OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION
WASHINGTON, D.C. 20460

MEMORANDUM

SUBJECT: Science Review of a Protocol for Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field

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REFERENCE: SAST-001 (2024). Submission of "*Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field"* March 22, 2024. Sponsored by Citrefine International Ltd. Moorfield Rd. Yeadon, Leeds. LS19 7BN U.K. MRID 523504. Protocol version 0.1 as amended March 11, 2024. IRB approved March 7, 2024. 117 p.

The study protocol dated March 22, 2024, was conditionally approved by the WCG Western Copernicus Group Institutional Review Board (IRB) on March 7, 2024. We have reviewed the referenced protocol, for a topically applied insect repellent against mosquitoes in the field from scientific and ethics perspectives.

This review assesses the scientific aspects of the proposed research for a product performance study to evaluate the efficacy of two skin-applied insect repellent spray products. The Environmental Protection Agency's (EPA) OCSPP 810.3700 Guideline, *Insect Repellents to be Applied to Human Skin* (2010) (https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0150-0011), product performance rule reference and the previous recommendations of the EPA Human Studies Review Board (HSRB) were used to evaluate the scientific merit of the proposed study. Evaluation of the ethical aspects of the proposed research are based on the standards defined by 40 CFR 26 subparts K and L and the recommendations of the HSRB.

EPA's OSCPP 810.3700 guidelines include the following topics.:

- Landing: A ***landing*** is the act of a flying or jumping insect or other arthropod alighting on human skin without probing or biting.

- Attractiveness testing landing pressure: Before the test, subjects should expose their untreated forearms to the target insects in a test cage to establish their attractiveness. Five mosquito landings in one minute or less.

- Field testing landing pressure: Field testing landing pressure of the target species is at least one mosquito landing within one minute or 5 landings in 5 minutes or less.

- First confirmed landing: One landing followed by second landing within 30 minutes.

- Complete protection time (CPT): CPT is the time from application of a repellent until efficacy failure as it is defined in each study—for example, the time from application until the first efficacy failure event confirmed within 30 minutes by a second similar event.

EPA's rule "Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests"[1] specifies testing in three genera (*Culex, Aedes,* and *Anopheles*) is required. This testing must include:

- One of the following *Culex* species: *Culex pipiens* OR *Culex quinquefasciatus* OR *Culex tarsalis*.
- AND one of the following *Aedes* species: *Aedes aegypti* OR *Aedes albopictus*.
- AND one of the following *Anopheles* species: *Anopheles albimanus* OR *Anopheles freeborni* OR *Anopheles gambiae* OR *Anopheles hermsi* OR *Anopheles punctipennis* OR *Anopheles quadrimaculatus* OR *Anopheles stephensi*.

[1] 40 CFR 158, Subpart R

A. Completeness of Protocol Submission

The submitted protocol was reviewed for completeness against the required elements listed in 40 CFR § 26.1125. The EPA's checklists are appended to this review. With EPA's recommendations addressed, the submission will be complete.

B. Summary of Scientific Aspects of the Proposed Research

This section provides a summary of the proposed research described in the materials submitted to EPA.

1. Objectives

The study (MRID 523504) is designed to assess longevity of repellency of two skin-applied repellent products at preventing mosquitoes from landing on treated human skin. The products will be tested at two field sites against mosquito species of public health relevance within the genera *Aedes*, *Anopheles*, and *Culex*, according to OCSPP 810.3700 Guidelines (Study Synopsis, p. 9 of 117, and 2. Objective, p. 15 of 117).

2. Compliance with Good Laboratory Practice Standards (GLP); 40 CFR, Part 160 and Quality Assurance

The following statements of compliance with GLP and quality Assurance is provided on p. 7 of 117 in study protocol:
"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.

"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."

3. Efficacy Endpoints and Definitions

In the Study Synopsis (p. 12 of 117), the efficacy endpoint is defined as the median Complete Protection Time (mCPT), measured as the First Confirmed Landing (FCL). The following is stated on p. 16 of 117: "*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* (p. 16 of 117)."

In other words, the efficacy endpoint is the CPT, measured by the first confirmed landing (FCL). CPT is defined as the time between product application and time point when FCL occurs. The FCL signals repellency failure or end of protection time. The FCL is defined as a landing confirmed by a second landing occurring within 30 minutes of the first. For 5-minute exposure periods occurring at 30-minute intervals, a confirmatory landing can occur as one mosquito landing on the treated leg in a 5-minute test period followed by a second landing during the same test period, or by a second landing occuring during the next test period.

4. Recruitment and Informed Consent

As described in, p. 19 of 117, recruitment will be conducted following IRB approval of amended versions. "*Subjects will be recruited from the local area, 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 (Appendix 1. Recruitment Advert). 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)."*

Recruitment will be limited to English speakers, who can read and understand the product labels. Interested volunteers will be informed of the inclusion/exclusion criteria. Those who qualify for study participation will be given a Participation Information Sheet (PIS) and schedule an appointment to meet with Study Director or research staff prior to the consent meeting. The consent meeting will take place either remotely or in-person. At the consent meeting, participants will be asked to show proper identification. They will be provided with PIS and Consent Form (included in Appendix 2 of study report). Research staff will explain the details of the study and be available to answer questions (pp. 19-20 of 117). If the participant wishes to enroll in the study, they will be asked a number of questions to ensure they have fully understood the information provided. The questions are provided in Table 2 (p. 20 of 117). The participants will be asked to sign the informed consent form if they can demonstrate their comprehension of the study procedures. Recruitment will be open until 30 consenting eligible subjects have been identified. If this group's demographics fall outside the parameters established in Table 3, p. 28 of 117 in the study protocol, 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.

5. Inclusion/Exclusion Criteria

Inclusion / exclusion criteria are listed in the Study Synopsis and pp. 21-22 of 117.

Inclusion criteria are listed as follows:

- Able and willing to give fully informed consent.
- Male or female.
- Aged 18 to 55 years old.
- 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 mosquitoes).
- 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 mosquitoes for periods of at least 5 minutes at a time.
    - Able to operate an aspirator.

Exclusion criteria are listed in study synopsis as follows:

- 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 study staff members and of the study Sponsor (Citrefine International Ltd.).
- Students of the Study Director or any other study staff members.
- 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 products or any of its ingredients, or any insect repellent products.
- Individuals who are not attractive to mosquitoes during mosquito attractiveness test.

- Individuals who have signs or symptoms related to COVID-19, have tested positive for COVID-19 within the last 10 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 10 days (subject to change, to be in line with CDC's guidance).

6. Pre-Test Activities

Prior to efficacy testing in the field, subjects undergo screening and pre-test activities, which include informed consent, a mosquito attractiveness test to determine subject's attractiveness to mosquitoes, and practice on the use of aspirators for proficiency in capturing mosquitoes before they bite. Attractiveness test and aspirator training will take approximately 2 hours. These activities will be conducted in the lab.

Attractiveness Test. This test will be conducted using arm-in-cage as specified in OCSSPP 810.3700 guidelines. The test will be supervised by study staff. If the subject fails to receive 5 landings in 1 minute, the test will be repeated two more times with a new batch of pathogen-free mosquitoes that never have had a blood meal. If the subject still fails to achieve the threshold of landing rate (5 landings in 1 minute), he/she will be disqualified for participation in the study. Mosquitoes will be tested prior to the test to confirm absence of mosquito-borne pathogens. Refer to p. 26 of 117.

Aspirator training. Subjects who prove to be attractive to mosquitoes will be trained in the use of aspirators for capturing mosquitoes before they probe or bite. The training will be conducted in a screened free-flight cage using pathogen-free colony-reared mosquitoes that have never had a blood meal. Mosquitoes will be tested prior to the test to confirm absence of mosquito-borne pathogens. Subjects will be paired with a partner and instructed to dress as for test days with long sleeved shirts and pants, gloves, and disposable head net. Pairs will practice for no longer than an hour until they skillfully aspirate mosquitoes from their partner's leg. If proficiency is not achieved after one hour, the subject will be disqualified for participating in the study. Refer to p. 26 of 117.

Measurement of Skin Surface Area and Individual Dose of Application. These measurements will be conducted in the lab. Skin surface of the left and right lower leg of each test subject will be calculated by multiplying the length from knee to ankle and the average circumference of the leg. The leg circumference will be measured from 2 equidistant points. The amount of product applied to each subject will be adjusted to their lower legs' surface area and calculated by dividing the area of the lower leg by 600 sq. cm and multiplying the result by 1 gram for a target dose of 1.67 mg/cm^2. Refer to p. 27 of 117.

7. Field Sites Qualifications and Monitoring

Field sites will be selected based on the presence of target mosquito species and absence of mosquito-borne pathogens within 25 miles of the proposed sites in the 4 weeks preceding field test initiation. Potential sites will be monitored for 4 weeks prior to test initiation using DC

Gravid Traps, CDC Light Traps baited with carbon dioxide, and/or BGS trap to document mosquito diversity, abundance, and activity. Closer to test days, temporal distribution of mosquito species will be assessed using BGS traps equipped with counters as a way to assess mosquito activity during the day (p. 28-29 of 117). In addition, the Study Director will coordinate with the local health department and mosquito control districts at least weekly for 2 months before field testing begins to confirm the absence of reported mosquito-borne disease cases in humans within 25 miles of planned test site, and again a week before each test day is conducted (p. 29 of 117).

