UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

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MEMORANDUM

SUBJECT: Science and Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing OLE

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REF: Logan, James, Study Director. (2017) Protocol for "A single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks." Unpublished document prepared by Citrefine International Ltd., Moorfield Road, Yeadon, Leeds, LS19 7BN, United Kingdom. 21st July 2017. 37 p. MRID 504422-01.

We have reviewed the referenced protocol for laboratory testing for a skin-applied repellent product containing Oil of Lemon Eucalyptus (OLE) against three species of ticks in a laboratory setting from both scientific and ethics perspectives. This protocol was submitted by arctec (Arthropod Control Product Test Centre), part of the London School of Hygiene and Tropical Medicine (LSHTM). The study is sponsored by Citrefine International, Ltd. This review assesses the scientific aspects of the proposed research for a product performance study to evaluate the efficacy of skin applied insect repellent products in terms of the recommendations of the EPA OCSPP 810.3700 Guideline, *Insect Repellents to be Applied to Human Skin*, and the EPA Human Studies Review Board (HSRB), concerning scientific merit of the proposed study. Ethical aspects of

the proposed research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L.

A. Completeness of Protocol Submission

The submitted protocol was reviewed for completeness against the required elements listed in 40 CFR §26.1125. EPA's checklist is appended to this review. All elements of required documentation are provided in the submitted protocol package and supplementary documentation of review by Western Institutional Review Board (WIRB).

B. Summary Assessment of Ethical Aspects of the Proposed Research

Here is a summary of our observations about the ethical aspects of the proposed protocol as amended to address EPA's comments and recommendations. EPA and arctec met in March to discuss EPA's review and recommendations; arctec reviewed and agreed to make all suggested changes, except deleting the consumer dose phase and using EPA's recommended standard dose during the repellency testing portion of the study. 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 and protection time of a topically topically-applied skin repellent against ticks. Efficacy will be expressed as Complete Protection Time (CPT), which is defined as the time between application of the repellent product and the time at which 1 tick crosses 3 cm of treated skin, followed within 30 minutes by a second confirmatory crossing. The study will also include a dosimetry phase to establish an expected "consumer dose" that will be used as the standard dose during the repellent efficacy trial. The research has societal value because people are at risk of contracting tick-borne diseases, and such risks can be mitigated by the use of insect repellent products. There are no data showing the necessary efficacy of OLE in human studies and no recommended dose for the proposed delivery mechanism for this product. The rationale for this testing is to collect data to estimate the CPT for this tick repellent product containing OLE. As intended, the data resulting from this proposed study will be used to support registration of topically-applied repellents containing OLE.

2. **Subject Selection:** With EPA's comments and recommendations incorporated, subjects will be recruited through emails, posters, and social media targeted to the London area, namely from the area in and around the London School of Hygiene and Tropical Medicine. The advertisements will be posted on bulletin boards and electronically in order to ensure that there is equity of access to participate in the study. The advertisement will provide a brief description of the study and an email address and phone number that interested persons can use to contact the arctec Trial Coordination Centre to get more information about the study and how to participate.

   Study staff will contact persons interested in participating by phone or email to provide additional information about the study and to determine whether they meet basic inclusion and exclusion criteria. Potentially qualified persons will be added to a recruitment list in the order of their enrollment. Once a pool of at least 75 potentially

qualified persons is assembled, the list will be randomized. The potentially qualified subjects will be contacted in order of the randomized list and invited to the arctec facility for a one-on-one consent meeting with a member of the study team.

The study will be conducted in two phases: a dosimetry phase, to determine a typical consumer dose, and the tick repellent efficacy testing phase. A total of 25 subjects will be necessary to complete the dosimetry phase. For the repellent efficacy testing phase, EPA recommends a test subject sample size of 25 for each of the three tick species that will be tested to generate statistically-sound data (see Attachment 3). As discussed above, the subjects will be contacted according to the order of the randomized list of qualified persons. At the end of the dosimetry phase, staff will confirm with enrolled and consented subjects whether they wish to participate in testing the repellent against one or more tick species. As necessary, additional subjects from the generated randomized list will be called on to enroll in testing against one or more tick species as necessary to ensure that a total of 25 subjects complete a repellency efficacy trial against each of the three species of ticks discussed in this protocol.

If the original pool of 75 subjects is not sufficiently large to complete the dosimetry phase and repellent efficacy trials against all 3 species of ticks, recruitment will be re-opened as described above until another pool of at least 25 potentially qualified subjects has been assembled. Then the same randomization process will be followed to add them to the end of the previously compiled randomized list. Then the potentially qualified subjects will be invited for a consent meeting and, if qualified, enrolled into the study in sequential order.

The inclusion and exclusion criteria, with EPA's recommendations addressed, are appropriate and complete.

3. **Risks to Subjects:** The protocol discusses potential hazards associated with these tests including adverse reactions to the test substance, adverse reaction to tick bites, potential transmission of tick-borne disease, potential stress from finding out the results of a pregnancy test, and unintentional release of confidential information. The protocol notes that risks will be minimized as follows. To minimize risks of adverse reactions to the test substance, subjects will be enrolled only if they do not have a known sensitivity or allergy to the test substance or any skin-applied insect repellents. In addition, subjects with localized skin disorders on the forearms that could be exacerbated by exposure to the test substance will be excluded.

   To mitigate risks from exposure to ticks, ticks will be placed one at a time on subjects' arms and monitored closely to minimize the chance for the tick to move further onto the host for a suitable location to attach (e.g., armpit). Ticks will be removed if they begin to bite and attach to the subject. Should a tick exhibit behavior indicating it is attaching to the subject, it will be removed immediately by the researcher with fine forceps. To eliminate the risk of transmission of tick-borne disease, ticks will be sourced from pathogen-free colonies at Oklahoma State University Tick Rearing Facility. Ticks will only be used once with a single subject, and ticks will be destroyed by immersion in alcohol after they are placed on a subject's arm.

EPA's suggested edits to the protocol incorporate additional protections to keep the subjects' identities and results of pregnancy testing private. Practical steps to minimize subject risks have been described in the protocol, and the remaining risks have a low probability of occurrence.

With EPA's recommendations addressed, the risks to subjects have been identified and appropriate steps to minimize risks are included.

4. **Benefits:** This research offers no benefits to subjects. Depending on the results of the research, it may provide indirect benefits to subjects and society by potentially leading to data that could be used by EPA to register insect repellent products containing OLE. These repellent products could lead to fewer tick bites and reduced incidents of tick-borne illnesses.

5. **Risk/Benefit Balance:** The protocol describes measures to further reduce risk to subjects while maintaining the robustness of the scientific design. Due to the risk mitigation measures put in place, the residual risk to subjects is low and reasonable in light of the potential benefits of the data to society.

   Subjects will be exposed to the test substance in two phases – the dosimetry phase, and the repellent efficacy phase. Although EPA recommends using a standard dose for repellent efficacy trials to avoid additional exposure of subjects during a consumer dose phase, OPPTS Guidelines 810.3700 provides guidance on conducting a "dose determination" phase to establish a typical consumer dose for use in a subsequent repellent efficacy trial (pp. 23-25). The Study Sponsor believes that the typical consumer dose for their product is higher than EPA's recommended standard dose (0.5 g/600 cm^2). Testing the product at a lower rate than consumers would typically use could result in an underestimation of the product's efficacy, which could result in consumers over-applying the product. The product being tested has already approved by EPA and has been on the market for more than a decade, the product is only being applied to a fraction of the skin that it would be in normal use, and the product would be removed immediately after each application so exposure is further limited. The minor incremental risks of additional exposure to subjects participating in the dosimetry phase are reasonable when considered with the potential benefits of ensuring the product labeling provides an estimate of protection time based on consumer practices and of avoiding over-application of the product by future consumers. When set against the far greater risk of consumer over application which would result from an underestimate of the product's efficacy in preventing tick bites, it suggests that the dose rate study is fully justified.

6. **Independent Ethics Review:** Western Institutional Review Board (IRB) has reviewed and approved the protocol, informed consent form, and recruitment materials. Western IRB is registered with the Office of Human Research Protections at the Department of Health and Human Services (IRB00000533). Western IRB has been accredited by the Association for the Accreditation of Human Research Protection Programs (AAHRPP) since 2003. Satisfactory documentation of the Western IRB procedures and

membership is on file with the Agency and has been provided to the HSRB members. Documentation regarding IRB approval of the protocol has been provided to the HSRB members with the background materials for this protocol.

7. **Informed Consent:** With the agency's comments addressed, the protocol contains 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 background materials; with EPA's recommendations and comments incorporated, it meets the requirements of 40 CFR §§26.1116 and 26.1117

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. The protocol will be revised to explain the provisions made for discrete handling of the pregnancy testing that is required of female subjects on each day of testing. Any photographs or videos of the subjects will not include their faces, tattoos, or other identifying features. Candidates and subjects will be informed that they are free to decline to participate or to withdraw at any time for any reason. Subjects will be compensated as described in the protocol. Breaks for subjects between exposures and provision of snacks and drinks for interested subjects will be incorporated into the study design.

C. Compliance with Applicable Ethical 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 EPA under the pesticide laws. Thus 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 as Attachment 1.

Although this research will take place in a foreign country, the Study Sponsor has agreed to conduct the research in compliance with the requirements of 40 CFR 26 Subpart K and L. In addition, the research will comply with the World Medical Association's Declaration of Helsinki (2003), which covers the principles of respect for individuals, beneficence, and justice. These are also the foundations of United States' regulations for the protection of human subjects.

