treat the injury, as long as:

1. The injury or illness occurs during the subject's participation in the study, and
2. The injury or illness results directly from the test substance or study-related procedures.

If an insect tests positive for a pathogen, then the subject that this mosquito was aspirated from will be contact and informed of the result and advised to consult with a medical practitioner. All other subjects will be notified of the result and recommended to seek medical assistance if they feel ill.

The Sponsor, Medical Monitor, and Study Director will determine whether the injury is related to the subject's participation in the study. To do this, they may request to consult with the person/facility that provided medical treatment following an adverse event, which could require the subject's consent.

No additional compensation for adverse events beyond payments for medical expenses related to participation in the study is routinely offered. The Study Director and others involved in this study may be LSU employees. As employees of the University, they are protected under State Law, which limits financial recovery for negligence.

8.5. Safety Reporting Procedures

All adverse events and serious adverse events should be reported. Depending on the nature of the event the reporting procedures listed below should be followed. Any questions concerning adverse event reporting should be directed to the chief investigator in the first instance.

All adverse events should be recorded on the Adverse Event Record Form (Appendix 5) and Adverse Event Monitoring Questionnaire (Appendix 6) and entered into a log. All data should be kept securely and to maintain confidentiality. All adverse events will be reported to the sponsor, Livful Inc. Depending on the nature of the event the reporting procedures below should be followed, see Appendix 7.

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the definitions in section 7.2. AE questionnaires meeting the SAE definition will be submitted to the medical monitor within 24 hours. SAEs that are assessed by the Study Director as being both related and unexpected must be reported to the LSU IRB within 15 days of the Study Director becoming aware of the event.

In the case of a severe reaction such as anaphylaxis, it will be treated as an emergency and an ambulance will be called immediately by dialling 911 directly from a mobile. In addition, a trained First Aider will be called.

8.6. Risk Assessment

The Study Director has determined this study to be “low-risk” when the hazards associated with trial design, randomization system and outcome measures, participating sites, study population, test product, trial supplied, data management systems, potential for adverse reactions, and oversight mechanisms are assessed. Day-to-day monitoring will be carried out at the study centre by a member of the study team with delegated responsibility.

Safety information regarding the repellent used in the trial have been assessed, material safety data sheets (MSDS) and labels have been read to be sure they are safe for human use. The active ingredient in the test product is IR3535. Subjects will be given an information sheet explaining the details of the ingredients and what to do if they have a reaction to the product or mosquito bites after completion of the test.

8.7. Data Management

Informed consent forms will contain the subjects' full names. The subjects' names will be entered onto an enrollment log and each subject will be assigned a subject identification number. All other data collection forms will use the subject identification number only (see Appendix 8).

During screening the subject will self-complete an eligibility questionnaire including their date of birth and some medical history. All other data collected will be recorded in the case report form (CRF) and signed on each page by the researcher responsible. The CRF is considered to be source data. Data to be collected on the CRF are: subject number a date of test on every page, confirmation of informed consent, eligibility details, attractiveness test data (number of mosquitos attracted to arm); training data (successful attempts to capture mosquitos); leg measurements; test visit data (product application details, mosquitos caught, times of testing); and adverse event monitoring.

Raw data from the CRF will be entered into a database, from which datasets can be extracted for analysis. This database and any datasheets or analysis produced will be saved in a location with access limited by password identification to members of the study team. Data will be double entered and verified to ensure accuracy. The Study Director will confirm that all data from all subjects have been entered to avoid analysing an incomplete data set. CRFs will be kept in secure storage after completion. Information in the database will be linked to a relevant SOP, risk assessment, contract, and files of statistical analysis and location of study report.

9. Ethics and Dissemination

9.1. Research Ethics Approval

This study will be conducted in accordance with the EPA's final regulation at 40 CFR 26 that establishes requirement for the protection of subjects in human research. The protocol, informed consent form, and other required documentation must be submitted to an independent Institutional Review Board (IRB) and submitted to the EPA as required by 40 CFR26.1125. These documents must be approved before any portion of the study is initiated, including recruitment of the subjects. To maintain scientific integrity in regards to testing procedure and clarity of the protocol, any revisions made to the protocol as a result of the protocol review process will be reflected directly in the protocol itself.