8. Pathogen Screening

Trapped mosquitoes captured during the site monitoring period, approximately 4 weeks prior to the field tests, will be screened for pathogens, based on genera. *"Mosquitoes will be tested in pools of up to 50 specimens for pathogens using PCR. Culex will be tested for WNV, SLEV and EEEV. Aedes mosquitoes will be tested for EEEV and ZIKV. Aedes will be tested for chikungunya and/or dengue if there is an identified threat."* (p. 29 of 117).

9. Subject Selection and Randomization

Twenty subjects will be randomly selected from a pool of 30 informed, consenting, and eligible volunteers who are representative of the demographics of the general population as established in Table 3, p. 28 of 117, in the study protocol. Of the 20 randomly selected subjects, 13 will be randomly assigned as treated subjects, 2 as controls, and the remaining 5 as alternates on the day of efficacy testing. Each product will be tested at both field sites on 2 separate days, one day per site. "Subjects will be able to take part in one or both test days. Therefore, a minimum of 20, and a maximum of 80 subjects will be required to complete the study." (p. 35 of 117).

10. Study Design

The experimental design is a Latin square design, described in Appendix 4 of study protocol. Nine collection stations ('A'- 'I') will be set up at the field site, with a minimum distance of 3 meters between each station. Paired test subjects will be randomly assigned to the collection stations as described below.

The randomization procedure is described in p. 30 of 117. By order of arrival, the first seven males and first seven females to arrive to the test facility, plus the 8th male or female, will be assigned as test subjects on repellency testing day. The remaining subjects arriving later will be assigned as alternates. The 15 test subjects will be randomized further as either 13 treated subjects or 2 control subjects. Application of treatment to either left or right leg of treated subjects will also be randomized (pp. 30-31 of 117; refer to Table 4 for an example of STATA randomized data set). The gender balance will be maintained by having at least 6 volunteers of each sex serve as test subjects and a 7th treated subject of either sex. Treated subjects will be paired and randomly assigned to the nine collection stations according to a randomization

procedure described in p. 32 of 117 and tabulated in Table 5 on p. 33 of 117. The 2 controls will not be paired together, but rather each control subject will be randomly paired with one staff member. Likewise, the unpaired treated subject will be randomly paired with a staff member. Study staff will perform counts but will not expose their own legs.

11. Compliance Check

Subjects will be contacted within 48 to 72 hours prior to test day for verification of their health and compliance with test conditions. Subjects will be reminded not to use insect repellents and scented soaps, shampoo, deodorant, perfumes, or cosmetics, drink alcohol, smoke, or chew tobacco, or engage in vigorous exercise 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. In addition, subjects will be asked to arrive at the test facility at a specific time, approximately 3 hours ahead of efficacy test initiation to allow time for registration, randomization, product application, and travel to test site.

12. Field Testing Day

On test day and upon arrival to the test facility, subjects will be checked for eligibility and for their compliance with test conditions. Subjects will also be reminded that they can withdraw at any time and request that their data not be used without affecting their remuneration.

Application of Standard Dose. Prior to product application, the lower leg of treated and control subjects will be washed with unscented soap and water and rinsed with a water solution of 70% isopropyl alcohol. The application procedure is described in p. 31 of 117 in the study protocol. A beaker will be weighed, and its weight will be recorded. The previously calculated and recorded individual dose per subject will be weighed by spraying an aliquot of the repellent into the beaker and placing the beaker on a balance. "*The specific gravity of the formulation will be used to convert from weight to volume (mg to ml). The volume of repellent will be measured using a micropipette and applied evenly over the lower leg from the ankle to the knee using a single gloved finger to ensure uniform coverage. If the product is a viscus liquid: the product will be removed from the beaker using a spatula until the required weight is achieved either on the spatula or in the beaker. The product will then be applied evenly over the lower leg from the ankle to the knee using a single gloved finger to ensure uniform coverage. The spatula/beaker will be reweighed until it contains less than 0.05 g product. The excess product will be weighed and recorded as "disposed" in the product accountability log. The weight of the glove before and after testing will be recorded*." (p. 31 of 117). Subjects will be instructed not to disturb the product after being applied.

Exposure Delay and Sequence of Exposures. Exposure delay is proposed to be 1 hour from product application. "*Human landing collection*" or 5-minute exposure periods will begin 1 hour after product application. The mosquito landing pressure will be determined before the treated volunteers expose their legs. First, 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. Once both control subjects have received 5 landings, and treated subjects will expose their treated skin for a 5-minute period at 30-minute intervals. This sequence of exposures will be repeated for each control and treated subject until he/she experiences FCL or reaches the end of the test day, whatever happens sooner. The time of the landings on control and treated subjects, and when the threshold number of 5 landings occur on control subjects will be recorded under the supervision of a staff member (p. 33 of 117). Mosquitoes landing on test subjects will be collected for taxonomical identification and pathogen screening (p. 33 of 117).

13. Criteria for Skipping Exposure Periods and Ending Testing

"*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."* (p. 33 of 117).

"If there are 4 non-consecutive exposure periods missed due to bad weather the test day should be stopped and testing re-arranged. If 3 consecutive periods are missed due to bad weather then, the test day should be stopped and testing re-arranged." (p. 34 of 117).

14. Criteria for Determination of CPT

"*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*." (p. 33 of 117). In the case of missing exposure periods, "*[i]f 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*" (p. 34 of 117).

15. Criteria for Use of Right Censored Data and Ending Test

"*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*" (p. 36 of 117).

Testing will end when more than half of the test subjects experience FCL. "*If treatment failure is not reached in more than half of subjects, the test will continue until 6 hours for 10-15% OLE aerosol or 10 hours for 30% OLE aerosol post application. 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*." (p. 34 of 117).

16. Determination of Sample Size and Statistical Analysis

The sample size of 13 treated subjects is employed according to EPA recommendations for sample size *(*Appendix 5: Sample Size Calculation; p. 47 of 117 in study protocol). "*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. 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*" (p. 35 of 117 in study protocol).

The mCPT from a sample of 13 subjects will be calculated using the Kaplan-Meier survival analysis. "*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*" (p. 36 of 117 in study protocol).

17. Toxicity Profile of Test Items and MOE Calculations

Subjects will be exposed to insect repellent products that are already registered with the EPA. The protocol submission did not include the complete Tier I mammalian toxicity data for the two end-product formulations and the mammalian toxicity profile of the active ingredient.

C. EPA Science Comments and Recommendations

If conducted according to the protocol with EPA's recommendations listed below addressed, this research is likely to result in scientifically valid research that conforms to the OPPTS 810.3700 Guideline. EPA has already discussed the recommendations with the sponsor, and the sponsor is amenable to the making the suggested changes.

EPA recommends that the study protocol be revised to address the following recommendations:

1. A more detailed plan for site monitoring is recommended. The protocol should include the proposed number of traps per site, distance between the traps, distribution, trapping

period, and trap collection frequency. In addition, the protocol should describe the proposed methodology for species identification in detail. The protocol should specify that abundance and distribution of mosquito species at each site will be reported in the study report.

2. It is recommended to include location of proposed field sites and ensure that the distance between sites is greater than the flight distance of mosquito species encountered at the site(s). For example, the reported flight range of some *Culex, Aedes,* and *Anopheles* species are ≥ 15 km (Verdonschot and Besse-Lototskaya, 2014); therefore, the distance between field sites should not be less than 15 km. Also, the habitats chosen should be distinct ecological areas per guideline recommendations. In the proposed repellent guidelines, we recommend at least two distinct habitats (e.g. forest, grassland, salt marsh, wetland, beach, or urban environments) where the predominant mosquito species differ.

3. Samples of raw data sheets for recording mosquito attractiveness data should be appended to the study protocol. Similarly, results of aspirator training should also be recorded and reported.

4. A sample of data sheets for difference in weight of finger cots before and after product application should be appended to study protocol.

5. The proposed label should be appended to the study protocol for review of label efficacy claims to be supported with data.

6. The criterion for determining proficiency/competence in the use of aspirators for catching landing mosquitoes should be established and included in the protocol.

7. Mosquito rearing procedure and maintenance condition for the lab experiment needs to be explained.

8. Specific procedures that will be employed to ensure that mosquitoes used in the lab for assessing subject's attractiveness and aspirator training are pathogen free, should be described in the protocol. In addition, the protocol should specify that mosquitoes used in lab testing are unfed females.

9. In section 6.5, it is mentioned that "the study director will make a documented decision… whether stratified selection of test subjects will be required". The applicant needs to explain how stratified selection will be performed while still maintaining randomness.