EPA's Ethics Comments

The London School of Hygiene and Tropical Medicine/arctec were notified that, before the research is conducted, the protocol and supporting documents must be revised to address EPA's comments and recommendations resulting from the review by the Human Studies Review Board (HSRB). They have already agreed to address EPA's comments. To facilitate the HSRB's review of the latest protocol, which incorporates the EPA's comments, the EPA is providing a separate file for the HSRB titled "OLE Revised Protocol with EPA Comments Incorporated." After the HSRB

completes its review of the protocol and relays its recommendations to the EPA, the EPA and London School of Hygiene and Tropical Medicine/arctec will reach agreement on implementation of the HSRB's recommendations; the revised protocol and supporting documents should be resubmitted for review and approval to Western IRB prior to initiating the research.

The EPA's ethics comments are provided below and presented in the order they are found in the protocol. Minor editorial comments and corrections of typographical errors have not been included here. In addition, EPA has provided ethics-related comments on the informed consent materials; these are provided to the HSRB as a separate file.

General Comments & Study Synopsis

1. Add the date of IRB approval to the protocol.
2. Revise the subject size throughout the protocol to reflect the statistically-supported sample size of 25 subjects for each of the tick repellent efficacy tests. In addition, indicate that 25 subjects will be enrolled for the dosimetry phase. Ensure that the protocol and consent form clearly state that subjects may enroll in one or more test days. Ensure that at least 10 subjects of each sex are recruited for each test, with a goal of having at least 12 subjects of each sex.
3. Revise the protocol to clearly state that at least 48 hours will elapse between a subject's participation in a 2nd test day; based on discussions between the researchers and EPA, a 1 week waiting period is unnecessary. Provide rationale for follow up at 48 hours and 1 week.
4. Separate "eligibility criteria" into "inclusion criteria" and "exclusion criteria".
5. Revise exclusion criteria to include the following:
    -
    - Known phobia of ticks or tick bites
    - Employees and spouses of employees of the Study Sponsor (Citrefine, Inc.), arctec, and those directly line managed by Professor James Logan (study director)
    - Students of the study director, principal investigator, or any other faculty members involved in this study

Section 4 – Trial Design

6. In Section 4.2, Risks and Benefits Assessment:
    - Separate out each risk and discuss the corresponding benefits.
    - Regarding potential for eye injury, please have eye wash available for subjects in the event they get the product in their eyes.
    - Provide specific information on how ticks will be destroyed.
    - Clarify whether tick paralysis is an actual risk to subjects if the ticks will not be allowed to attach/bite.
    - Again, revise period between a subject's participation to at least 48 hours.
    - Add risk of unanticipated loss of confidential information and psychological risks associated with pregnancy testing, and corresponding risk mitigation measures.

Section 5 – Participant Entry

7. In Section 5.1, Recruitment:
    - Revise the estimated maximum number of subjects needed for the study to reflect the revised number of subjects for the dosimetry and repellent efficacy phases.
    - Expand recruitment to the London area – limiting recruitment to the London School of Hygiene and Tropical Medicine is too narrow and could result in a skewed pool of subjects.
    - Provide to EPA copies of the materials that will be used during recruitment, e.g., Product Information Sheet, sample email and telephone script that will be used to follow up with those interested in participating.
    - Include a randomization process to avoid biasing the pool towards those who respond immediately. (Note: This could also be included in Section 6.2.) For example:
      "Recruitment will initially be open for a 2-week period. The goal is to have at least 75 potentially qualified subjects. If this target is not reached after 2 weeks, recruitment will continue for an additional week at a time, until the target is reached. The total number of qualified subjects will each be assigned a unique and consecutive number, starting at OLE-01 based on the order of their enrollment. The numbers will then be randomized using the randomize function in Microsoft Excel. The first 25 subjects in the generated randomized list will be consented for the dosimetry phase and will be offered the opportunity to consent for one or more repellent efficacy trials. If needed, subjects from the generated randomized list will be called on in sequential order to replace any subject who withdraws from the dosimetry phase.

      "At the end of the dosimetry phase, staff will confirm with enrolled and consented subjects whether they wish to participate in testing the repellent against one or more tick species. As necessary, additional subjects from the generated randomized list will be called on to enroll in testing against one or more tick species as necessary to ensure that a total of 25 subjects complete a repellency efficacy trial against each of the three species of ticks discussed in this protocol.

      "If the original pool of 75 subjects is not sufficiently large to complete the dosimetry phase and repellent efficacy trials against all 3 species of ticks, recruitment will be re-opened as described above until another pool of at least 10 potentially qualified subjects has been assembled. Then the same randomization process will be followed to add them to the end of the previously compiled randomized list and if they are qualified and interested, they will be enrolled into the study in sequential order."
8. For Section 5.2, Eligibility Criteria:
    - Separate the list into inclusion and exclusion criteria.
    - Add to the inclusion criteria "Non-smoker or willing to refrain from smoking for 24 hours prior to and during each test".
    - Revise the exclusion criteria listed below as follows:
        - Known or suspected history of tick bite paralysis or tick bite allergies
        - Known phobia of ticks or tick bites

- Known allergy or sensitivity to Oil of Lemon Eucalyptus, any other repellent ingredients, or skin-applied repellent products
- Employees and spouses of employees of the Study Sponsor (Citrefine, Inc.), arctec, and those directly line managed by Professor James Logan (study director)
- Students of the study director, principal investigator, or any other faculty members involved in this study

9. Clarify that non-scented soap will be provided to subjects at the consent meeting.
10. For Section 5.3, Consent Process:
    - Provide a thorough description of what will be covered during the consent meeting, including a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the repellent to which subjects will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with the dosimetry test should be demonstrated step-by-step to all subjects who participate in the training prior to the dosimetry phase. The study team member should explain and demonstrate how the test substance will be applied and give a step-by-step description of how a 15-minute exposure interval during the tick repellent efficacy portion of the trial will be conducted.
    - Pregnancy testing on each day of participation must be explained to female subjects. This discussion should cover how and when testing will occur and how the female subject's privacy will be respected.
    - Revise consent process as necessary to reflect recruitment and enrollment in both dosimetry and repellent efficacy phases.
    - Ensure that subjects are informed that they can speak with the Study Director in private during or after the consent meeting if they have any questions.
    - Include questions that will be asked by the person conducting the consent meeting to ensure that subjects demonstrate comprehension of the materials presented prior to giving informed consent to enroll in the study.
    - Revise this section to note that pregnancy testing must be conducted on each day of the study.
11. In Section 5.4, Withdrawal Criteria, add the following language immediately following the first sentence: "Participants will be told during the consent process and again at the initiation of each test day in which they participate that they may withdraw at any time without giving a reason and without forfeiting benefits to which they are entitled."

Section 6 - Intervention

12. Separate the discussion of the interventions into a description of the test substance, randomization, dosimetry phase, and repellent efficacy phase.

Section 7 – Participant Timeline

13. Revise this section to align with all other edits to the protocol (timing, recruitment, enrollment, number of subjects, follow up/waiting periods).

Section 8 – Test Methodology

14. Revise instructions to participants for dosimetry phase. Subjects should be provided the label and instructed to read the label and apply according to the instructions. Researchers should not influence the amount of product applied during the dosimetry phase.
15. Explain when and how the subjects for the dosimetry phase will be provided with safety glasses/goggles to protect their eyes during application.
16. Prior to initiating the dosimetry phase, both forearms of each participant should be washed with unscented soap and water and dried with paper towels.
17. State explicitly that on each day of testing, females will complete a pregnancy test prior to being exposed to the test substance.
18. Prior to both phases, a study team member or medical professional should inspect the subject's forearms for disqualifying skin conditions. Include this step in the protocol and consent form.
19. For Section 8.2, Assessment of complete protection time:
    - Provide information about how subjects' comfort will be accommodated during the testing. For example, provide information about how/where subjects will be seated, and that adequate time will be allowed between trials for subjects to stand and stretch, to use the restroom, to eat or drink, etc.

Section 10 – Safety Reporting and Data Monitoring

20. Explain how the medical monitor will participate in the study. Will he be on-site or on-call during monitoring events? How will he respond in the event of an adverse effect? Ensure that the medical monitor is familiar with the protocol prior to study initiation.
21. Provide on-call first aider a copy of final approved protocol, brief them on study process and test substances, and if applicable, contact on-call first aider at initiation of each test day to confirm that testing has begun for that day.
22. Provide to subjects during the consent meeting instructions on how to report adverse events as described in this section.
23. For reporting adverse events, ensure that the protocol includes language specific to the reporting requirements for Western IRB as well.

Section 11 – Ethics & Dissemination

24. In section 11.1, include WIRB requirements for reporting changes to the protocol made to eliminate apparent immediate hazards to human participants, e.g., must be reported in writing within X days of the change.
25. In section 11.2, include the following language: "All deviations (including minor corrections) will be documented by the Study Director, reported to the IRB as required, and included in the final study report provided to EPA."
26. Revise section 11.3 as follows: "Consent to enter the study must be sought from each volunteer only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed volunteer consent ~~should~~ must be obtained. The right of the participant to refuse to participate without giving reasons must be respected. Participants must be informed of their right to withdraw from participation at any point in the study without forfeiting benefits to which they are entitled."

27. Compensation for participants should be revised. Participants should be compensated for participating in an in-person screening appointment/consent meeting. Volunteers in the tick trial, who could spend over 10 hours at the test facility, should be compensated adequately for their inconvenience and loss of employment income. EPA recommends compensating subjects for the consent meeting at a flat rate (e.g., £20 each for taking part in the screening and consent meeting, which could take up to an hour). EPA also recommends compensating subjects on an hourly basis, at close to the minimum wage (note: in the UK, minimum wage varies by age), £7.50/hour for the lab-based repellent testing, rounded up to the next hour.

D. Summary Assessment of Scientific Aspects of the Proposed Research

1. Objectives

The main objectives of the proposed study are to determine the complete protection time (CPT) of the skin-applied repellent products, EPA Reg. No. 84878-2 and 305-62, containing 30% Citriodiol® (Oil of Lemon Eucalyptus (OLE); CAS No. 1245629-80-4; PC Code: 040522) as the active ingredient, against ticks, and to determine the duration of 90% repellency. The products are proposed to be tested on 3 tick species, *Ixodes scapularis, Amblyomma americanum,* and *Rhipicephalus sanguineus* at the typical consumer dose. The secondary objective is to estimate the average dose applied by consumers (a.k.a. typical consumer dose) measured as ml/cm^2. (Study Synopsis (pg. 3) Section 1. a. Objectives (pg. 7)).