The report of the completed research is subject to the requirements at 40 CFR 26.1303 to provide documentation related to ethical conduct of the study.

The IRB responsible for approval and continuing review of this research is:

Elizabeth Cadarette, IRB Coordinator
130 David Boyd Hall
Louisiana State University
Baton Rouge
LA 70803

9.2. Protocol Amendments

All amendments and deviations will be reported to the study sponsor in a timely manner. All amendments and deviations to the protocol will be reported to the IRB consistent with their standard reporting guidance. Protocol amendments may not be initiated without prior IRB review and approval except where necessary to eliminate apparent immediate hazards to human subjects.

All amendments, deviations, and any adverse events will be documented in the final study and reported consistent with Good Laboratory Practices (GLP) 40 CFR 26, Subpart K, and IRB reporting procedures. Documentation will include a description of the change, the reason for the change, the effect of the change on the conduct and outcome of the study, and whether or not the IRB approved each amendment prior to implementation.

9.3. Consent

Consent to enter the study must be sought from each volunteer only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed volunteer consent must be obtained. The right of the subject to refuse to participate without giving reasons must be respected. Subjects must be informed of their right to withdraw from participation at any point in the study without forfeiting benefits to which they are entitled.

9.4. Confidentiality

Subjects' identification data will be required for the enrollment process. This data will be linked on a single form (subject enrollment log) to their unique identification number. The subject identification number will be used on all other data collection forms, which will not contain any other identifiable

data. The subjects' names will not appear anywhere on the data sheet, or in the reports. The Test Facility will preserve the confidentiality of subjects taking part in the study.

The information obtained from subjects taking part in this study will be used by the researchers, and the Sponsor/Funder, and will become part of the report. All reports (as well as all study-related records) will be kept as confidential as possible under US and State law. The results of the study are not intended for publication; however, if any of the study-related data are published, subjects' identities will remain confidential.

All efforts will be taken to maintain the confidentiality of the pregnancy test results. The test results will not be recorded, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director.

The study records will be maintained at the Test Facility in locked cabinets, and electronic files kept on a password protected computer server. No one outside researchers, Sponsor, IRB, or certain governmental agencies will have access to subjects' personal information.

9.5. Record Retention

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

9.6. Payment for Participation

Subjects will be paid to compensate for their time when participating in testing. Subjects will be paid $10 each for the consent and enrollment appointment, estimated to take around 1 hour, regardless of whether they choose to enroll in the study. Subjects will be paid $40 each for taking part in training, estimated to take approximately 2 hours, regardless of whether they continue their enrollment in the study. For the field test, subjects will be paid $10 per hour for taking part in the field test, for the first 8 hours, and $15 per hour for participation beyond the first 8 hours. These payments will be rounded up to the next hour. If field test days are truncated, such as due to bad weather, payments will be made per hour.

An alternate who shows up to the test site and who is not needed to replace a test subject will be paid $20. The decision as to whether an alternate is needed will occur within the first two hours of the test, before all the treatments have been finished, at which point they can leave. If an alternate is asked to replace a subject, he or she will be paid at the same rate as other test subjects, as described above.

All payments will be made in cash or pre-paid card in person at the end of each event.

The Study Director, or other delegated staff may end a particular subject's participation on a test day at any time (see section 4.4). If the Study Director or other delegated staff ask a subject to withdraw from the test and they have complied with all of their requests, or if a test subject needs to withdraw early because of a health or emergency reason, full payment will still be made even if the test subject has participated for less than the full test day. This will not affect payment for any previous test days that have been completed.

If a subject is asked to withdraw from the test because they have not followed all the directions, or if they choose to withdraw from testing early on a test day for a non-health related or non-emergency reason, full payment will not be made if the test subject participates in less than the full test day. Instead, they will be paid for the number of hours (rounded to the nearest hour) at a rate of $10 per hour ($15 per hour beyond the first 8 hours). This will not affect payment for any previous test days that have been completed.