10. The statement, "…*test the products against natural populations of mosquito species of public health importance within the genera Aedes, Anopheles, and Culex*" should be amended throughout the document and replaced with the specific representative mosquito species required for testing as per Efficacy Testing for Pesticides Targeting Certain Invertebrate Pests | US EPA. Testing in three genera (*Culex, Aedes,* and *Anopheles*) of

mosquitoes is required. One species should be *Culex*: *Culex pipiens* OR *Culex quinquefasciatus* OR *Culex tarsalis*. Other one should be *Aedes* species: *Aedes aegypti* OR *Aedes albopictus*. The third one should be one of the following *Anopheles* species: *Anopheles albimanus* OR *Anopheles freeborni* OR *Anopheles gambiae* OR *Anopheles hermsi* OR *Anopheles punctipennis* OR *Anopheles quadrimaculatus* OR *Anopheles stephensi*.

11. According to protocol, subjects who withdraw or are removed before the ending of the test will not be replaced. The applicant should consider the adequacy of replacing subjects into testing who withdraw thus to minimize right censoring and avoid reducing sample size.

12. The concentration of active ingredient in one of the products is given as range from 10-15 % OLE. The specific concentration of active ingredient should be reported in the study protocol.

13. The endpoint and the definition of CPT is inconsistent and incorrect. The endpoint is not the mCPT but the CPT, measured by the FCL. Please address this is in the study synopsis, p. 12 of 117, and p. 16 of 117.

14. The protocol needs to be amended to include the formula to convert from weight to volume on p. 31 of 117.

15. The number of trained technicians applying the test substances to subject's legs needs to be disclosed.

16. Transportation of the subjects to the field site and product application before the start of the study needs to be explained in detail. Information is needed to describe whether the product is being applied before they reach the test sites, who is applying the products, and how untreated control subjects will be transported. Section 6.7.6 needs more detail.

17. The protocol should include results from Tier I mammalian toxicity data for each product formulation. This information on human health data is important concerning the test material to which test subjects will be exposed.

18. The MOE calculation for each proposed formulation must be addressed in the protocol. The Agency has the following information concerning risk assessment of OLE that should be added to the protocol:

    "*The risk assessment for OLE is based on the EPA risk assessment for p-Menthane-3,8-diol (PMD), which is the active component in OLE. Citriodiol contains 65% PMD. A 90-Day dermal study in rats (MRID 444387-10) tested PMD (98.3 % pure) at increasing doses, 0, 1,000 and 3,000 mg/kg/day. The NOAEL = 1,000 mg/kg/day, and the LOAEL = 3,000 mg/kg/day. The endpoints for NOAEL and LOAEL are based on treated skin observations, erythema, edema, eschar, and histological observations in treated skin, increased acanthosis, and inflammation at the highest dose of 3,000 mg/kg/day. No dermal absorption data are*

required for Tier I Toxicity data for registration of biochemical products therefore, without these data, dermal absorption is assumed to be 100%. Risk characterization for infants and children is based on data from one developmental study (MRID 444387-11) in which the NOAEL =3,000 mg/kg/day. No LOAEL was established, and thus, a 10-fold safety factor is applied for risk characterization. Based on this information, MOEs are not calculated because there are no endpoints of concern for the dermal route of exposure. The Agency concluded that there is reasonable certainty of no harm to populations or subpopulation (infants and children) from the use of PMD in insect repellent products applied to human skin."

19. In the protocol notes that the test subjects will be paired with each other, except for the odd number (13th subject) who will be paired with a staff member. However, it is not clear what happens to the partner of one treated subject who gets FCL or withdraws before end of testing. It is unclear whether the partner of this subject will be paired with a staff member to continue testing. This needs clarification in the protocol.

20. It is mentioned in section 6.7.8 that "Human landing collections will start 1 hour after product application". It is not clear if this first hour will be counted as repellent efficacy time since there are no mosquitoes present during this time. Also, it is mentioned that "If a first confirmed landing occurs within the first three exposure periods, the CPT will be counted as 0 hours". Explanation needs to be provided if this account for the first hour of no exposure.

D. Ethics Review

Here is a summary of the EPA's observations about the ethical aspects of the proposed research. Attachment 1 provides supporting details and a point-by-point evaluation of this protocol.

1. Societal Value of Proposed Research

This study is designed to determine the efficacy of two topically-applied mosquito repellents, which contain OLE. The aim of this research is to provide data resulting from this proposed study will be used to support registration of a skin-applied repellent by EPA.

Efficacy at preventing mosquitoes from landing on each subject will be expressed as CPT, which is defined as the time between application of the repellent product and the occurrence of the first mosquito landing on the treated skin followed by a second landing within 30 minutes. The CPT data from each subject will be combined and analyzed to determine a median CPT (mCPT), which will be used to develop product labeling. Research with human subjects is justified because sufficiently reliable non-human methods for testing the efficacy of topically-applied repellents have not been developed.

The research has societal value because people are at risk of contracting mosquito-borne diseases, and such risks can be mitigated by the use of insect repellent products. The testing will include natural populations of mosquito species of public health importance, which can transmit diseases to humans. Registration of these products, if effective, would offer consumers a product that would repel mosquitoes.

2. Subject Selection

The protocol calls for testing each product with 13 subjects, with an approximately equal number of males and females. An additional two individuals will participate in the testing as untreated controls, monitoring mosquito landing pressure immediately prior to each exposure period. In addition, five subjects will be enrolled as alternates, to take the place of any test subjects who withdraw before or on the day of testing (at least two subjects of each gender). A total of 20 individuals (13 test subjects, 2 untreated controls, and 5 alternates) will be selected to test each product on each test day. Therefore, a total of 80 subjects would be needed assuming each individual participates only in a single test day with a single product.

The protocol notes that testing locations will be in the United States and will be selected based on preliminary trapping data and information from local monitoring programs. The sites will be selected based on the presence of mosquito species of public health importance, as well as data showing consistent mosquito pressure throughout the testing duration.

Regardless of the testing location or timing, similar recruitment procedures will be followed. Recruitment will be conducted in the local area to the test site using traditional, digital, and social media. The protocol notes that all study-related recruitment and activities will be conducted in English only. Advertisements will provide basic information about the study, and an email address and phone number where interested candidates can get more information. The Study Director or member of the research team will contact interested individuals by email or phone. Candidates will receive more information about the study and will be asked to screen themselves against the most common exclusion criteria.

The results of testing this product should be as generalizable as possible to the target population of skin-applied insect repellent users. Every effort will be made to achieve an appropriate demographic composition of the pool of recruited and enrolled subjects. The protocol includes a table of demographics to consider in recruitment. The Study Director will review the demographics of the 30 individuals who consent to participate and determine whether additional recruitment is necessary or whether stratified selection of test subjects will occur. The final study report will include demographic information about the subjects who participated, based on age and gender, due to availability of test subjects on each test day. Recruitment will be open until at least 30 individuals who meet the criteria to consent and participate in the study have been identified.

Prior to field testing, subjects will participate in a mosquito attractiveness test and training on how to use an aspirator. To verify subjects' attractiveness to mosquitoes, they will place an arm

into a 45 cm x 45 cm x 45 cm cage with 78 female, non-bloodfed *Aedes aegypti* mosquitoes (density of 1 mosquito per 1,160 cm3). The forearm will be untreated and exposed to the mosquitoes. Mosquitoes who land during this attractiveness assessment will not have a chance to bite the candidates; they will be blown off or captured by study staff. Candidates must receive 5 landings in a 1-minute period in order to qualify as attractive to mosquitoes and to continue enrolling in the study. Candidates can perform this test up to 3 times with a new batch of 78 mosquitoes during each attempt, at which point they are disqualified from continuing if they have not received 5 mosquito landings in 1 minute during any of the trials.

Following the attractiveness assessment, subjects will be trained to aspirate mosquitoes in a screened free-flight cage populated with non-bloodfed female *Aedes aegypti* mosquitoes. For this training, subjects will dress as if they are going into the field for testing, wearing long-sleeved shirts, pants, gloves, and head net. Research staff will train subjects on how to watch mosquitoes approach and land, and how to quickly remove landing mosquitoes. Subjects will be paired and asked to aspirate mosquitoes that land on their partner's pant leg. Training will continue until subjects demonstrate that they can successfully aspirate a mosquito from a partner's leg before it begins to probe. If the candidate is not successful after 1 hour of training and practice, they will be withdrawn from the study.

3. Informed Consent

During the recruitment period, interested candidates will contact study staff via phone or email to learn more about the study and to self-evaluate whether they meet the eligibility criteria. Those who are interested in continuing with enrollment will be invited to meet with the study staff remotely or in a private location and will receive a participant information sheet to review prior to the meeting. When the session begins, the candidate will be asked to provide documentation to verify their name and age. Additionally, they will be provided with a copy of the consent form and asked to read it before the discussion begins. The study staff will provide information about the study orally and to describe the elements of study participation step by step. Female candidates will be informed of the pregnancy testing requirements. Candidates will be reminded that they are not obligated to consent to enroll and that they are free to withdraw from participation at any time without penalty, as well as how the subjects' privacy will be protected. The candidates will have an opportunity to ask questions of the study staff conducting the session. After all questions are answered, the candidate will answer a series of questions to demonstrate that they comprehend the study procedures. Those who the research determines have an adequate comprehension of the materials and proposed study will be invited to complete the consent process. All individuals will be provided a signed copy of their consent form.