2. Endpoints

Efficacy endpoints are defined on pg. 4, Study Synopsis**,** as "*Time to complete protection time measured as a single tick crossing 3 cm into treated skin, and time to 90 % protective efficacy measured as more than 10% of ticks crossing treated skin***."**

Efficacy endpoints are defined on pg. 9, Section 4.1 Trial Endpoints**,** as "*The primary endpoint measure is the complete protection time (CPT), which is defined as the time between application of the repellent product and the time at which 1 tick crosses 3cm of treated skin, followed within 30 minutes by a second confirmatory crossing. The median CPT will be calculated for each species using Kaplan-Meier survival curves. The first secondary endpoint measure is the average dose rate applied by consumers when using EPA Reg. No. 84878-2 (alt) formulated insect repellent according to label instructions for purposes of repelling ticks. Other secondary endpoint measures will be the time to 90% protective efficacy of EPA Reg. No. 84878-2 (alt) formulation against Ixodes scapularis, Amblyomma americanum and Rhipicephalus sanguineus.*"

The endpoint for estimation of CPT is also defined on pg. 18, Section 9.2. Statistical methods as the time to failure, which is the time when a first tick crossing into 3 cm of treated skin is followed by a second confirmatory crossing by a different tick 30 minutes

apart from the first crossing. *"The endpoint for CPT will be time to treatment failure for each participant. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the time at which 1 tick crosses 3 cm of treated skin, followed within 30 minutes by a second confirmatory crossing."*

The endpoint for dosimetry is defined in in Study Synopsis, pg. 4, and on pg. 9, Section 4.1 Trial Endpoints as *"The typical consumer dose of one insect repellent product measured as ml/cm^2."* And It is also defined on pg. 18, Section 9.1. Statistical methods as **"***The endpoint for the consumer dose assessment is the mean dose from all 21 volunteers in mg/cm^2. This will be converted to ml/cm^2 taking into account the specific gravity of the test material."*

3. Sample size

The study protocol proposes to test the product on 30 participants (50:50 sex ratio), which corresponds to a sample size of 10 subjects per tick spp. A sample size of 21 participants is proposed for determination of consumer dose. "*Twenty-one participants will be required for the consumer dose evaluation whereas repellent product testing requires up to 30 participants (preferably with a 50:50 ratio of males to females). Participants will test the product at a single dose against at least one of the three* [tick] *species*." (pg. 8, Section 4. Trial Design).

"Twenty-one participants will be required for the consumer dose study and ten participants (at least three of each gender) will be required per tick species. All participants will be 18 to 65 years of age. Participants can take part in multiple experiments if they wish but can only do each test once. Therefore, a minimum of 21, and a maximum of 51 volunteers will be required to complete the study." "Volunteers will be given the option to consent to the determination of the consumer dose and/or to the determination of the CPT of one or more insect tick species." (pg. 11, Section 5. Participant Entry, and Subsection 5.1. Recruitment Number of Participants). Justification for a sample size of 21 participants for determination of consumer dose is provided on pg. 29, Section 14.2 Sample Size Calculations of the study protocol. The level of precision is 0.1 µl/cm^2 with a 95% confidence interval, for example 1.5 – 1.6 µl/cm^2. The 1.6 µl/cm^2 value is the standard deviation of doses applied to dosimeters derived from previous studies (references not provided). The formula used to calculate sample size at the given level of precision was taken from the publication of Kirkwood and Stern (2003). According to the sample size of 61 subjects was corrected for clustering as a single participant would contribute up to 9 data points (Hayes and Bennet, 1999), resulting in a sample size of 21 participants (See Table A1, *Sample size calculations to determine the consumer dose with a specified level of precision* on pg. 29 of the Study protocol, and Table 2 at the end of this review).

4. Study duration

The study is anticipated to last 12 weeks to have sufficient time to conduct screening, dosimetry testing, and repellent testing against all three tick species. Prior to efficacy testing, participants will undergo screening and dosimetry testing, which are estimated to last 1 hour, followed by a follow up period of 48 hours. Efficacy testing is scheduled for up to 10 hours from the time of product application, with follow ups of 48 hours and 1 week between test days (for adverse effects). (Pg. 13 Section 7. Participant timeline).

5. Determination of consumer dose and determination of CPT

The study is split into two parts; the first part is for determination of consumer dose, and the second part is for determination of CPT. Efficacy testing is designed to assess the longevity of consumer dose against three tick species. Consumer dose determination (Dosimetry) will employ 21 volunteers; each volunteer will perform 3 product applications to calculate the average dose per participant. The consumer dose, measured in mg of applied product /cm^2 skin surface area, is based on the grand mean of triplicates application across 21 subjects. The consumer dose, measured in mg/cm^2, will be converted to ml/cm^2 using the specific gravity of the formulation. Dosimetry phase is described on pg. 15, Section 8. Test Methodology. Subsection 8.1. Consumer dose determination. "*Participants will be asked to carry out a practice application to one forearm in order to allow them to get a feel for the spray bottle and formulation. The repellent will then be washed off with soap and water and patted dry with paper towels. Three pre-weighed gauze bracelets 3cm wide will be spaced evenly along the participant's forearm, and the arm circumference at these points recorded. Participants will be asked to apply the repellent to their forearm only as if they were about to enter a tick-endemic area. Two more applications will then be performed by each participant with the arm washed between each repetition. The weight gain of the bracelets will be used to determine the application rate applied. The "typical dose" in mg/cm^2* [converted to ml/cm2 skin, using specific gravity of the formulation] *will be calculated as the* [overall or grand mean from the] *mean of multiple applications* [3 applications per subject] *by each of the participants and* [converted to volume by] *the specific gravity of the test material*."

5.1 Estimation of Skin Surface Area

The skin surface area of both forearms (left and right) will be calculated for every subject forearms as the product of the sum of Wrist circumference + Elbow circumference multiplied by ½ of the forearm Length. The procedure is described on pg. 15, Section 8.2. Assessment of complete protection time. "*Participants' forearms (left and right) will be measured, and the surface area calculated using the formula:*

Forearm area = (Wrist circumference + Elbow circumference) x ½ Length forearm.

The dose required [for testing efficacy] *will then be calculated based on the outcome of the consumer dose test.*"

6. Randomization

Treatment will be applied to either left or right forearm depending on a randomization schedule. The schedule will randomly assign treatment to either left or right forearm for each of the 3 tick species tested for each participant.
(pg. 13. Section 6.2. Randomization).

7. Dose Application

Consumer dose for testing efficacy will be applied by the same research assistant, using a micropipette and applied with a single nitrile gloved finger to the whole right or left forearm from the boundary line up to the elbow, as described on pg. 13, Section 6. Interventions, "*The repellent will be carefully rubbed evenly onto the skin using a single finger by the same researcher wearing nitrile gloves. Participants will be asked to avoid rubbing or washing the arm for the first 20 minutes after application. If accidental or deliberate removal does occur, the test will be terminated for that day and repeated on another*."

8. Study Design

Efficacy testing for estimation of CPT will be conducted under laboratory conditions of 20-25 °C and over 35 % relative humidity. The treatment will be randomly applied to either the left or right forearm of each participant for each test. Each subject will act as their own control. There is no blinding employed since the outcome measures are based on tick behavior, and no positive controls are proposed. Both forearms of each participant, control and treated forearm, will be arranged in identical fashion, washed with unscented soap and water, dried with paper towels, and marked with 3 lines, 3 cm apart form each other, starting at the wrist toward the elbow. One line is drawn around the wrist, the "release" line, another is drawn 3 cm above the release line toward the elbow, that is the "boundary" line, marking the edge of the treated skin area, and a third line, drawn 3 cm above the boundary line toward the elbow, that is the crossing line (Figure 2 on pg. 16, Section 8.2. Assessment of complete protection time). These lines are used to define the criteria for repellency. Ticks crawling beyond the boundary line within 3 minutes and remaining on treated skin for 1 minute, are considered not repelled. Ticks that do not cross the boundary line, or that do but turn back or fall off are classified as repelled. Only ticks that are actively questing will be selected for repellency testing. Ticks will be screened for testing by placing individual ticks, oriented toward the elbow, at the release line on the untreated forearm. The untreated forearm will be resting at a 30° angle on a flat surface. Ticks that crawl upward across the boundary line toward the elbow will be selected for efficacy testing, and those that fail will be discarded by immersing them in isopropyl alcohol. The same procedure will

be repeated on the treated arm with actively questing ticks. The movement of the tick on the treated forearm will be timed for 3 minutes from the moment the tick is released on the release line (pg. 16, Section 8.2. Assessment of complete protection time).