Subjects that have reached treatment failure and are no longer part of the testing, but who are unable to leave the test site as they are reliant on trial staff for transport will be paid until they are transported back to the Test Facility.

9.7. Dissemination Policy

The results of the trial will be analysed and a report submitted to Livful Inc. This report will be used to support efficacy claims when requested through a submission to the United States Environmental Protection Agency.

10. References

1. United States Environmental Protection Agency. Product Performance Test Guidelines. OPPTS 810.3700: Insect Repellents to be Applied to Human Skin. 2010.

2. United States Environmental Protection Agency O. Biopesticide Active Ingredients. https://www.epa.gov/ingredients-used-pesticide-products/biopesticide-active-ingredients. Published 2021. Accessed January 29, 2021.

3. Merck. Insect Repellent - FAQ. https://www.merckgroup.com/en/expertise/cosmetics/care-solutions/insect-repellent/faq.html. Published 2021. Accessed January 29, 2021.

4. ICH. *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2).*; 2016.

5. World Medical Association. *WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects*.; 2013.

6. WHO. *Report of the Fourth WHOPES Working Group Meeting*.; 2001.

7. US EPA Office of Pesticide Programs. *3-[N-Butyl-N-Acetyl]-Aminopropionic Acid, Ethyl Ester (113509) Technical Document.*; 1999.

8. US Census Bureau. QuickFacts: United States.

9. Patel R V., Shaeer KM, Patel P, et al. EPA-Registered Repellents for Mosquitoes Transmitting Emerging Viral Disease. *Pharmacotherapy*. 2016;36(12):1272-1280. doi:10.1002/phar.1854

11. Appendices

11.1. Appendix 1: Recruitment Advert

FACEBOOK RECRUITMENT USING LSU AGCENTER FACEBOOK PAGE

Targeting Criteria

The ad will target the following:

- Male and Female
- 18-55
- Within 30 miles of Baton Rouge, LA

Text:

Heading

- Healthy volunteers are being recruited for a research study at the Louisiana State University AgCenter

Content

- Researchers at the Louisiana State University are evaluating two types of mosquito repellent. We are seeking healthy volunteers between 18 and 55. Compensation will be provided. For more information, please contact the study director, Dr. Kristen Healy, 225-578-7386, khealy@lsu.edu

Photo to include in advertisement

NEWSPAPER RECRUITMENT

VOLUNTEERS NEEDED FOR INSECT REPELLENT RESEARCH STUDY AT THE LOUISIANA STATE UNIVERSITY AGCENTER

IRB#

We are seeking volunteers for participation in a study of insect repellents and how they will prevent bites from mosquitoes in a field setting. Participants must be between the ages of 18 and 55. The study will take up to 6 visits to complete (compensation is provided per visit). If you would like to participate or you would like more information please contact the study director Dr. Kristen Healy, Phone 225-578-7386, email: khealy@lsu.edu

11.2. Appendix 2: Participant Information Sheet and Consent Form

(Provided separately)

11.3. Appendix 3. Latin square for test station designation

Example Latin square-based rotation for determining the test stations to be used by test subject pairs on a test day. The Latin square design ensures that pairs rotate between stations throughout their test visit. Note that with 9 test stations, the Latin square is only balanced up to 18 exposure periods or 10.5 hours post repellent application. After this point pairs will start repeating the square rotation, but will not complete another full randomised block.