With the EPA's comments addressed, the protocol will contain a complete and satisfactory description of the process by which potential subjects will be recruited, informed, and trained in preparation for the test day, and the process for seeking subjects' consent to participate. A copy of the IRB-approved consent document is included in the materials provided to the HSRB.

4. Risks to Subjects

The protocol discusses potential hazards associated with these tests including risks from exposure to the test material, biting mosquitoes, and vector-borne pathogens; risk of contracting SARS-CoV-2; physical risks from being outside during the test day; potential reaction to the test substances; unanticipated loss of confidential information; and psychological risks related to pregnancy testing.

To mitigate risks from exposure to the test material, the test product uses materials that have been evaluated by EPA for toxicity and found to have low acute risk profiles. Individuals have had allergic reactions to insect repellents and cosmetics in the past will be excluded from participation. Subjects with a history of or who are suffering from rashes or other skin conditions that could be exacerbated by exposure to the test substance are also excluded from participation. Any subject who shows signs of adverse reactions to the test substance during the course of the study will be removed from participation.

To mitigate risks from exposure to mosquitoes and disease vectors, subjects will be trained to aspirate landing mosquitoes before they probe or bite. During the attractiveness testing and aspirator training, the mosquitoes used will be non-bloodfed and will be screened for pathogens prior to their use in the study. All mosquitoes used during the attractiveness testing and aspirator training will be used for one subject encounter and destroyed. For control subjects, aspiration during field testing will be done by a member of the research team. The protocol identifies the potential vector-borne diseases to which subjects could be exposed. The field testing sites will be monitored weekly for a month prior to the testing for relevant vector-borne viruses, and mosquitoes captured during each week of the monitoring phase will be pooled and tested for pathogens. All mosquitoes captured during the field trials will be tested for relevant pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified by the local health authorities. The absence of reported mosquito-borne disease cases in humans within 25 miles of the test site for the 4 weeks preceding the test day will be necessary for the field trial to be carried out.

To minimize the discomfort associated with mosquito bites, candidates known to be sensitive to or phobic of mosquito bites will be excluded. Topical antihistamines will be available to subjects at the end of the test day at no charge. In addition, participants will be instructed to wear clothing that covers their bodies, and will be provided with gloves and a head net to wear during any period when they will be exposed to mosquitoes. Only the area to be treated with the repellent will be exposed to mosquitoes during the test period. In addition, untreated control subjects will only expose their lower leg until the requisite number of mosquito landings have been observed or for up to five minutes for each period during the testing.

To protect subjects against the physical risks associated with the test environment, subjects will be protected from biting insects while not actively participating in a test period. Each field site will have a large, screened shelter, along with fans. Subjects will have access to food and beverages, and restrooms. The Study Director will provide water, other drinks, and snacks. A

cooler will be available for subjects to store meals and snacks that they bring to the field trial. Individuals who are in poor physical health, which could make the challenges of participating in a test day in an outdoor environment will be excluded during the screening process. A certified first aider will be present during training and testing to provide emergency assistance if required.

To ensure that no subject experiences adverse effects and continues to participate, subjects will only be eligible to participate in additional test days after 72 hours elapse from the prior test day to allow them to recover and to identify any adverse effects that could have been associated with their participation. The research team will follow up with all subjects 48-72 hours after each test day.

Pregnancy testing will be conducted in private and only a single female member of the research team will discuss the results with the subject. Only women of childbearing potential will participate in the pregnancy testing. The protocol describes the process for a subject to self-certify that they are not considered to be of childbearing potential and to exempt themselves from the pregnancy testing process.

Practical steps to minimize subject risks have been described in the protocol, and the remaining risks have a low probability of occurrence.

5. Benefits

This research offers no benefits to subjects. Depending on the results of the research, it may benefit society by generating reliable repellency efficacy data that could be used by the EPA to register insect repellent products containing OLE. Registration of effective repellent products could lead to fewer mosquito bites and reduced incidents of vector-borne illnesses.

6. Risk/Benefit Balance

The protocol describes measures to minimize risk to subjects while maintaining the robustness of the scientific design. With to the risk mitigation measures put in place and the EPA's comments addressed, the residual risk to subjects is low and reasonable in light of the potential benefits of the data to society.

7. Independent Ethics Review

WIRB reviewed and approved the protocol, informed consent form, and eligibility questionnaire. The IRB is registered with the Office of Human Research Protections (IRB00000533) and the organization holds a Federal-wide assurance (FWA00033319). Satisfactory documentation of the IRB procedures and membership is on file with the Agency. Documentation regarding IRB approval has been provided to the HSRB members with the background materials for this review.

8. Respect for Subjects

The subjects' identities will be protected as follows: each subject will be assigned a code number/identifier. The study records will be maintained in locked cabinets, and electronic files kept on a password-protected computer server or encrypted electronic storage devices. Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing.

Throughout the recruitment and consent processes, and again at the start of each test day, candidates and subjects will be informed that they are free to decline to participate or to withdraw at any time for any reason.

The protocol notes that subjects will be compensated for their time spent participating in the study as follows: $10 for the participating in the consent meeting, $40 for participating in the attractiveness testing and aspirator training session, and the minimum wage in the state where the field test is conducted for taking part in the field test, rounded up to the next hour. The timing for this payment is from the time the subject registers at the start of the test day until the product failure or end of testing. Alternates are expected to stay at the test site for up to 2 hours and will be compensated $20 for their time if they are not needed to replace a test subject.

Breaks for subjects between exposures and provision of snacks and drinks have been incorporated into the study design.

Any expenses for injury or illness incurred as a result of study participation will be paid by the study sponsor.

E. Compliance with Applicable Ethics Standards

This is a protocol for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to the EPA under the pesticide laws. The primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply. A point-by-point evaluation of how this protocol addresses the requirements of 40 CFR 26 Subparts K and L and the criteria recommended by the HSRB is appended to this review as Attachments 1-4.

With the EPA's comments on the consent form and protocol addressed, the consent materials and process will meet the requirements of 40 CFR 26.1116 and 26.1117. With the protocol and all associated materials revised according to recommendations from the EPA and the HSRB and approved by WIRB, the research will likely meet the applicable requirements of 40 CFR part 26, Subparts K and L.

F. EPA Ethics Comments and Recommendations

The EPA notified the study team that before the research is conducted the protocol and supporting documents should be revised to address the EPA's comments and recommendations resulting from the review by the HSRB. After all necessary changes have been made, the revised protocol and supporting documents must be resubmitted for review and approval to the overseeing IRB prior to initiating the research.

The EPA's ethics comments are provided below. Minor comments on typographical errors have not been included here.

Protocol

1. Ensure that the recruitment and enrollment reflect the principles of justice, including equity and balanced enrollment. The protocol notes that "Current repellent product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitoes. 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." Regardless of the language on labeling, it is important to discuss the equitable selection of subjects. This is research with no direct benefits to subjects, so there is no significant unfair sharing of the burdens or the benefits of the research. However, excluding those who do not speak English denies fair access to participation in this research to those individuals. Justice and equity mean that "research should be open to members of populations that will be affected by the application of the knowledge gained (e.g., policy and program development)".[2]

2. Provide information on the aspirators to be used. Will they be mechanical or manual?

3. Clarify the protocol to indicate that for each control subject landing pressure assessment period, both of the control subjects must receive 5 landings within 5 minutes for the landing pressure to be considered adequate.

4. To ensure that subjects do not have an active skin condition that would disqualify them from participating in the study, consider revising the protocol to include a check of the skin by the first aid person present at the test site upon the subjects' arrival.

5. The protocol should indicate that if the two sites are geographically distinct, two different pools of at least 30 participants will be enrolled.

[2] Office of Human Research Protections. Consideration of the Principle of Justice under 45 CFR part 46. Secretary's Advisory Committee on Human Research Protections. July 22, 2021. https://www.hhs.gov/ohrp/sachrp-committee/recommendations/attachment-a-consideration-of-the-principle-of-justice-45-cfr-46.html

6. Although the EPA's guidelines recommend that subjects are between 18 and 55, feedback from the HSRB and discussions of previous protocols have changed the recommendation to remove upper age limit or provide rationale for excluding subjects over 55 years old. Please revise the protocol to address.

7. Provide details in the protocol about who will serve as the study's medical monitor and the individual's qualifications.

8. As required by EPA's Human Studies Rule, before the initiation of the study provide EPA with the email template, phone script, advertisements and any other documents that will be used at any point during subject recruitment and consent phases.

9. Clarify the compensation for subjects. The protocol notes that the compensation period for a test day ends at product failure or the end of the test day. If subjects have been transported to the field site by the study team and are not free to leave, their compensation period should extend until they are returned to the study site.