9. Statistical Analysis and Sample Size Calculation

The median CPT within 95% confidence intervals, will be estimated from the CPT for each participant per tick species, using Kaplan-Meier survival analysis (pg. 18. Section 9. Statistical Analysis). Sample size calculations on pg. 18, under Section 9.1 Sample Size Calculations, have been revised and amended according to EPA's power simulation for determination of sample size in Attachment 3). The following is a brief summary of EPA's comments on sample size determination:

- EPA obtained the raw data from the authors of the Büchel et al. (2015) cited in the LSHTM protocol, corrected the data to reflect endpoints determined according to EPA's guidelines (first crossing vs. first confirmed crossing) and analyzed those data to determine the P5MR for the three repellents tested. The P5MRs ranged from 0.269 for EBAAP to 0.539 for Icaridin. The P5MRs for DEET products fell within that range.
- Given the small sample size (10 subjects), EPA did not find it appropriate to attempt to establish a confidence interval around these P5MR values.
- For EPA to accept a study as valid for showing a product's efficacy, the P5MR should be greater than or equal to 0.4. Study results showing P5MRs of 0.3-0.4 will generally be considered on a case by case basis. Any results where the P5MR is lower than 0.3 are unlikely to meet EPA's criteria for efficacy of a repellent product. The P5MR value indicates the "peakedness" of the CPT distributions (or the distributions of point of efficacy failure), with higher P5MRs indicating that more of the distribution is closer the median CPT. It is a measure of the "spread" of distribution of CPT for a given product, with flatter distributions suggesting greater expected variability in CPTs among users of the product. The lower the P5MR, the higher the spread or variation in CPT, and the greater the proportion of the population of users likely to experience protection times substantially less indicated on the product label. The protection time on the product label is generally the median CPT, and a small P5MR value indicates that a substantial portion of the population will realize a significant departure from that labelled protection time, or the median CPT.
- Based on the results of EPA's simulations, where the desired K=0.7, the median CPT is 4 or 6 hours, and where the expected P5MR is 0.4, the number of subjects likely to achieve sufficient precision with adequate power of at least 80% precision is 23-28 (Table A5).
- EPA (science, statistics, and ethics) and LSHTM agreed that a sample size of 25 would be adequate to ensure that the study includes enough subjects to return reliable results without unnecessarily including more subjects than necessary.

In addition, the study protocol proposes to pool all the data from all tick species tested on all volunteers to calculate period of lasting repellency, defined as to the time point at which less than 90% of the ticks are repelled. *"The data from all tick* [species] *on all volunteers will be pooled and the protective efficacy will be calculated for each time point using the formula below. The time at which protective efficacy fall below 90% will be reported.*

Protective efficacy = (Total ticks tested – Total ticks not repelled) x 100
Total ticks tested"

(pg. 18, Section 9.2 Statistical methods). However, it is recommended that this objective be removed from the protocol.

10. Withdrawal criteria:

Participants can withdraw at any time without giving a reason for withdrawing. Data collected to the point of withdrawal will be used in the statistical analysis of the data "*unless the participant requests that their data is not used, in which case it will be removed from the database. Participants may also be removed at the discretion of the Chief Investigator, where continued participation may affect the safety of the participant or where there is a development of any condition which might interfere with study participation*." (pg. 13, section 5.4. Withdrawal Criteria).

11. How and to what will human subjects be exposed/ Product description:

The products, are registered products (EPA Reg. No. 84878-2, 305-62), containing the active ingredient Citriodiol, also known as Oil of Lemon Eucalyptus (OLE). Citriodiol® contains a minimum of 65% *p*-Menthane-3,8-diol (PMD), which is the active component in OLE, as well as other naturally occurring constituents present in the essential lemon eucalyptus oil from which it is derived. Copies of submitted Master label include Sublabel A for liquid pump spray packaging, and Sublabel B for pressurized (bag-on-valve) (BOV) packaging**.**

Subjects will be exposed to the repellent product, EPA Reg. No. 84878-2 or EPA Reg. No. 305-62 for 10 hours of testing against each of 3 species of pathogen-free adult ticks from laboratory-reared tick colonies. Proposed exposure periods consist of exposing ticks to untreated human skin for screening, and exposing 1 individual tick to treated forearms for 3 minutes, at intervals of 15 minutes for a maximum of 10 hours of testing per species, or until the time point when repellent breakdown or CPT is reached by subject, whatever happens sooner.

EPA Reg. Nos. 84878-2, and 305-62, containing 30 % OLE, are categorized as Toxicity Category III for acute oral toxicity (LD_{50} > 2,000 mg/kg), acute dermal toxicity (LD_{50} > 2,000 mg/kg), and eye irritation (moderate irritant), and toxicity category IV for dermal irritation. OLE is not a skin sensitizer.

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 according to EPA's risk assessment (1999). 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**.** MOEs were 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.

12. GLP and QA

"This study will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, Good Laboratory Practices (as defined by 40 CFR part 160), protocol and all applicable local regulations." (pg. 2).

Arctec will assign the independent Quality Assurance Unit (QAU) person, who is independent from the study sponsor and will perform all QA duties.

13. Good Laboratory Practice (GLP) Compliance and Quality Assurance

"Good Laboratory Practices, as defined by 40 CFR part 160, will be followed throughout this study. arctec will be a member of the UK GLP compliance monitoring programme (GLP-CMP) before any trial procedures are undertaken. The UK is part of the OECD GLP Mutual Acceptance of Data agreement with the USEPA. Under this agreement, UK laboratories that are members of the UK GLP-CMP have met the US GLP burden of compliance, and data produced as such will be accepted as would data generated in the US under the USEPA GLP regulations. The UK GLP-CMP is run by the UK GLP Monitoring Authority (UK GLPMA). The GLPMA will inspect the test facility every 12-30 months, including an audit of completed and on-going studies to check that the principles of GLP have been complied with. Under the UK GLPMA, the QA unit will be a person employed by arctec, who is independent of study conduct and directly responsibly to management. The QA representative 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, positive and negative findings, 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 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. Under UK GLPMA, the responsibility for producing a statement of compliance rests with the Study Director." (pg. 8, Section 3).

14. Compliance with EPA, FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), and Good Laboratory Practice Standards (GLP); 40 CFR, Part 160 (October 1989):

The UK is part of the OECD GLP Mutual Acceptance of Data agreement with the USEPA. Under this agreement, UK laboratories that are members of the UK GLP-CMP have met the US GLP burden of compliance, and data produced as such will be accepted as would data generated in the US under the USEPA GLP regulations. Arctec will be a member of the UK GLP compliance monitoring programme (GLP-CMP). The UK GLP-CMP is run by the UK GLP Monitoring Authority (UK GLPMA). Under the UK GLPMA, the QA unit will be a person employed by arctec, who is independent of study conduct and directly responsibly to management. The QA representative will conduct critical phase inspections at intervals adequate to ensure study integrity, and maintain written and signed records of each inspection.

Study site location and testing facility: Arthropod Control Product Test Center (arctec), a division of Chariot Innovations Limited. A wholly-owned subsidiary of the London School of Hygiene & Tropical Medicine. (pg. 4)

Study Director: Professor James Logan, arctec, LSHTM, Chief Investigator (pg. 3)

Study Sponsor: Citrefine International Ltd. Moorfield Rd., Yeadon, Leeds. LS19 78N, UK

E. Compliance with Applicable Scientific Standards

This protocol adequately addresses the following elements according to applicable scientific standards:

- Experimental design
- Data analysis
- Risk minimization

F. EPA Science Comments

The study sponsor has agreed to make changes per the recommendations below with the exception of using the standard dose of 0.5 mg product/600 cm^2. EPA's recommendation and rationale for using the standard dose is presented in the science comments below. Instead of the standard dose, the sponsor intends to conduct a dosimetry phase to determine a typical consumer dose.

In summary, the following sections in the study protocol should be revised according to the following recommendations before the research goes forward:

1. Objectives: Determination of 90% repellency duration should be removed from study objectives in Study Synopsis, pg.3, and in Section 1.a. Objectives, pg. 7, and from Section 9.2 Statistical methods, pg. 18, in the protocol. The objective of the proposed study is to estimate lasting efficacy of the repellent product, expressed as complete protection time. This measure of efficacy is defined as the period of time between product application and first confirmed crossing. The first confirmed crossing signals repellency failure or CPT. First confirmed crossing is the endpoint used to measure lasting efficacy. Therefore, to be consistent, lasting efficacy doesn't need to be measured, or expressed, by sustained 90% repellency. In addition, update this section of the protocol by adding product EPA Reg. No. 305-62 to the objectives, and revise EPA Reg. No. 84878-2 (alt). Identify "(alt)," and explicitly state the registration numbers of the products proposed for testing throughout the document.

2. Endpoints: The definition of endpoints should be revised. The endpoint for estimation of CPT should be identified as the time to first confirmed crossing, signaling the time point of repellency failure or complete protection time.

Efficacy endpoint is defined on pg. 4, Study Synopsis; on pg. 9, Section 4.1 Trial Endpoints, and on pg. 18, Section 9.2. Statistical methods, as complete protection time, which is a measure of lasting repellency. The efficacy endpoint however, is the First Confirmed Crossing, which is defined in Section 9.2. Statistical methods, as 1 tick crossing 3 cm of treated skin, followed by a second confirmatory crossing [by another tick] within 30 minutes apart. The CPT used for the product should be the tick species which results in the lowest CPT.

Dosimetry endpoint, or dose determination is defined in Study Synopsis, pg. 4, and on pg. 9, Section 4.1 Trial Endpoints. It is defined in Section 9.1. Statistical methods, pg. 18, as the mean dose from a sample of 21 volunteers, measured as mg/cm^2 and converted to volume using the specific gravity value of the formulation. Endpoint of dosimetry should be accurately defined throughout the document consistently with the definition given in Section 9.1. Statistical methods, pg. 18, "*as the mean dose from a sample of 21 volunteers, measured as mg/cm^2 and converted to volume using the specific gravity value of the formulation*." In addition, update sample size to 25 subjects.

3. Sample size: LSHTM has agreed with EPA that a sample size of 25 would be adequate to ensure that the study includes enough subjects to return reliable results without including more subjects than necessary (Attachment 3 of this review; Table A5). Thus, the protocol should be updated to a sample size of 25 test subjects for efficacy testing, and the number of alternate subjects in section 6.2. Randomization, pg. 13, should be increased accordingly (See item #4. Number of alternate participants). In addition, using the same number of subjects (25) is also recommended for dosimetry testing.

4. Number of alternate participants: Update Section 6.2. Randomization, on pg. 13 of the protocol to state the number of alternate subjects, including distribution of their sex ratio according the amended sample size of 25 subjects. The total number of participants (75 participants) in section 6.2. Randomization, pg. 13, includes alternates, that number should be updated according to amended sample size of 25 test subjects per tick species. In addition, consider recruiting alternate subjects during recruitment process, and describe how participants will be randomly assigned as either test subjects or alternates.