Exposure period	Time after repellent application (hours)	Test Station								
		1	2	3	4	5	6	7	8	9
1	2	A	B	FC	C	MC	D	G	E	F
2	2.5	G	F	MC	E	FC	D	A	C	B
3	3	C	D	B	E	A	F	FC	G	MC
4	3.5	FC	MC	A	G	B	F	C	E	D
5	4	E	F	D	G	C	MC	B	FC	A
6	4.5	B	A	C	FC	D	MC	E	G	F
7	5	G	MC	F	FC	E	A	D	B	C
8	5.5	D	C	E	B	F	A	G	FC	MC
9	6	FC	A	MC	B	G	C	F	D	E
10	6.5	F	E	G	D	MC	C	FC	B	A
11	7	B	C	A	D	FC	E	MC	F	G
12	7.5	MC	G	FC	F	A	E	B	D	C
13	8	D	E	C	F	B	G	A	MC	FC
14	8.5	A	FC	B	MC	C	G	D	F	E
15	9	F	G	E	MC	D	FC	C	A	B
16	9.5	C	B	D	A	E	FC	F	MC	G
17	10	MC	FC	G	A	F	B	E	C	D
18	10.5	E	D	F	C	G	B	MC	A	FC
19	11	A	B	FC	C	MC	D	G	E	F
20	11.5	G	F	MC	E	FC	D	A	C	B
21	12	C	D	B	E	A	F	FC	G	MC
22	12.5	FC	MC	A	G	B	F	C	E	D
23	13	E	F	D	G	C	MC	B	FC	A
24	13.5	B	A	C	FC	D	MC	E	G	F
25	14	G	MC	F	FC	E	A	D	B	C

11.4. Appendix 4: Sample Size Calculation

Objective
To determine the sample size N such that the proposed IR3535 mosquito repellency study has sufficient power to obtain a given degree of **precision** in the estimate of Median Complete Protection Time (mCPT). This precision – designated as "K" -- will be expressed by 95% LCL_{mCPT}/estimated mCPT

The simulation used to estimate varying sample sizes will require that that 95% LCL_{mCPT}/estimated mCPT<K , given the true **variation** of the Complete Protection Time (CPT) distribution, expressed by a the Weibull distribution family and a parameter, P5MR, defined as the 5^{th} percentile/mCPT.

Additional Details: Two important considerations in developing an estimate of the CPT as a measure of repellency are the **precision** of the estimate and the **variability** of CPT in the population. The precision of the estimate -- designated as "K" -- is defined here as follows:

K = 95% LCL_{mCPT}/estimated mCPT

Where: mCPT= median protection time

95% LCL_{mCPT} = 95% lower confidence limit on mCPT

The degree of variation of the mCPT distribution will be defined as the P5MR which is defined here as the ratio between the mCPT of the 5^{th} percentile of the population to the Median CPT (mCPT) of the population:

$P5MR = CPT_{5th\ \%ile}$ /mCPT

Where:

mCPT= median protection time

$CPT_{5th\ \%ile}$ = 5^{th} percentile of the distribution of CPT

The simulation performed by EPA estimates the power of a mosquito repellency study design as a function of the following parameters:

- Sample size
- (Required) precision K of estimated mCPT
- mCPT
- P5MR

Table 1 below presents the power estimates from simulations in which the data were randomly generated from Weibull distributions for K = 0.6. These are shown for various values of mCPT (ranging from 2 to 8 hours), P5MR (ranging from 0.2 to 0.8), and Sample Size (ranging from 10 to 20). Note that K reflects the precision of the estimate of mCPT. For example, the K value of 0.6 requires that the 95% LCL on a median protection of 10 hours be no less than 6 hours while a K value of 0.8 requires that the

95% LCL on that same median protection time be no less than 8 hours. The K of 0.8, then, requires a more precise estimate of the MPT than a K of 0.6.

Full tables and graphs of the simulations are available in trial files.