10. Generally, the EPA recommends that the Study Director have limited discretion to withdraw subjects from the study as outlined in the protocol. For example, such discretion could be characterized as: "*Participants' enrollment in the study may be ended at the discretion of the Study Director where continued participation may affect the safety of the participant or where there is a development of any condition that might interfere with study participation.*" EPA recommends reconsidering the inclusion of the general stopping condition if the Study Director believes it's in the best interest of the study data and the permission for the Study Director to withdraw a subject at any time and for any reason. More specific, limited conditions should be specified in the protocol.

11. There is a discrepancy related to the use of withdrawn subjects' data. The protocol notes in the section on withdrawal criteria that "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" (p. 22 of 117). Under the statistical methods section, the protocol states "Participants that withdraw during the test day will have their data included in the statistical analysis as right censored data" (p. 36 of 117), which would depend on the subject not withdrawing consent for their data to be used in the study. Revise the protocol to reflect a consistent approach to handling the data of subjects who withdraw, which should respect the autonomy of subjects.

12. Describe how subjects who choose to take the study-provided transportation will be returned from the field to the test center. Subjects should be informed in advance (i.e., during the consent process) whether transportation will only be provided at the start and end of the test period. Also describe how subjects who experience failure before the end of the full test day will be handled, e.g., will they remain with the

group until all subjects experience product failure, will they be returned to the test facility, or will they be free to leave in their own transportation from the test site.

13. Revise section 9.2 to include the following language: "*Proposed changes deemed necessary to eliminate apparent immediate hazards to the human subjects may be implemented without prior IRB approval; these must be reported to the IRB within the timeframe specified in the IRB's written policies and procedures, within 5 days. Any deviation to the protocol related to subject encounters will be reported to the IRB. Documentation of deviations will include a description of the deviations, the reasons for the deviations, and the effect of the deviations on the outcome of the study*."

Consent form

14. The consent form must be revised to reflect all changes made to the protocol.

15. The consent form should be revised to match the protocol in terms of the formulations of the two products being tested (i.e., aerosol, lotion, or pump spray).

16. Revise the consent form to include the provision for female subjects to self-certify if they are not considered to be of child-bearing potential.

17. The consent form should match the protocol in indicating that subjects will receive the minimum wage in the state where the research is being conducted. Alternatively, you can get IRB approval of different versions of the consent form reflecting the minimum wage at the field test location prior to enrolling subjects.

18. The consent form needs to include one of the following statements about any research that involves the collection of identifiable private information or identifiable biospecimens: (i) a statement that identifiers might be removed from the identifiable private information or identifiable biospecimens and that, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from the subject, if this might be a possibility; or (ii) a statement that the subject's information or biospecimens collected as part of the research, even if identifiers are removed, will not be used or distributed for future research studies.

Attachments:

1. EPA Protocol Review
2. Checklist General Requirements for Informed Consent § 26.1116
3. Checklist Documentation of informed consent § 26.1117
4. Submission of Proposed Human Research for EPA review 40 CFR § 26.1125
5. Basis for EPA Standard Dose in Skin-Applied Repellent Testing

Attachment 1
EPA Protocol Review

EPA Protocol Review

Title: Citrefine International Limited, (2024). Study Protocol for "*Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field*." p. 1-117. MRID 523504. Protocol version 0.1 as amended March 11, 2024. IRB approved March 7, 2024

Submission Date: March 22, 2024

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Sponsor: Citrefine International Limited
Moorfield Rd. Yeadon, Leeds LS19 7BN U.K.

IRB: Institutional Review Board (IRB): Western Copernicus Group Institutional Review Board (WCG IRB).

1. Societal Value of Proposed Research

(a) What is the stated purpose of the proposed research?

EPA requires efficacy testing of products claiming efficacy against disease vectors to support registration and efficacy claims on product labels. This study is designed to determine the complete protection time (CPT) of 2 skin-applied aerosol formulations intended to repel mosquitoes. "*The aim of this study is to provide longevity and efficacy data for two topically applied insect repellents*" (p. 15 of 117 in study protocol).

(b) What research question does it address? Why is this question important? Would the research fill an important gap in understanding?

Lasting efficacy of the newly proposed formulations for repelling mosquitoes does not currently exist. The study is designed to estimate the duration of repellency of two skin-applied mosquito repellents containing oil of lemon eucalyptus (OLE) (CAS: 1245629-80-4) (Citriodiol) as active ingredient at concentrations of 10-15% OLE or 30% OLE. Longevity of repellency will be tested at 2 distinct mosquito habitats, where different mosquito species of public health significance predominate. The products will be tested at the EPA standard dose of 1 g/600 cm^2 on a sample of 13 human subjects.

(c) How would the study be used by EPA?

EPA will consider the study to satisfy product specific efficacy data requirements for use in support of efficacy claims against mosquitoes on the repellent product label.

(d) Could the research question be answered with existing data? If so, how? If not, why not?

EPA requires product-specific efficacy data to support product registration. No previous testing of these formulations against mosquitoes under the proposed use pattern has been conducted.

(e) Could the question be answered without newly exposing human subjects? If so, how? If not, why not?

Human subjects are required because they represent the target system for the test material, and sufficiently reliable non-human models for repellency testing have not been developed. "*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 mosquitoes, 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 mosquitoes 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"* (p. 18 of 117 in study protocol).

2. Study Design

(a) What is the scientific objective of the study? If there is an explicit hypothesis, what is it?

The aim of this study is to determine the duration of repellency of 2 mosquito repellents, one containing 30 % OLE and the other 10-15% OLE as aerosol formulation as the active ingredient against field mosquitoes within the genera *Aedes, Anopheles* and *Culex* at an application rate of 1g/600cm^2 (1.67mg/cm^2) (p. 25 of 117 in study protocol). "*The study will be conducted in line with EPA Product Performance Test Guidelines* [OCSPP 810.3700, Insect Repellents to be Applied to Human Skin]. *The proposed study is intended to test the products against natural populations of mosquito species of public health importance within the genera Aedes, Anopheles, and Culex, across two field sites in the United States (US)*" (p. 9 of 117 in study protocol).

(b) Can the study as proposed achieve that objective or test this hypothesis?

The objective may be achieved by the study as proposed if the protocol is revised and amended according to EPA recommendations on the scientific and ethical aspects of the study protocol.

2.1 Statistical Design

(a) What is the rationale for the choice of sample size?

The sample size of 13 subjects is in accordance with the EPA's recommended sample size of 13 test subjects for testing repellency against mosquitoes. The rationale is that a sample size of 13 human subjects "*with a minimum of six of either sex*" (p. 15 of 117 in study protocol) "*allows for over 90% power at a range of expected median CPTs from 2-8 hours (Appendix 5: Sample size calculation)"* (p. 35 of 117 in study report). This sample size would be adequate to ensure that the study includes enough subjects to return reliable results without including more subjects than necessary.

(b) What negative and positive controls are proposed? Are proposed controls appropriate for the study design and statistical analysis plan?

Two untreated subjects will monitor mosquito landing rate at the field to ensure adequate mosquito landing pressure throughout the test. A positive control will not be used.

(c) How is the study blinded?

The study is not blinded. Two products will be tested once, each on separate days, and observations are based on frequency of mosquito landings over time.

(d) What is the plan for allocating individuals to treatment or control groups?

Twenty out of 30 informed and consenting eligible subjects will participate in the study either as test subjects or controls or alternates [3 treated subjects (6 of one sex and 7 of the other); 2 controls (1 male and 1 female), and 5 alternates]. The first 15 participants (7 males and 7 females plus an 8th male or female participant) arriving at test facility will be assigned as test subjects. This group of 15 test subjects will be randomly assigned as either treated or control subjects on the day of field testing. The remaining 5 subjects will be assigned as alternates for replacing subjects that withdraw prior to test initiation. A randomization list will be created for generating a random allocation of six or seven treatments and a single control to the list of male and female participants (Figure 2 on p. 30 of 117). It will also be used to assign the treatment to the right or left leg (Table 4 on p.31 of 117; p. 30 of 117 in study protocol). In addition, 9 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 randomly assigned to the collection stations according to a randomization procedure described in p. 32 of 117 of study protocol. The pairs will not change throughout the test day. Test pairings will be randomly assigned to test stations through a Latin square design described in Appendix 4 of study protocol. Control and repellent treated individuals will not be paired together. The 2 controls will not be paired together, but with study staff. Unpaired treated subjects will also be paired with a staff member in the same manner. Study staff will perform counts but will not expose their own legs. Table 5 on p. 33 of 117 shows randomization of test subjects pairing.

(e) Can the data be statistically analyzed?

Yes.

(f) What is the plan for statistical analysis of the data?

The mCPT from a sample of 13 subjects will be calculated using the Kaplan-Meier survival analysis. "*The times to treatment failure will be analysed 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*" (p. 36 of 117 in study protocol.

(g) Are proposed statistical methods appropriate to answer the research question?

The median CPT will be estimated from the CPTs of individual subjects using Kaplan-Meier survival analysis. The Kaplan-Meier procedure is a non-parametric method for survival analysis; this method does not require or assume the data to follow a particular parametric distribution. This method can also account for censored observations. Kaplan-Meier estimator has been accepted by EPA and the HSRB for mCPT calculation in past repellent efficacy studies and is also recommended by the World Health Organization for CPT calculation from these non-parametric data sets.