5. Estimation of skin surface area: Describe in more detail how to measure the forearm surface area. For example, the narrative could include the use of 4 or 3 equidistant bracelets (i.e., 4 or 6 cm wide) placed above the wrist to determine the average circumference of the forearm at more than 2 points, wrist and elbow as proposed, and multiply the average circumference by the length of the forearm. Wrist and elbow are not enough to estimate the average circumference of the forearm. (OCSPP 810.3700 Guidelines, (i) *Specific guidance for Dose Determination***,** (3) *Measuring subject's skin area*). **"***The surface area of subject's limb in* cm^2 *can be estimated by measuring the circumference of the forearm in centimeters at the wrist and elbow, or of the leg at the ankle and knee, and in either case at one or two equally spaced intermediate poin*ts"

6. Dose application and time to first exposure: The statement "*Participants will be asked to avoid rubbing or washing the arm for the first 20 minutes after application"* in Section 6. Interventions, pg. 13**,** is unclear. Explain why participants are not asked not to disrupt treated skin throughout the entire period of testing. In addition, clarify whether the 20 minutes after application (as stated in Section 6. Interventions, pg. 13), indicates that testing (exposure of ticks to treated skin) will begin 20 minutes post-application. It is not clear whether exposure of ticks to treated skin will start 20 minutes post-application, or whether exposure of ticks to treated skin will begin immediately after application.

7. Definition of "crossing" and criteria for repellency: Define "crossing" as stated in the OCSPP 810.3700 Guideline, (b) Definitions (i) (iv) "*A* ***crossing*** *is the act of passage by a tick or chigger from an area of untreated skin to an area of treated skin. A crossing may be quantified either or both by the distance the tick or chigger moves onto treated skin or by how long the tick or chigger remains on treated skin."* Add the criteria for repellency to Section 8.2. Assessment of complete protection time on pg. 16 of the study protocol. If the crossing line is eliminated from the design then, the criteria for no repellency should be

amended as "a tick is classified as not repelled when it crosses the boundary line and spends 1 minute on treated skin." In addition, describe the criteria for determination of first confirmed crossing: Breakdown of the product occurs when a tick crossing unto treated skin is not repelled, and it is followed by a second tick that is also not repelled, or it is followed by a second tick that is repelled, but if the second tick is followed by the next tick (the third tick), and that third tick is not repelled then, the third tick is considered a confirmatory crossing within a 30 minute period for the first tick that was repelled.

8. Statistical analysis and sample size calculation: EPA (science, statistics, and ethics) and LSHTM agreed that a sample size of 25 would be adequate to ensure that the efficacy study includes enough subjects to return reliable results without including more subjects than necessary. The same sample size and the same subjects should also be employed for dosimetry.

9. Alternate subjects and subjects' withdrawal: Specify that alternate subjects will replace individual subjects who withdraw from the study. If a subject withdraws after a full day of testing with one tick species, they will be replaced for testing with the next tick species. The data for the completed test day will be used. However, if subjects withdraw before completing a test day their data will not be used and they will be replaced with an alternate subject.

10. Standard versus consumer dose: *Standard dose (EPA recommendation):* Although EPA's product performance guidance includes steps for a dose determination phase as part of an insect repellent efficacy study, to minimize the exposure of test subjects, EPA recommends using a standard dose based on previously reviewed and accepted studies. Based on an analysis of dosimetry results from repellent studies reviewed by EPA and HSRB since 2006 (Table 1), EPA considers the dose of 1 g product/600 cm^2 of skin to be an appropriate product dose for testing aerosol, wipes, and lotion type products and 0.5 g (± 10%) product/600 cm^2 of skin for testing pump spray type products under this protocol.

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) ± 1 SD**	**Dose range (g/600 cm^2)**
Lotion	112	0.933 ± 0.299	0.63-1.23
Pump spray	102	0.417 ± 0.120	0.28-0.56
Aerosol	25	0.815 ± 0.262	0.55-1.08

Reasons for using a set standard application rate rather than Dosimetry:

1. Minimize variability. Variable application rates between studies make it impossible to determine whether performance of similar formulations (pump, lotions or aerosols) tested at different consumer doses is driven by variable application rates or by the test products per se.
2. Dosimetry testing introduces additional test subjects for an adequate sample size.
3. The standard application rates for pump spray products are based on dosimetry data from 10 repellent studies previously reviewed by EPA/HSRB (Table 1). One of these studies was conducted using EPA Reg. No. 305-62, one of the products proposed for testing. In the dosimetry phase for EPA Reg. No. 305-62, the average typical dose applied to subject's legs (n = 10) was 0.28 g/600 cm^2. Although the registrant submitted a rationale indicating that pump sprays will be applied at higher rates to subject's arms, EPA's analysis contains five studies conducted with pump sprays applied to subject's arms and the mean typical consumer dose (± SD) from only these six studies is 0.496 ± 0.09 g product/600 cm^2. Also, as seen in Table 1 the high end of the dose range for pump sprays is 0.56 g/600 cm^2.

Therefore, EPA expects the results of a dosimetry phase conducted with the proposed products to be consistent with the results of previous studies.

Dosimetry phase (Sponsor preference): The study sponsor expects the typical consumer dose rate to be substantially higher than EPA's recommended standard dose, and therefore believes the recommended standard dose will result in an underestimate of the actual protection time against ticks offered by this product for the following reasons.

1. The data relied on to support EPA's standard dose as based in whole or in part upon dose rate studies that only measure self-application to the lower leg. The relevance of these data to this tick study is questionable because this study will assess application to the consumer's forearm.
2. The pump spray mechanism functions optimally when used upright or at a slight angle, and flow-through is reduced when consumers angle the pump spray mechanism significantly as naturally occurs to apply to areas below the waist.
3. Risk associated with exposure of human subjects during a dosimetry phase de minimus; the product is already registered by EPA and has been on the market for over a decade. Additionally, in this study the product is only being applied to a fraction of the skin area to which a consumer would be expected to apply.

10. Calculation of consumer dose: If dosimetry testing is preferred, the protocol should describe the method employed for quantification of applied product, including how the average consumer dose is calculated. For example, the study protocol should show the calculations that will be employed for quantification of applied product, the mean dose applied by each subject to each limb, and the grand mean across all subjects' means. (OCSPP 810.3700 Guidelines, (i) *Specific Guidance for Dose Determination (5) Calculating standard dose for use in repellency trials*).

11. Remove the reference to previous consumer dose testing with this product because EPA cannot rely on those data.

12. Mode of application for determination of consumer dose: The statement on pg. 15, Section 8. Test Methodology, Subsection 8.1. Consumer dose determination, "*Participants* [for dosimetry testing] *will be asked to apply the repellent to their forearm only as if they were about to enter a tick-endemic area*" should be removed from the protocol. Subjects should be instructed to follow label recommendations for application of the product to their forearms.

13. Please include copies of the data collection sheets as part of the study protocol in a separate Appendix.

14. On pg. 29, §14.2 Sample size calculation, it is not explained how the level of precision for a 95% confidence interval of 0.1 µl/cm^2, e.g., example 1.5-1.6 µl/cm^2, was calculated (Table 2 below corresponds to Table A1 under the same section) to determine sample size of 21 subjects. Consider omitting this part in the protocol. This sample size calculation could be eliminated if the same sample size for 25 subjects already determined for efficacy testing is also employed for dosimetry testing.

Table 2. *Sample size calculations to determine the consumer dose with a specified level of precision* [0.1 µl/cm^2] (reproduced from Table A1, pg. 29 of the Study protocol).

Size of 95% confidence interval	Uncorrected sample size [A]	Cluster size [B]	Number of participants
0.4	4	9	2
0.3	7	9	3
0.2	16	9	6
0.1	61	9	21
0.075	109	9	37
0.05	244	9	82

[A] according to the publication of Kirkwood and Stern (2003)
[B] according to the publication of Hayes and Bennet (1999)

15. Add the following to section 8.1 Consumer Dose Determination on pg. 15: "the typical consumer dose in mg/cm^2 will be converted to volume using the specific gravity of the test material."

16. On pg. 15, § 8.2, state that control arm will be arranged in an identical fashion as treated arm, but without repellent. In addition, questing behavior on the control arm should be described as "a tick that moves steadily from the release line across boundary line and upward."

17. On pg. 16, § 8.2, the statement: "*This will be repeated so that five actively questing ticks will be exposed to the treated arm one at a time, at 30 minute intervals (timed from product application) for 10 hours or until treatment failure*" - should be changed if the exposure model is changed to 1 tick every 15 minutes. Support this change with the following rationale:

"EPA revised the model discussed with LSHTM in December to include simulations for up to 30 subjects doing testing with 5 ticks every 30 minutes. EPA also developed simulations for up to 30 subjects doing testing with 1 tick every 15 minutes, another option for testing listed in EPA's guidelines (https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0150-0011)."

18. Formulation of Products on pg. 13, § 6, subsection 6. Information about product composition is considered confidential business information and should be removed from the protocol.

19. Risks and Benefits - In section 4.2, make the statement clear that for individual subjects, at least 48 hours will lapse between test days involving exposure to ticks.

20. Please make the following corrections on the data sheets collection provided to EPA:

a) For dosimetry phase, change the directions for the practice applications to read as follows "Give the participant the product bottle and ask them to read the label instructions and then practice spraying the product onto their forearm. DO NOT offer advice on the application method."

b) When calculating the consumer dose rate on the forearm measurement & dose calculation sheet, report the amount in weight units.

c) In the section on preparation of participant's arms, make sure that release and boundary lines reference the correct locations (e.g., boundary line will be drawn at the wrist, and release line will be located 3 cm below boundary line.)