Table 1: Results of power analysis when the lowest acceptable ratio 95% LCL/mCPT = 0.6 (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		_10	_11	_12	_13	_14	_15	_16	_17	_18	_19	_20
2	0.2	0.071	0.291	0.207	0.473	0.362	0.369	0.502	0.494	0.637	0.626	0.521
	0.4	0.297	0.691	0.594	0.841	0.804	0.779	0.893	0.898	0.939	0.945	0.932
	0.5	0.498	0.850	0.802	0.942	0.938	0.921	0.968	0.977	0.964	0.982	0.986
	0.6	0.733	0.949	0.943	0.962	0.971	0.955	0.954	0.979	0.915	0.951	0.971
	0.7	0.893	0.945	0.955	0.875	0.918	0.852	0.855	0.893	0.810	0.859	0.886
	0.8	0.819	0.786	0.826	0.666	0.734	0.591	0.637	0.708	0.558	0.632	0.689
4	0.2	0.043	0.208	0.146	0.356	0.289	0.254	0.432	0.380	0.567	0.516	0.435
	0.4	0.241	0.595	0.521	0.783	0.737	0.709	0.849	0.842	0.930	0.920	0.884
	0.5	0.412	0.795	0.730	0.921	0.901	0.888	0.956	0.964	0.986	0.988	0.973
	0.6	0.648	0.938	0.899	0.987	0.980	0.976	0.995	0.997	0.997	0.999	0.998
	0.7	0.869	0.988	0.986	0.993	0.995	0.994	0.992	0.998	0.977	0.992	0.996
	0.8	0.975	0.982	0.987	0.948	0.970	0.949	0.934	0.968	0.887	0.932	0.954
6	0.2	0.075	0.204	0.153	0.339	0.280	0.252	0.426	0.369	0.557	0.490	0.424
	0.4	0.227	0.572	0.504	0.759	0.743	0.689	0.851	0.826	0.929	0.916	0.885
	0.5	0.408	0.779	0.729	0.914	0.905	0.873	0.963	0.958	0.987	0.981	0.978
	0.6	0.645	0.925	0.906	0.984	0.980	0.977	0.997	0.997	1.000	0.999	0.999
	0.7	0.874	0.990	0.988	0.998	0.999	0.999	1.000	1.000	0.999	1.000	1.000
	0.8	0.986	0.998	0.999	0.993	0.995	0.994	0.990	0.997	0.975	0.989	0.995
8	0.2	0.323	0.346	0.362	0.457	0.443	0.361	0.537	0.453	0.636	0.564	0.522
	0.4	0.314	0.586	0.552	0.769	0.753	0.700	0.858	0.836	0.934	0.919	0.891
	0.5	0.421	0.779	0.732	0.914	0.904	0.875	0.960	0.956	0.989	0.985	0.979
	0.6	0.638	0.927	0.906	0.983	0.979	0.974	0.997	0.997	1.000	0.999	1.000
	0.7	0.874	0.990	0.989	0.999	1.000	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.985	0.999	1.000	0.997	1.000	0.999	0.998	1.000	0.994	0.998	0.999

NOTE: Yellow indicates power > 0.8; orange indicates power > 0.9 blue indicates unusual power when median complete protection time = 2 hours and P5MR = 0.8.

11.5. Appendix 5: Adverse Event Record Form

Study Title:

Volunteer reference number	**Date**	**Side effect/adverse event**	**Related to the product**	**Comments**	**Action taken**
			Y/N/don't know		