(h) Does the proposed design have adequate statistical power to definitively answer the research question?

The sample size of 13 treated subjects is employed according to EPA recommendations for sample size recommendation *(*Appendix 5: Sample Size Calculation in study protocol). "*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. 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*" (p. 35 of 117 in study protocol).

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to 2 new skin-applied repellent products containing OLE. Toxicity profile for EPA Reg. No. 84878-2 (Aerosol): Toxicity category IV for acute dermal, acute inhalation, and dermal irritation; toxicity category III for acute oral and eye irritation. The product is not a dermal sensitizer. The products will be tested at the EPA standard dose for aerosol applications. EPA standard dose of application is derived from dosimetry data following

label directions for use. "*One product formulation that is the subject of this study, US EPA Reg. No. 84878-2, contains 30% OLE and was registered with the EPA by Citrefine in 2008, as a pump spray formulation. The product for testing is intended to be sold in an aerosol format. The second product contains 15% OLE and is a liquid emulsion formulation also to be sold in an aerosol format based closely on a formulation currently sold in Europe"* (p. 25 of 117 in study protocol). The active ingredient in the proposed formulations is registered with the Agency for its use as active ingredient in skin-applied repellents. "*The active ingredient of each product is Oil of Lemon Eucalyptus (OLE, contained in an EPA registered repellent product EPA reg. no. 84878-2, and sold under the trade name Citriodiol. Citriodiol® contains approximately 65% p-Menthane-3,8-diol (CAS: 42822-86-6), lesser amounts of isopulegol and citronellol, as well as all other naturally occurring constituents present in the essential lemon eucalyptus oil from which it is derived*" (p. 15 of 117 in study protocol).

A full risk assessment for p-Menthane-3,8-diol (PMD) technical was conducted in 1999. The technical grade active ingredient, PMD, is placed into Toxicity Category IV for acute oral toxicity (LD_{50} > 5,000 mg/kg), acute dermal toxicity (LD_{50} > 5,000 mg/kg), and skin irritation (slight irritant), and Toxicity Category I for eye irritation (severe irritant). It was not a skin sensitizer. A 90-Day dermal study in rats (MRID 444387-10) tested PMD (98.3 % pure) at increasing doses, 0, 1,000 and 3,000 mg/kg/day. The NOEL = 1,000 mg/kg/day, and the LOEL = 3,000 mg/kg/day. The endpoints for NOEL and LOEL are based on treated skin observation, erythema, edema, eschar, and histological observations in treated skin, increased acanthosis, and inflammation at the highest dose of 3,000 mg/kg/day. No dermal absorption data is required for Tier I Toxicity data for biochemicals. Without these data, dermal absorption is assumed to be 100%. Risk characterization for infants and children is based in one developmental study (MRID 444387-11). The NOEL =3,000 mg/kg/day. No LOEL was established, and a 10-fold safety factor is applied for risk characterization. MOEs were not calculated because there are no endpoints, level of concern. The Agency concluded that there were no risks of concern. (U.S. EPA, 2000, https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/related_PC-011550_1-May-00.pdf

(a) What is the rationale for the choice of test material and formulation?

EPA requires submission of product performance data for all products claiming efficacy against public health pests. EPA requires efficacy data for registration of these skin-applied mosquito repellents claiming repellency against mosquitoes of public health importance on their labels.

(b) What is the rationale for the choice of dose/exposure levels and the staging of dose administration?

The dose used for testing repellency (1g/600 cm^2) is EPA standard dose (1.0 g/600 cm^2) used for testing repellency of aerosol spray products. EPA has recommended that the study use the EPA standard dose of 1.0 g/ 600 $cm^{2,}$ which is based on dosimetry data for estimation of typical consumer dose when the product is applied from its commercial container according to label use directions.

(c) What duration of exposure is proposed?

One day of testing will take up to 6 hours for the formulation containing 10-15% OLE and another day of testing will take up to 10 hours for the formulation containing 30% OLE from time of product application. Repellency evaluation will take up to 4 days of testing per subject to complete one day of testing for each product at 2 field sites. That is, one day for testing each formulation at 2 field sites. Proposed exposure periods consist of exposing untreated human skin to mosquitoes for 5 minutes at 30-minute intervals to monitor landing pressure and exposing treated skin to mosquitoes for 5 minutes at 30-minute intervals until end of test day or until the time point when repellent breakdown or CPT is reached by subject, whatever happens first.

2.3 Endpoints and Measures

(a) What endpoints will be measured? Are they appropriate to the question(s) being asked?

The efficacy endpoint is the CPT time measured by the First Confirmed Landing (FCL). The FCL signals the time point of repellency failure. CPT is measured by the FCL as a single time value for each subject. "*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*" (p. 16 of 117 in study report). The endpoints are appropriate to the questions being asked.

(b) What steps are proposed to ensure measurements are accurate and reliable?

• Good Laboratory Practices, as defined by 40 CFR part 160 is proposed to be followed throughout the study.
• More than one trained technician may apply the test substances to subject's legs. Technicians will ensure consistency as best as possible (p. 31 of 117).
• Research staff and study director will monitor testing, and data recording. Study staff paired with control subjects and paired with the unpaired treated subject will perform counts but will not expose their own legs.
• Alternate subjects (2 alternate subjects) will be enrolled to ensure adequate sample size.
• A Quality Assurance Unit will be in place to monitor all study activities and data collection.
• There will be three test days for subjects testing all three tick species.
• Stopping rules and criteria for subject withdrawal are established.

(c) What QA methods are proposed?

An independent Quality Assurance Unit (QAU) unit [Compliance Services International] will perform all QA duties. The QA representative will inspect the study and maintain written and signed records. Inspections will include observations of study conduct and data collection, and

compliance and no compliance with study protocol. All inspection findings will be reported to management and the Study Director. 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 (p. 7 of 117 in study protocol).

(d) How will uncertainty be addressed? Will point estimates be accompanied by measures of uncertainty?

Sources of variation include mosquito species, mosquito landing activity, and subjects' attractiveness to mosquitoes. These uncertainties will be addressed by each subject being tested for their attractiveness to mosquitoes, trained in the use of aspirators for capturing landing mosquitoes, and for selecting the lowest mCPT of the two field sites for the duration of efficacy on label. For rate of application, "*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"* (p. 24 of 117 in study protocol).

3. Subject Selection

3.1 Representativeness of Sample

(a) What is the population of concern?

The population of concern is people who would purchase and use skin-applied insect repellents.

(b) From what populations will subjects be recruited?

Volunteers will be recruited who meet the eligibility criteria listed in p. 21-22 of 117, including speaking English, being between 18 and 55 years old, and attractive to mosquitoes. Demographic conditions for age, gender and ethnicity are presented on Table 3, p. 28 of 117 in study protocol.

(c) Are expected participants representative of the population of concern? If not, why not?

Yes. Based on the proposed recruitment for this study, participants should be relatively representative of the population of concern.

(d) Can the findings from the proposed study be generalized beyond the study sample?

Yes, if EPA recommendations are adopted.

3.2 Equitable Selection of Subjects

(a) What are the inclusion/exclusion criteria? Are they complete and appropriate?

The protocol's eligibility criteria are included below:

Inclusion criteria are listed as follows:

- Able and willing to give fully informed consent.
- Male or female.
- Aged 18 to 55 years old.
- 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 mosquitoes).
- 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 mosquitoes for periods of at least 5 minutes at a time.
    - Able to operate an aspirator.

Exclusion criteria are listed in study synopsis as follows:

- 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 study staff members and of the study Sponsor (Citrefine International Ltd.).
- Students of the Study Director or any other study staff members.
- 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 products or any of its ingredients, or any insect repellent products.
- Individuals who are not attractive to mosquitoes during mosquito attractiveness test.
- Individuals who have signs or symptoms related to COVID-19, have tested positive for COVID-19 within the last 10 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 10 days (subject to change, to be in line with CDC's guidance).

(b) What, if any, is the relationship between the investigator and the subjects?

There should be no relationship between the investigator and the subjects. The protocol specifies that employees, managers, and spouses of employees of the study staff members and of the study Sponsor (Citrefine International Ltd.), as well as students of the Study Director or any other study staff members are not eligible to participate.

(c) Will subjects be recruited from a vulnerable population?

Recruitment does not target specifically any vulnerable populations.

(d) What process is proposed for recruiting and informing potential subjects?

The recruitment process is described in p. 19 of 117.
"Subjects will be recruited from the local area, 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 (Appendix 1. Recruitment Advert)."
Consent Process is described in section 4.2 on p. 19-20 of 117. Questions posed to candidates to confirm their understanding are listed on Table 2, p. 20 of 117 in study protocol.

(e) If any subjects are potentially subject to coercion or undue influence, what specific safeguards are proposed to protect their rights and welfare?