Attachments:

1. EPA Protocol Review (Protocol dated July 21, 2017)
2. Completeness checklists
3. EPA's Analysis & Support for Sample Size
4. EPA's comments on the IRB-approved protocol
5. EPA's comments on the IRB-approved informed consent materials
6. IRB approval of protocol dated July 21, 2017
7. IRB approved protocol & informed consent materials
8. IRB correspondence
9. IRB minutes

Attachment 1 - EPA Protocol Review

Title: A single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks

Date: July 21, 2017

Principal Investigator and any sub-investigators: Dr. James Logan, arctec

Participating Laboratory: Arthropod Control Product Test Centre (*arctec*), a division of Chariot Innovations Limited, a wholly-owned subsidiary of the London School of Hygiene & Tropical Medicine (LSHTM).

Sponsor: Citrefine International Ltd. Moorfield Rd., Yeadon, Leeds. LS19 78N, UK

Trial Monitoring Center:
arctec
Room LG38, LSHTM
Chariot Innovations Ltd.
Keppel St.
London WC1E 7HT
UK

IRB:
Western Institutional Review Board
1019 39th Avenue SE Suite 120
Puyallup, WA 98372-2115

1. Societal Value of Proposed Research

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

This study is designed to determine the complete protection time (CPT) of EPA registered repellents, EPA Reg. No. 84878-2 and 305-62, containing 30% Citriodiol® (Oil of Lemon Eucalyptus (OLE)) as its active ingredient, against 3 adult tick species, (pathogen free) *Amblyomma americanum, Rhipicephalus sanguineus,* and *Ixodes scapularis,* at an average consumer dose. The product will be tested on human subjects in a laboratory setting for up to 10 hours. EPA requires efficacy testing of products claiming efficacy against disease vectors to support efficacy claims on product labels. "*The primary objectives are to determine the complete protection times of the formulation corresponding with EPA Reg. No. 84878-2 (alt) insect repellent against adult Ixodes scapularis, Amblyomma americanum and Rhipicephalus sanguineus applied at the consumer dose rate*." Study (Synopsis, pg. 3). The aim of the proposed study is to determine the duration of efficacy of an insect repellent containing 30% OLE against three tick species. The secondary objective is to determine the average consumer dose for assessment of efficacy. (Section 1. a. Objectives (pg. 7).

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

The purpose of the study is to determine the mCPT of a personal, skin-applied tick repellent product, containing the active ingredient OLE. This information does not currently exist.

The rationale for testing is to collect data to establish a median complete protection time. The data supporting currently registered products are not sufficient to establish a median complete protection time for the product discussed in the protocol.

A standardized protocol will enable the EPA to receive consistent and scientifically reliable data about the complete protection time for the product. The laboratory testing data will provide information about: 1) a "consumer dose" derived from 25 subjects each making 3 applications of the product to one forearm, and 2) the length of time after treatment before the first confirmed crossing by a tick. Ticks will not be permitted to bite subjects. The endpoint is crossing, and ticks attempting to attach will be removed before they can bite a subject.

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

EPA requires product-specific efficacy data conducted to assess skin applied insect repellent products in terms of the recommendations of the EPA OPPTS 810.3700 Guideline. These products have not been evaluated for their performance against ticks. EPA will review the study to satisfy product specific efficacy data requirements and acceptable label claims for repellent efficacy for the test products.

(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 this product against ticks 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.

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 efficacy of an insect repellent containing 30% OLE against three tick species.
The primary objectives are to:

1. *Determine the complete protection time (CPT) of EPA Reg. No. 84878-2 and 305-62 formulated insect repellents against adult Ixodes scapularis applied at the consumer dose rate.*
2. *Determine the CPT of EPA Reg. No. 84878-2 and 305-62 formulated insect repellents against adult Amblyomma americanum applied at the consumer dose rate.*
3. *Determine the CPT of EPA Reg. No. 84878-2 and 305-62 formulated insect repellents against adult Rhipicephalus sanguineus applied at the consumer dose rate.*

The EPA has established consumer dose rates for pump sprays of 0.83 mg/cm^2. As these two figures are so different, a consumer dose rate study would allow this protocol to test a realistic dose rate, rather than relying on rates established for other products. The secondary objectives are to: 1) determine the average dose rate applied by consumers when using EPA Reg. No. 84878-2) formulated insect repellent to repel ticks" (pg.7, §2).

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

The objective cited may be achieved by the study if the protocol is revised and amended in accordance with EPA's comments on the ethical and scientific aspects of the protocol.

2.1 Statistical Design

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

The original protocol submitted arctec proposed that 10 individuals serve as test subjects. However, the justification for the proposed sample size is not supported statistically. After consultation with EPA, arctec and the Study Sponsor have agreed to use EPA's recommended sample size of 25 test subjects, the methodology for which is described in Attachment 3 to EPA's science and ethics review of the protocol. A sample size of 25 would be adequate to ensure that the study includes enough subjects to return reliable results without unnecessarily including more subjects than necessary (pg. 18, § 9).

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

One arm from each subject will remain untreated and serve as the untreated control for the purpose of screening active questing ticks, and the other arm will be treated with the test product and serve as the treatment group. A positive control will not be used (pg.16, § 8.2).

(c) How is the study blinded?

The study is not blinded. There is only one product tested at a time, and observations are based on tick behavior.

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

"The treatment will be applied to either the left or the right arm of the participants depending on a randomization schedule to be produced by the trial statistician. The schedule will assign treatment to either the left or right forearm for each of the three tick species for each participant up to eighty participants. This number should be sufficient to cover the participant numbers of volunteers who consent, but fail screening or who withdraw or are withdrawn before taking part in efficacy testing" (pg. 13, § 6.2).

(e) Can the data be statistically analyzed?

Yes. See (f) below.

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

The median CPT of all test subjects for each tick species will be calculated using the Kaplan-Meier survival analysis. The duration of protection for the repellent product will be the lowest median CPT of the 3 tick species (pg. 18, §9). EPA recommends rounding down to the nearest whole number. For example, three hours and 45 minutes would be listed on the label as three hours

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

The median CPT, will be estimated from the CPT for each participant per tick species, 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 median CPT 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 has been revised and updated according to EPA recommendations for a sample size of 25 subjects. This recommendation is based on the results from EPA's simulations, where the desired K=0.7, the median CPT is 4 or 6 hours, and where the expected P5MR is 0.4, the number of subjects likely to achieve sufficient precision with adequate power of at least 80% precision is 23-28. For EPA to accept a study as valid for showing a product's efficacy, the P5MR should be greater than or equal to 0.4. Study results showing P5MRs of 0.3-0.4 will generally be considered on a case by case basis. Any results where the P5MR is lower than 0.3 are unlikely to meet EPA's criteria for the efficacy of a repellent product. The P5MR value indicates the "peakedness" of the CPT distributions (or the distributions of point of efficacy failure), with higher P5MRs indicating that more of the distribution is closer to the median CPT. It is a measure of the "spread" of distribution of CPT for a given product, with flatter distributions suggesting

greater expected variability in CPTs among users of the product. The lower the P5MR, the higher the spread or variation in CPT, and the greater the proportion of the population of users likely to experience protection times substantially less indicated on the product label. The protection time on the product label is generally the median CPT, and a small P5MR value indicates that a substantial portion of the population will realize a significant departure from that labelled protection time, or the median CPT. For detailed information on the statistical simulation see Attachment 3 of this review.

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to an EPA-registered repellent product, either EPA Reg. Nos. 84878-2 and 305-62 containing 30% oil of lemon Eucalyptus (OLE). The active ingredient in OLE is 65 % p-Methane-3,8-diol (PMD). The application of the product to the skin of subjects in the proposed study will be consistent with the label directions for use. The active and inert ingredients of a currently registered formulation have undergone EPA review and the requirements are fulfilled for EPA registration of repellent product.

Subjects will be exposed to ticks during the laboratory-based repellent testing. To eliminate the risk of transmission of tick-borne disease, ticks will be sourced from pathogen-free colonies at Oklahoma State University Tick Rearing Facility. Ticks will only be used once with a single subject, and ticks will be destroyed by immersion in alcohol after they are placed on a subject's arm.

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

Efficacy data to satisfy product performance requirements and to support label claims of repellency against ticks for this product are required by EPA for registration. EPA requires submission of product performance data for all products claiming efficacy against public health pests.

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

The rationale for testing is to collect data to establish a median CPT for a product containing OLE. The data supporting currently registered OLE products do not provide this information.

The dose for the repellency phase of the study will be the grand mean of the 3 doses applied by each of the 25 subjects during the dosimetry phase.

Standard dose (EPA recommendation): Although EPA's product performance guidance includes steps for a dose determination phase as part of an insect repellent efficacy study, to minimize the exposure of test subjects, EPA recommends using a standard dose based on previously reviewed and accepted studies. Based on an analysis of dosimetry results from repellent studies reviewed by EPA and HSRB since 2006 (Table 1), EPA considers the dose of 1 g product/600 cm^2 of skin to be an

appropriate product dose for testing aerosol, wipes, and lotion type products and 0.5 g (± 10%) product/600 cm^2 of skin for testing pump spray type products under this protocol.

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) ± 1 SD**	**Dose range (g/600 cm^2)**
Lotion	112	0.933 ± 0.299	0.63-1.23
Pump spray	102	0.417 ± 0.120	0.28-0.56
Aerosol	25	0.815 ± 0.262	0.55-1.08

Reasons EPA recommends using a set standard application rate rather than dosimetry:

1. Minimize variability. Variable application rates between studies make it impossible to determine whether performance of similar formulations (pump, lotions or aerosols) tested at different consumer doses is driven by variable application rates or by the test products per se.
2. Dosimetry testing introduces additional test subjects for an adequate sample size.
3. The standard application rates for pump spray products are based on dosimetry data from 10 repellent studies previously reviewed by EPA/HSRB (Table 1). One of these studies was conducted using EPA Reg. No. 305-62, one of the products proposed for testing. In the dosimetry phase for EPA Reg. No. 305-62, the average typical dose applied to subject's legs (n = 10) was 0.28 g/600 cm^2. Although the registrant submitted a rationale indicating that pump sprays will be applied at higher rates to subject's arms, EPA's analysis contains five studies conducted with pump sprays applied to subject's arms and the mean typical consumer dose (± SD) from only these six studies is 0.496 ± 0.09 g product/600 cm^2. Also, as seen in Table 1 the high end of the dose range for pump sprays is 0.56 g/600 cm^2.