11.6. Appendix 6: Adverse Event Monitoring Questionnaire

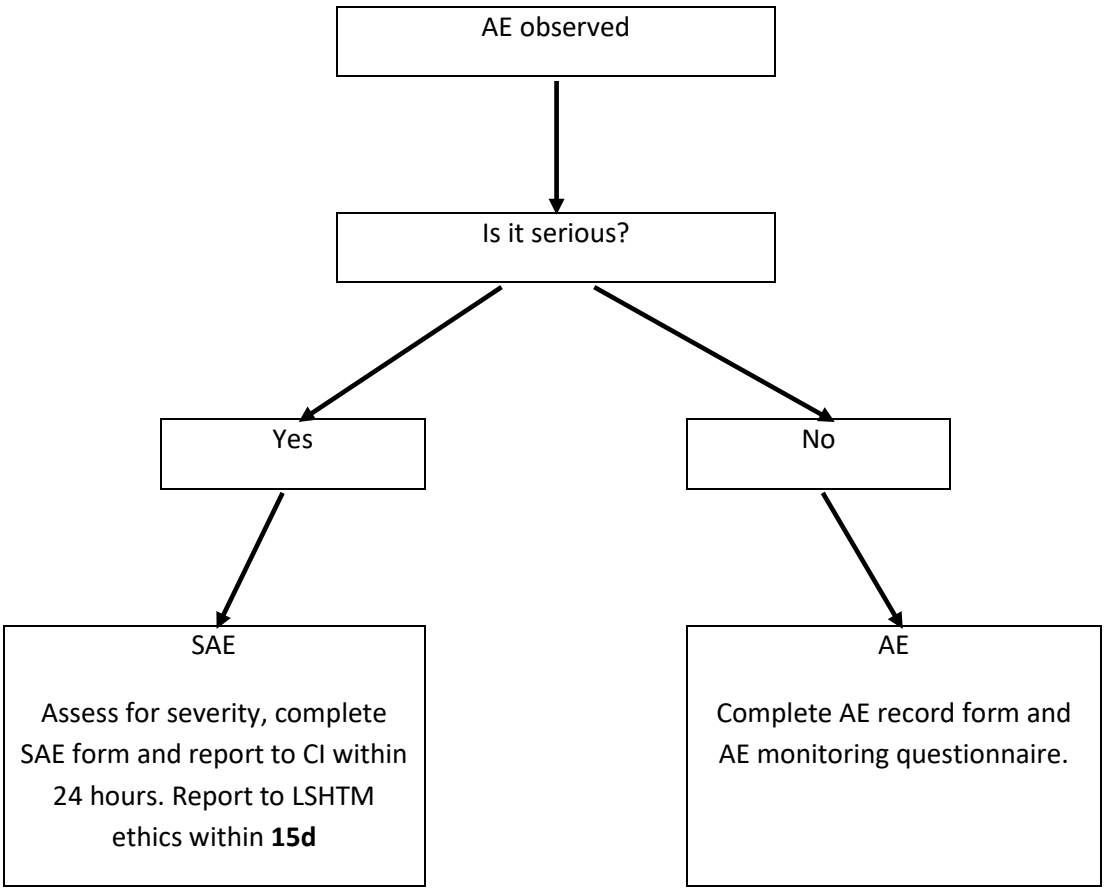
To be completed by person who experienced the Adverse Event	
Name (first name SURNAME)	
Date of birth dd/mm/yyyy	
Phone number	
Mobile number	
E-mail address	
What kind of adverse event did you experience? e.g. skin rash, burning sensation, severe allergic reaction	
How long did the adverse event last?	
How serious was the event? mild / moderate / severe / life threatening	
Did you take any action to resolve the event?	
Was any treatment required?	
Did you visit A&E? Please enter details	
Did you stay in hospital overnight? Pease enter details (no. nights/admission)	
Outcome unresolved / resolved / resolved with sequel	
To be completed by arctec trial manager	
Subject ID	
Study title	

Study code	
Type of study e.g. repellent, impregnated clothing, after-bite cream	
Exposure type e.g. chemical, mosquito bites, bed bug bites	
Exposure area e.g. forearm, legs	
Active ingredient	
Report date dd/mm/yyyy	
Other event Please enter details	
Likelihood of Adverse Event being related to study unrelated / unlikely / possible / probably / definite	
Serious Adverse Events	
Was the event serious? yes / no	
Admitted to Intensive Care Unit? yes / no	
Seriousness criteria (please tick)	
life threatening	
required hospitalisation	
prolonged hospitalisation	
congenital anomaly	

disabling/incapacitating	
important medical event	
required intervention to prevent impairment or damage	
Fatal	
If fatal, date of death dd/mm/yyyy	
Primary cause of death	
Was a post-mortem performed? yes / no	
Date adverse event become serious dd/mm/yyyy	
Possible contributing factors to SAE other than study participation or underlying disease being studied Please give details	
None apparent	
Concurrent illness, disease or other external factors	
Concurrent medication	
Study procedure	
Accident, trauma, or other external factors	
Other	
Relevant concomitant medication at time of SAE yes / no – if yes please provide details	
Treatments/procedures for SAE yes / no – if yes please provide details	