Subjects will be recruited through print, digital and social media advertisements. There will be no connection or communication between the researchers and the potential subjects' employers, which minimizes the potential for coercion or undue influence. In addition, students or employees of the study director or other faculty and researchers involved in the study are excluded from participation. Finally, any employees, managers, and spouses of employees of arctech and the study sponsor are excluded from participation.

3.3 Remuneration of Subjects

(a) What remuneration, if any, is proposed for the subjects?

The protocol notes that subjects will be compensated for their time spent participating in the study as follows: $10 for the participating in the consent meeting, $40 for participating in the attractiveness testing and aspirator training session, and the minimum wage in the state where the field test is conducted for taking part in the field test, rounded up to the next hour. The timing for this payment is from the time the subject registers at the start of the test day until the product failure or end of testing. Alternates are expected to stay at the test site for up to 2

hours and will be compensated $20 for their time if they are not needed to replace a test subject.

(b) Is proposed remuneration so high as to be an undue inducement?

No.

(c) Is proposed remuneration so low that it will only be attractive to economically disadvantaged subjects?

No.

(d) How and when would subjects be paid?

The protocol calls for compensating subjects at the end of each encounter in cash or a pre-paid card.

4. Risks to Subjects

4.1 Risk characterization

(a) Have all appropriate prerequisite studies been performed? What do they show about the hazards of the test material?

Subjects will be exposed to 2 new formulations of skin-applied mosquito repellent product, containing active ingredient, OLE, that is registered with the Agency for use in skin-applied repellent products. The active ingredient in the proposed formulation have undergone EPA review and fulfilled the requirements needed for EPA registration as repellent products to be applied to human skin. The rate of application of these aerosol products to the skin of subjects in this study will be consistent with typical consumer dose derived from dosimetry data used for estimating EPA standard dose of application for aerosol products. Dosimetry studies follow the directions for use on product label. Toxicity profile for EPA Reg. No. 84878-2 (aerosol): Toxicity category IV for acute dermal (LD_{50} > 5000 mg/kg), acute inhalation (LC_{50} > 2.06 mg/L), and dermal irritation (slight irritation); toxicity category III for acute oral (LD_{50} = 2408 mg/kg combined) and eye irritation (Reversible irritation clearing in 7 days). The product is not a dermal sensitizer.

(b) What is the nature of the risks to subjects of the proposed research?

The protocol discusses the following risks to subjects as a result of study participation: effects from exposure to the test material, exposure to field mosquitoes, physical stress due to environmental test conditions, unintentional release of confidential information, and psychological risks associated with disclosure of pregnancy testing results.

(c) How do proposed dose/exposure levels compare to the established NOAELs for the test material?

The test material is an EPA-registered product to be used as skin applied repellent and it will be used consistent with the Directions for Use on the product label. Because there is no endpoint of toxicological concern for the dermal route of exposure (section 4.1 (a) above) the dose and exposure levels are lower than any NOAEL or LOAEL for OLE, EPA considers the exposure of the subjects to the tested levels of the test substance not to pose an unreasonable risk of adverse effects.

(d) What is the probability of each risk associated with the research? How was this probability estimated?

No numerical probability is estimated, but risks have a low probability of occurrence. Practical steps to minimize subject risks have been described in the protocol.

(e) If any person with a condition that would put them at increased risk for adverse effects may become a subject in the proposed research, is there a convincing justification for selection of such a person and are there sufficient measures to protect such subjects?

Individuals who may be at an increased risk for adverse effects are not eligible to become subjects in this study, including individuals known to be allergic or sensitive to skin applied insect repellents, and those with known skin conditions (Individuals with localized skin disorders or problems affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes (Exclusion criteria, p 11 – 12 of 117 in study protocol).

4.2 Risk minimization

(a) What specific steps are proposed to minimize risks to subjects?

The protocol outlines risks and risk minimization measures in table on pp. 16-18. To mitigate risks from exposure to mosquitoes and disease vectors, subjects will be trained to aspirate landing mosquitoes before they probe or bite and will wear clothing, gloves, and head net to protect untreated areas from bites during the test periods. To minimize the risk of contracting any mosquito-borne diseases during the lab-based mosquito attractiveness test, the cages will be populated with mosquitoes from a colony reared in the laboratory and screened for diseases. The testing sites will be monitored weekly for a month prior to the testing, and mosquitoes captured during the monitoring phase will be tested for pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified. The Study Director will work with the local authorities to ensure that testing is not conducted in an area where vector-borne illnesses have been identified within 25 miles for at least 4 weeks prior to the test day.

To minimize the discomfort associated with mosquito bites, candidates known to be sensitive to or phobic of mosquito bites will be excluded and topical antihistamines will be available to subjects at the end of the test day at no charge. In addition, participants will be instructed to wear light, loose-fitting clothing that fully covers their bodies and will be provided with a head net and gloves to wear during the testing. Only the area to be treated with the repellent will be exposed to mosquitoes during the test period. In addition, untreated control subjects will only expose their lower leg until the requisite number of mosquito landings have been observed for each period during the testing.

To protect subjects against the risks associated with a long test day and with being outside for extended periods in a hot, humid climate, subjects will be provided with snacks, water, and other drinks. A shaded, screened area with chairs will be available for subjects' use during the periods between the test periods. To protect against the risk of irritation from exposure to the test substance, people who are sensitive to insect repellents and those with open cuts, scrapes, skin disease and skin problems will be excluded. A certified first aider will be present during training and testing to provide emergency assistance if required.

(b) What stopping rules are proposed in the protocol?

Test will be stopped if Study Director believes this is in the best interests of the trial subjects, study data or both. Testing will stop immediately if adverse events are apparent (p. 37 of 117). Stopping rules are described on p. 33 to 34 of 117 in study protocol as follows: If no CPT is reached for more than half of the sample, the test will continue until 6 or 10 hours post application (depending on the product being tested) or will end when more than half of sample experience FCL or CPT. A median CPT can be established when CPT occurs for half the subjects, so at that point there would be no need to risk exposure of subjects who have not reached treatment failure. Early stopping criteria due to low landing pressure of bad weather are described in section 6.7.9 as follows: "*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 and testing re-arranged. If there are 3 consecutive exposure periods with low-landing pressure the test day should be stopped and testing re-arranged. Likewise, if there are 4 non-consecutive exposure periods missed due to bad weather the test day should be stopped and testing re-arranged. If 3 consecutive periods are missed due to bad weather then, the test day should be stopped and testing re-arranged*" (p. 34 of 117 in study protocol).

(c) How does the protocol provide for medical management of potential illness or injury to subjects?

The medical management procedures are described in the protocol in p. 36-39 of 117.

(d) How does the protocol provide for post-exposure monitoring or follow-up? Is it of long enough duration to discover adverse events which might occur?

Follow up procedures are described in p. 35 of 117 as follows:
"*Subjects will be followed up within 24-72 hours after any exposure to mosquitoes or test product to assess any possible adverse events. 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 recorded.*
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."

(e) How and by whom will medical care for research-related injuries to subjects be paid for?

"If a subject is injured or becomes ill as a direct results [sic] 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." (p. 38 of 117)

5. Benefits

(a) What benefits of the proposed research, if any, would accrue to individual subjects?

"*The research provides no direct benefit to subjects. Indirect benefits to society will be additional products available to consumers to repel mosquitoes, thereby reducing the potential for mosquito bites and transmission of vector-borne illnesses. The results of this study will inform the product labeling*" (p. 16 of 117 in study protocol).

(b) What benefits to society are anticipated from the information likely to be gained through the research?

This study is designed to determine median CPT of 2 skin-applied mosquito repellents containing OLE as active ingredient. The data collected in the study will be used to support product registration. The research has societal value because people are at risk of contracting mosquitoes-borne diseases**.**

(c) How would societal benefits be distributed? Who would benefit from the proposed research?

One beneficiary will likely be the sponsor who is seeking EPA-registration for skin- applied repellent products containing OLE. Indirect beneficiaries would include the general public who may benefit from the availability of another effective skin-applied mosquito repellent.

(d) What is the likelihood that each identified societal benefits would be realized?

EPA cannot predict the outcome of the testing results; the testing could demonstrate that the formulation is effective at providing hours of protection by preventing mosquito bites and the likelihood of mosquito-borne disease pathogens.

6. Risk/Benefit Balance

(a) How do the risks to subjects weigh against the anticipated benefits of the research, to subjects or to society?

The likely benefit to society in general, in the form of more products to prevent biting by insects that can transmit diseases to humans, must be weighed against the risks to study participants. Mosquitoes can transmit a variety of diseases to humans. Data involving human subjects must be generated to support registration of this new insect repellent products because no reliable alternatives to human testing exist for evaluating the efficacy of skin-applied products. Because the EPA has determined that there is not a dermal risk of concern with the product proposed for use in this research study, subjects are unlikely to experience adverse effects. With procedures put in place to minimize the risks associated with exposure to the product and other risks to participants such as mosquito-borne pathogens, the likelihood of serious adverse effects is very small. In summary, the risks to study subjects from participating in this study are reasonable in light of the likely benefit to society from the knowledge to be gained.

7. Independent Ethics Review

(a) What IRB reviewed the proposed research?