Dosimetry Phase (Sponsor Preference): The study sponsor expects the typical consumer dose rate to be substantially higher than EPA's recommended standard dose. The Study Sponsor proposes to conduct a dosimetry phase to derive a realistic consumer dose for this product, which impact the repellent testing. The Study Sponsor expects that the dose derived from the dosimetry phase would result in an accurate CPT for this product, thereby preventing a significant underestimate of the efficacy, which in turn could result in millions of consumers over-applying the product. The Study Sponsor believes using EPA's recommended standard dose

would result in an underestimate of the actual protection time against ticks offered by this product for the following reasons.

1. The data relied on to support EPA's standard dose as based in whole or in part upon dose rate studies that only measure self-application to the lower leg. The relevance of these data to this tick study is questionable because this study will assess application to the consumer's forearm.
2. The pump spray mechanism functions optimally when used upright or at a slight angle, and flow-through is reduced when consumers angle the pump spray mechanism significantly as naturally occurs to apply to areas below the waist.
3. Risk associated with exposure of human subjects during a dosimetry phase de minimus; the product is already registered by EPA and has been on the market for over a decade. Additionally, in this study the product is only being applied to a fraction of the skin area to which a consumer would be expected to apply.

(c) What duration of exposure is proposed?

The exposure is a 3-minute period at 15 minute intervals. The tick is placed on the untreated arm to determine if it is attracted to the subject's skin. After a tick is deemed to be attracted to the subject, and actively questioning, the tick is then placed on the treated arm for 3 minutes. This exposure process is repeated every 15 minutes with a different questing tick, and thereafter for 10 hours or until the test substance fails to repel the tick (first confirmed crossing takes occurs). (pg. 16, § 8.2).

2.3 Endpoints and Measures

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

Efficacy endpoints are defined on pg. 4, Study Synopsis, as "*Time to complete protection time measured as a single tick crossing 3cm into treated skin, and time to 90 % protective efficacy measured as more than 10% of ticks crossing treated skin***."** Efficacy endpoints are defined on pg. 9, Section 4.1 Trial Endpoints**,** as "*The primary endpoint measure is the complete protection time (CPT), which is defined as the time between application of the repellent product and the time at which 1 tick crosses 3cm of treated skin, followed within 30 minutes by a second confirmatory crossing. The median CPT will be calculated for each species using Kaplan-Meier survival curves. The first secondary endpoint measure is the average dose rate applied by consumers when using EPA Reg. No.84878-2 formulated insect repellent according to label instructions for purposes of repelling ticks. Other secondary endpoint measures will be the time to 90% protective efficacy of EPA Reg. No. 84878-2 formulation against Ixodes scapularis, Amblyomma americanum and Rhipicephalus sanguineus*." The endpoint for estimation of CPT is correctly defined on pg. 18, Section 9.2. Statistical methods, as the time to failure, which is the time when a first tick crossing into 3 cm of treated skin is followed by a second confirmatory crossing from a different tick within 30 minutes from the first crossing. "*The endpoint for CPT will be time to treatment failure for each participant test. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the*

time at which 1 tick crosses 3 cm of treated skin, followed within 30 minutes by a second confirmatory crossing." (pg. 18, § 9.2. Statistical methods. If the crossing line is removed from the design, the criteria for repellency is changed to a tick that crosses the boundary line and spends 1 minute on treated skin.

The endpoint for dosimetry is defined in the same section as *"The typical consumer dose of one insect repellent product measured as ml/cm^2."* And It is also defined on pg. 18, Section 9.1. Statistical methods as *"The endpoint for the consumer dose assessment is the mean dose from all 21 volunteers in mg/cm2. This will be converted to ml/cm^2 taking into account the specific gravity of the test material."*

Using the Kaplan-Meier estimator, the Median CPT will be calculated for all test subjects exposed to each tick species.

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 will be followed throughout all studies.
- Study staff will prepare forearm for dose application, and will measure skin surface area in advance, and perform dose application for testing.
- Study staff will prepare both forearms to assess tick behavior on untreated and treated forearms.
- Study staff will place ticks on the untreated and treated arms.
- Study staff will monitor and record tick movement and the start and stop times for each exposure period.
- Study staff and study director will closely monitor the testing, and data recording.
- 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 two calendar days in between test days for subjects participating in more than 1 test.

(c) What QA methods are proposed?

"Arctec will be a member of the UK GLP compliance monitoring programme (GLP-CMP) before any trial procedures are undertaken. The UK is part of the OECD GLP Mutual Acceptance of Data agreement with the USEPA. Under this agreement, UK laboratories that are members of the UK GLP-CMP have met the US GLP burden of compliance, and data produced as such will be accepted as would data generated in the US under the USEPA GLP regulations.

"The UK GLP-CMP is run by the UK GLP Monitoring Authority (UK GLPMA). The GLPMA will inspect the test facility every 12-30 months, including an audit of completed and on-going studies to check that the principles of GLP have been complied

with. Under the UK GLPMA, the QA unit will be a person employed by arctec, who is independent of study conduct and directly responsibly to management. The QA representative 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, positive and negative findings, 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 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. Under UK GLPMA, the responsibility for producing a statement of compliance rests with the Study Director." (pg. 8, §3).

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

Sources of variation include tick species, source of ticks, tick activity, and attractiveness of subjects. These uncertainties will be addressed by each subject serving as their own control for qualifying ticks, using a single source for a single tick species, qualifying ticks to ensure they are questing, and using the lowest mCPT for the three tick species for the duration of efficacy.

3. Subject Selection

3.1 Representativeness of Sample

The population of repellent users is presumed to be diverse in age, gender, physical size, general health, attractiveness to biting insects, and other characteristics. EPA recommended that the protocol state explicitly that eligible subjects will be selected to be representative by gender.

(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 from the London area, specifically from the area including and surrounding the London School of Hygiene and Tropical Medicine.

EPA recommends that the protocol be amended to expand the recruitment area (i.e., include the London area), add a phone number that people can use to express an interest in participating, and describe in detail the recruitment process. For example, where will advertisements be posted and for how long? What steps will arctec take to ensure that the recruited population is representative of the general public/users of skin-applied insect repellents? Who will make contact to with people who express an interest in participating?

Participants will be recruited from a pool of interested candidates who meet the inclusion and exclusion criteria in Section 5.2 of the protocol, as outlined below (EPA recommendations incorporated):

Inclusion criteria

- Able to understand and comply with the study procedures
- Able to speak and understand English
- Able and willing to give fully informed consent
- Aged 18 to 65 years
- Consider themselves to be in good general health
  - no serious cardiac disorder (whether active or inactive)
  - no serious respiratory disorder
  - no compromised immune system
  - no history of anaphylaxis
- Non-smokers or willing to refrain from smoking for 24 hours prior to and during each test

Exclusion criteria

- Localised skin disorders affecting the forearms where the product will be applied (including but not restricted to open wounds, eczema, psoriasis, dermatitis or open wounds)
- Participation in another clinical intervention study (excluding biting insect challenge studies*) in the previous 3 months
- Participation in another biting insect challenge study* in the previous 72 hours
- Known or suspected history of tick bite paralysis or tick bite allergies
- Known phobia of ticks or tick bites
- Known history of anaphylaxis as a result of insect bite
- Known allergy or sensitivity to Oil of Lemon Eucalyptus or any other repellent ingredients
- Employees and spouses of employees of the Study Sponsor (Citrefine, Inc.), arctec, and those directly line managed by Professor James Logan (study director)
- Students of the study director, principal investigator, or any other faculty members involved in this study

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

The participants should be representative of the population of concern, with EPA's recommendations about recruitment addressed. Further, EPA recommends that arctec strive to balance the number of males and females consented to participate in the study.

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

Yes.

3.2 Equitable Selection of Subjects

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

The inclusion/exclusion criteria are complete and appropriate assuming EPA's comments, identified in red below, are incorporated. (Section 5.2).

Inclusion criteria

- Able to understand and comply with the study procedures
- Able to speak and understand English
- Able and willing to give fully informed consent
- Aged 18 to 65 years
- Consider themselves to be in good general health
  - no serious cardiac disorder (whether active or inactive)
  - no serious respiratory disorder
  - no compromised immune system
  - no history of anaphylaxis
- Non-smokers or willing to refrain from smoking for 24 hours prior to and during each test

Exclusion criteria

- Localised skin disorders affecting the forearms where the product will be applied (including but not restricted to open wounds, eczema, psoriasis, dermatitis or open wounds)
- Participation in another clinical intervention study (excluding biting insect challenge studies*) in the previous 3 months
- Participation in another biting insect challenge study* in the previous 72 hours
- Known or suspected history of tick bite paralysis or tick bite allergies
- Known phobia of ticks or tick bites
- Known history of anaphylaxis as a result of insect bite
- Known allergy or sensitivity to Oil of Lemon Eucalyptus or any other repellent ingredients

- Employees and spouses of employees of the Study Sponsor (Citrefine, Inc.), arctec, and those directly line managed by Professor James Logan (study director)
- Students of the study director, principal investigator, or any other faculty members involved in this study

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

None. EPA has suggested that the protocol exclude from participation employees and spouses of employees of the Study Sponsor (Citrefine, Inc.), arctec, and those directly line managed by Professor James Logan (study director). In addition, EPA recommends excluding students of the study director, principal investigator, or any other faculty members involved in this study.

(c) Are any potential subjects from a vulnerable population?