Relevant medical history (include only relevant past or concurrent medical disorder, surgeries, etc that might help explain the SAE) yes / no – if yes please provide details	
Relevant laboratory testing yes / no – if yes please provide details	
If relationship to study participation was unrelated, provide causality Please give specific details	
Discontinuation of study participation	
Concurrent disorder	
Concomitant medications	
Other	
If action taken with study participation, was study interrupted or discontinued? Provide date (dd/mm/yyyy)	
Did SAE abate after study was stopped? yes / no / not applicable / unknown	
Did SAE reoccur after reintroduction of study participation? yes / no / not applicable / unknown	
Narrative/Comments Please describe the SAE including a chronological clinical presentation and evolution of the SAE and associated signs/symptoms	
Please submit this questionnaire to Dr James Logan. SAEs that are assessed by the CI as being both related and unexpected must be reported to the LSHTM EC within 15 days of the CI becoming aware of the event.	

11.7. Appendix 7: Flowchart for Safety Reporting



11.8. Appendix 8: Data Collection Sheet for Control and Treatment Subjects.

Protocol number:	**Subject Number**		**Date**	Test Visit One
	Subject Initials		mmm/dd/yyyy	

CONTROL SUBJECT LANDING PRESSURE *(Record times in 24 h clock)*

Start of exposure time (hh:mm)	Landing time 1	Landing time 2	Landing time 3	Landing time 4	Landing time 5	Number of landings	Threshold met? (5 landings/5 minutes)	Time threshold achieved (hh:mm)	% exposure periods when threshold not met

Print Name	
Sign	
Date	

Protocol number:	Subject Number		Date	Test Visit One
	Subject Initials		mmm/dd/yyyy	

CONTROL SUBJECT LANDING PRESSURE *(Record times in 24 h clock)*

Start of exposure time (hh:mm)	Landing time 1	Landing time 2	Landing time 3	Landing time 4	Landing time 5	Number of landings	Threshold met? (5 landings/5 minutes)	Time threshold achieved (hh:mm)	% exposure periods when threshold not met

Were all mosquitos collected and labelled with participant ID and collection date? Yes ☐ No ☐

Was the test completed according to the protcol? Yes ☐ No ☐

If no, please provide a reason:

- ☐ AE (please complete AE form)
- ☐ Participant withdrawal (please complete withdrawal information on 'Completion' form)
- ☐ Other (please complete a 'Deviation' form)

Print Name	
Sign	
Date	

Protocol number:	Subject Number		Date	Test Visit One
	Subject Initials		mmm/dd/yyyy	

TREATMENT SUBJECT LANDING PRESSURE *(Record times in 24 h clock)*

Time of product application hh:mm

Time period between time of application and first field exposure hh:mm

Start of exposure time (hh:mm)	Landing time 1	Landing time 2	Number landing	End of exposure time (hh:mm)	Start of exposure time (hh:mm)	Landing time 1	Landing time 2	Number landing	End of exposure time (hh:mm)

Print Name	
Sign	
Date	

Protocol number:	Subject Number		Date	Test Visit
	Subject Initials		mmm/dd/yyyy	One

TREATMENT SUBJECT LANDING PRESSURE *(Record times in 24 h clock)*

Start of exposure time (hh:mm)	Landing time 1	Landing time 2	Number landing	End of exposure time (hh:mm)	Start of exposure time (hh:mm)	Landing time 1	Landing time 2	Number landing	End of exposure time (hh:mm)

Time period from first field exposure to first landing hh:mm

Time period from first field exposure to first comfirmed landing, or end of test hh:mm

Time period from time of test substance application to first landing hh:mm

Time period from time of test substance application to first confirmed landing hh:mm

Were all mosquitos collected and labelled with participant ID and collection date? Yes ☐ No ☐

Was the test completed according to the protcol? Yes ☐ No ☐

If no, please provide a reason:

- ☐ AE (please complete AE form)
- ☐ Participant withdrawal (please complete withdrawal information on 'Completion' form)
- ☐ Other (please complete a 'Deviation' form)

Print Name	
Sign	
Date	