WCG IRB.

(b) Is this IRB independent of the investigators and sponsors of the research?

Yes.

(c) Is this IRB registered with OHRP?

Yes.

(d) Is this IRB accredited? If so, by whom?

Yes, by the Association for the Accreditation of Human Research Protection Programs (AAHRPP).

(e) Does this IRB hold a Federal-Wide Assurance from OHRP?

Yes.

(f) Are complete records of the IRB review as required by 40 CFR 26.1125 provided?

Yes.

(g) What standard(s) of ethical conduct would govern the work?

This is a protocol for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to the EPA under the pesticide laws. The primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply.

8. Informed Consent
(a) Will informed consent be obtained from each prospective subject?

Yes.

(b) Will informed consent be appropriately documented, consistent with the requirements of 40 CFR 26.1117?

Yes.

(c) Do the informed consent materials meet the requirements of 40 CFR 26.1116, including adequate characterization of the risks and discomforts to subjects from participation in the research, the potential benefits to the subject or others, and the right to withdraw from the research?

Yes.

(d) What is the literacy rate in English or other languages among the intended research subjects?

Recruitment and enrollment are limited to English speakers in the current protocol. All subjects will be required to read English, but no information on literacy rates will be collected. EPA has recommended that enrollment not be limited to English-language speakers.

(e) What measures are proposed to overcome language differences, if any, between investigators and subjects?

Both the investigators and subjects will speak English.

(f) What measures are proposed to ensure subject comprehension of risks and discomforts?

Subjects will be asked specific questions following the presentation of information during the consent meeting to ensure their comprehension of the risks and discomforts.

(g) What specific procedure will be followed to inform prospective subjects and to seek and obtain their consent?

Consent will be obtained from subjects after they have a one-on-one meeting with a member of the research staff, learn about the study, and a research team member reads through the consent form with them. Subjects will be reminded that they are free to ask questions of the researcher or Study Director at any time. They will also be reminded that they are free to withdraw from the study at any time for any reason, without forfeiting any benefits to which they are entitled.

(h) What measures are proposed to ensure fully voluntary participation and to avoid coercion or undue influence?

Participants will be informed at the consent meeting orally and in writing, via the consent form, that they are free to withdraw from the study without any penalty and without forfeiting any benefits to which they are entitled.

To avoid coercion or undue influence in an individual's decision to enroll in the study, the eligibility criteria exclude employees, managers, and spouses of employees of the Study Director and of the study Sponsor, as well as students of the Study Director.

9. Respect for Subjects

(a) How will information about prospective and enrolled subjects be managed to ensure their privacy?

The protocol outlines confidentiality measures. Interviews for eligibility and consent are held one-on-one. All records with personal information are kept in a locked file, separate from main study records and with limited access. Individual subjects will be identified by number, not by name. Pregnancy test results will be shared only with a single female member of the research group and will not be recorded.

(b) How will subjects be informed of their freedom to withdraw from the research at any time without penalty?

Subjects will be told orally and in writing during the consent meeting that they are free to withdraw from the research at any time. The EPA recommends that subjects are reminded of this freedom during any pre-testing reminder calls and at the start of each test day before any test substance is provided.

(c) How will subjects who decline to participate or who withdraw from the research be dealt with?

Subjects who decline to participate or who withdraw during the test day will be compensated for their time and inconvenience for the amount of time they participated, e.g., attending a consent meeting, time from arrival to withdrawal.

Attachment 2

Checklist - §26.1116 General requirements for informed consent

Criteria		Y/N	Comment/Page Reference
No investigator may involve a human being as a subject in research covered by this subpart unless the investigator has obtained the legally effective informed consent of the subject		Y	
An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence		Y	
The information that is given to the subject or the representative shall be in language understandable to the subject or the representative		Y	All subjects speak English, all materials in English – see EPA recommendations
No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence		Y	p. 76 of 117
(a) In seeking informed consent the following information shall be provided to each subject	(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental	Y	p. 61-78 of 117
	(2) A description of any reasonably foreseeable risks or discomforts to the subject	Y	p. 61-78 of 117
	(3) A description of any benefits to the subject or to others which may reasonably be expected from the research	Y	p. 61-78 of 117
	(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject	Y	p. 61-78 of 117
	(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained	Y	p. 61-78 of 117

	(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained	Y	p. 61-78 of 117
	(7) An explanation of whom to contact for answers to pertinent questions about the research and research	Y	p. 61-78 of 117
	(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled	Y	p. 61-78 of 117
(b) When appropriate, one or more of the following elements of information shall also be provided to each subject	(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject may become pregnant) which are currently unforeseeable	Y	p. 69 of 117
	(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent	Y	p. 71 of 117
	(3) Any additional costs to the subject that may result from participation in the research	Y	p. 71 of 117
	(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject	Y	p. 109 of 117
	(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject	Y	p. 70 of 117
	(6) The approximate number of subjects involved in the study	Y	p. 69 of 117
(e) If the research involves intentional exposure of subjects to a pesticide, the subjects of the research must be informed of the identity of the pesticide and the nature of its pesticidal function.		Y	p. 64 of 117

Attachment 3

Checklist - §26.1117 Documentation of informed consent

Criterion	Y/N	Comment/Page Reference
(a) Informed consent shall be documented by the use of a written consent form approved by the IRB and signed by the subject. A copy shall be given to the person signing the form.	Y	p. p. 61-78 of 117
(b)(1) The consent form may be a written consent document that embodies the elements of informed consent required by §26.1116. This form may be read to the subject, but in any event, the investigator shall give either the subject adequate opportunity to read it before it is signed; or	Y	p. 109 of 117
(b)(2) The consent form may be a short form written consent document stating that the elements of informed consent required by §26.1116 have been presented orally to the subject. When this method is used, there shall be a witness to the oral presentation. Also, the IRB shall approve a written summary of what is to be said to the subject. Only the short form itself is to be signed by the subject. However, the witness shall sign both the short form and a	N/A	

Attachment 4

Checklist - §26.1125 Submission of proposed human research for EPA review

Requirement			Y/N	Comments/Page Refs
The following Information, to the Extent not already included:	§1125(a) a discussion of:	(1) The potential risks to human subjects	Y	Protocol document
		(2) The measures proposed to minimize risks to the human subjects;	Y	Protocol document
		(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	Protocol document
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	Protocol document
		(5) The balance of risks and benefits of the proposed research.	Y	Protocol document
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.			Requested to be provided prior to initiation of the study
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		Y	Protocol document; final advertisements and recruitment scripts requested
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human		Y	Protocol document; recruitment scripts requested
	§1125(e): All correspondence between the IRB and the investigators or sponsors.			Provided to EPA
	§1125(f): Official notification to the sponsor or investigator... that research involving human subjects has been reviewed and approved by an IRB.			Protocol document
	(1) Copies of • all research proposals reviewed by the IRB, • scientific evaluations, if any, that accompanied the proposals reviewed by the IRB, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.		Y	Requested to be provided prior to initiation of the study

all information relevant to the proposed research specified by § 26.1115(a)	(2) Minutes of IRB meetings . . . in sufficient detail to show: • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; and • a written summary of the discussion of controverted issues and their resolution.	Y	Provided to EPA
	(3) Records of continuing review activities.	N/A	
	(4) Copies of all correspondence between the IRB and the investigators.		Provided to EPA
	(5) A list of IRB members identified by name; earned degrees; representative capacity; indications of experience such as board certifications, licenses, etc., sufficient to describe each member's chief anticipated contributions to IRB deliberations.		Provided to EPA
	(6) Written procedures for the IRB in the same detail as described in §26.1108(a) and §26.1108(b).		Provided to EPA by WIRB
	(7) Statements of significant new findings provided to subjects, as required by §26.1116(b)(5).	N/A	

Attachment 5
Basis for EPA Standard Dose in Skin-Applied Repellent Testing

The information below is excerpted from the EPA's presentation to the HSRB in April 2015 of its review of a mosquito repellency protocol submitted by SC Johnson. EPA's complete review of the protocol is available at https://www.epa.gov/sites/production/files/2015-04/documents/epa_science_and_ethics_review_-_scj_protocol.pdf.

Based on an analysis of the dosimetry results from repellent studies reviewed by EPA and HSRB since 2006 (Table 1), EPA considers the dose of 1g product/600 cm^2 of skin to be an appropriate product dose for testing aerosol, wipes, and lotion type products and 0.5g product/600 cm^2 of skin for testing pump spray type products.

Table 1. Combined results of dosimetry testing from skin-applied repellent studies reviewed by EPA and HSRB since 2006 for three formulation types.

Formulation Type	**Total No. of Subjects in Dosimetry Phase for Mosquito Tests**	**Mean Dose (g/600 cm^2) $\pm$ 1 SD**	**Dose range (g/600 $cm^{2)}$**
Lotion	112	0.933 $\pm$ 0.299	0.63-1.23
Pump spray	92	0.434 $\pm$ 0.113	0.32-0.55
Aerosol	25	0.815 $\pm$ 0.262	0.55-1.08