The protocol does not propose to target recruitment to vulnerable populations.

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

"*Volunteers will be recruited through emails, posters, and social media to staff and students of LSHTM. All adverts, whether sent by email or on posters will contain a brief description of the trial methods, an invitation to take part and an email address with which to contact the Trial Coordination Centre (Appendix 14.3).*

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

"*Volunteers will be fully informed before the study and it will be made clear that they can withdraw from the study at any time. Volunteers will be given and asked to read the Participant Information Sheet and Product Information Sheet which describes the tests which they will take part in, and a consent form which must be signed before the test begins.*

"Volunteers will be consented prior to consumer dose determination being undertaken or being exposed to ticks. Volunteers will be given the option to consent to the determination of the consumer dose and/or to the determination of the CPT of one or more insect tick species.

"Volunteers who do not meet the criteria for eligibility will be excluded. Individuals who express an interest in participating in response to the recruitment materials will be contacted by telephone or e-mail to determine whether they meet the basic inclusion criteria. If they are interested in enrolling in the study, they will be given a time, date and location to meet with arctec staff for a private face-to-face meeting to learn more about the study and their potential role in it, go over the

inclusion/exclusion criteria and receive answers to any questions they may have. Volunteers who do not meet the criteria for eligibility will be excluded. The volunteer will complete an eligibility questionnaire and discuss their answers with the researchers if necessary. Contact information is included on the consent form for any individual who has additional questions or if further clarification is desired." (Section 5.2)

EPA recommends that the protocol be amended to expand the recruitment area (i.e., include the London area), add a phone number that people can use to express an interest in participating, and describe in detail the recruitment process. For example, where will advertisements be posted and for how long? What steps will arctec take to ensure that the recruited population is representative of the general public/users of skin-applied insect repellents? Who will make contact to with people who express an interest in participating?

EPA also recommends that the protocol be revised to include a more detailed section covering the information to presented to participants and how it will be presented. For example, "This meeting will cover a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the pesticide to which they will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with the dosimetry test, as well as a 15-minute exposure interval for the tick repellent testing will be explained and demonstrated step-by-step to all subjects who participate in the training. The subjects will be shown how the test substances will be applied to their arm for the future testing."

(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 emails, posters, and social media postings. 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 and spouses of employees of the study sponsor are excluded from participation.

3.3 Remuneration of Subjects

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

The protocol initially proposed "*Volunteers will be paid to compensate for their time when participating in testing. Volunteers will not be paid for consent and screening appointments. Volunteers will be paid £20 each for taking part in the consumer dose test, which is estimated to take approximately 30 minutes. Volunteers will be paid £60 for taking part in a repellent efficacy test which involves approximately 20 minutes*

each half hour up to 10 hours." (Section 11.6) EPA recommends compensating subjects for the consent meeting at a flat rate (e.g., £20 each for taking part in the screening and consent meeting, which could take up to an hour). EPA also recommends compensating subjects on an hourly basis, at close to the minimum wage (note: in the UK, minimum wage varies by age), £7.50/hour for the lab-based repellent testing, rounded up to the next hour.

(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?

With EPA's recommendations addressed, no.

(d) How and when would subjects be paid?

"Participants will be paid in cash after each test session." (Section 11.6)

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 a tick repellent product (either EPA Reg. No. 84878-2 or 305-62) that is registered by the US EPA. The application of these product to the skin of subjects in this study will be consistent with the directions for use on these products, and therefore is determined to be no toxicological concern. The active and inert ingredients in a currently registered formulation have undergone EPA review and fulfilled the requirements needed for EPA registration as repellent products to be applied to human skin.

The active component in Oil of Lemon Eucalyptus (OLE) is 65 % p-Methane-3,8-diol (PMD). A full risk assessment for PMD technical which is used to formulate end-use products was conducted in 1999. A 90-Day subchronic 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 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 are 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 on one developmental study assessing dermal application in rabbits. In rabbits, the NOAEL = 3,000 mg/kg/day. No LOAEL was established, and a 10-

fold safety factor is applied for risk characterization. MOAEs were 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 humans from the use of PMD in insect repellent products applied to human skin.

The toxicity profile for EPA Reg. Nos. 84878-2 and 305-62 is: toxicity category III for acute oral, acute dermal, and acute eye irritation, and acute toxicity category IV for acute inhalation and acute dermal irritation. Acute oral toxicity (LD_{50} > 2,000 mg/kg), acute dermal toxicity (LD_{50} > 2,000 mg/kg). For eye irritation, the product is moderate irritant. For dermal irritation, the product is not a skin irritant, and for skin sensitization, the product is not a skin sensitizer.

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

Risks to subjects include the risk of exposure to ticks, the risk of exposure to the test material, risks related to receiving an unexpected result on a pregnancy test, and the risk of a loss of confidentiality.

(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 measured?

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; risks are minimized by excluding candidates known to be hypersensitive to or phobic of tick bites; using pathogen-free colony-raised ticks; excluding candidates known to be sensitive to insect repellents; excluding subjects with open cuts, scrapes, skin disease and skin problems; including medical monitoring procedures; and incorporating procedures to keep the subjects' identities and results of pregnancy testing private, and to permit discrete withdrawal.

4.2 Risk minimization

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

Participants who do not meet the eligibility criteria will be excluded from participating. Pathogen-free ticks will be used in the study, discarded after use with a single subject, removed immediately upon the appearance of trying to attach, and counted in and out of the lab. Study team members will follow up with all subjects

48 hours after each instance of their participation in the study. Subjects in the dosimetry phase will use eye protection when applying the repellent to their arms to avoid eye irritation.

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

"*Volunteers will be monitored throughout the duration of the tests by investigational staff for any adverse events. If any adverse events related to tick bites or the repellent product are apparent at any time during the trial, testing will stop immediately and details of how to access treatment will be offered.*" (Section 10.2)

"*The Study Director or other designated study staff may end a particular participant's participation on a test day, at any time, for any reason.*" (Section 11.6)

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

Study staff will follow up with subjects 48 hours after their participation to assess whether any adverse effects have occurred.

(d) How does the protocol provide for safety monitoring?

"*Volunteers will be monitored throughout the duration of the tests by investigational staff for any adverse events. If any adverse events related to tick bites or the repellent product are apparent at any time during the trial, testing will stop immediately and details of how to access treatment will be offered.*" (Section 10.2) In addition, the study team includes a Medical Monitor.

(e) 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?

Study staff will follow up with subjects 48 hours post-exposure to the test substance. In addition, the consent form provides contact information for the Study Director and another study team member that a subject can use in the event they suspect they are experiencing a study-related adverse effect.

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

"*The London School of Hygiene & Tropical Medicine (LSHTM) and Citrefine International Ltd hold insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that the LSHTM or Citrefine International Ltd is at fault. This does not affect your legal rights to seek compensation."* (Consent Form)

5. Benefits

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

There are no direct benefits to subjects.

(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 a skin-applied tick repellent containing OLE. 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 tick-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 tick 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 the target level of tick repellency.

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 risk mitigation measures proposed in the protocol, with EPA's recommendations incorporated, reduce risks to subjects without reducing the robustness of the scientific design. No reasonable opportunities to further reduce subject risk have been overlooked. The resulting residual risk to subjects is very low. The potential benefits from availability of a wider variety of effective skin-applied tick repellents are likely to be realized, and the residual risks to subjects in this proposed research are reasonable in light of the expected benefits.

7. Independent Ethics Review

(a) What IRB reviewed the proposed research?

Western Institutional Review Board

(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?

WIRB has full AAHRPP accreditation.

(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 what EPA has interpreted to be intentional exposure of human subjects to a pesticide. The study is being conducted with the intention of submitting the resulting data to EPA under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Thus, 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?

With EPA's recommendations addressed, yes.

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

Ability to speak and read English is a requirement for participation.

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

N/A

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

With EPA's recommendations addressed, there will be adequate comprehension of risks and discomforts. The written consent form and consent meeting will address risks and discomforts. In addition, there will be frequent opportunities to ask questions during the consent process, as well as before and during the study. Before a participant signs the consent form, the person conducting the meeting will ask a series of questions based on the materials covered to ensure the participant's comprehension.

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

See section 3.2(d).

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

See section 3.2(e).

9. Respect for Subjects

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

The subjects' identities will be protected as follows: each subject will be assigned a code number, and only subjects' code numbers will appear on data sheets. The subjects' names will not appear anywhere on the data sheet, or in the reports. Care will be taken to only show participants' hands and forearms in videos taken during the study, and particular care will be taken to not show faces or other identifiable marks. The identity of the participants will not be disclosed with the videos. Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing. The test results will not be disclosed to anyone other than the test subject, the verifying female employee, and/or the Study Director.

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

Subjects will be informed about this during the informed consent meeting and at the beginning of each test day. In addition, the consent form includes language reminding subjects of their freedom to withdraw without penalty, and each consented subject will receive a signed copy of this form.

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

Subjects will be compensated if they withdraw from the research. The Study Director or other designated study staff may end a particular participant's participation on a test day, at any time, for any reason. If the Study Director or other designated study staff ask a participant to withdraw from the test and they have complied with all of their requests, or if a test participant needs to withdraw early because of a health or emergency reason, full payment will still be made even if the test participant has participated for less than ten hours. This will not affect payment for any previous test days that had been completed.

If a test participant is asked to withdraw from the test because they have not followed all their directions or if they choose to withdraw from testing early on a test day for a non-health related or non-emergency reason, full payment will not be made if the test participant participates in less than ten hours. Instead, they will be paid for the number of hours worked (rounded to the nearest hour) at a rate of £7.50 per hour. This will not affect payment for any previous test days that had been completed.

Attachment 2: Protocol Completeness Checklists
A single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against 3 species of ticks

Note: The responses in the four checklists below are based on the protocol and consent materials that reflect EPA's recommendations.

40 CFR § 26.1111
Criteria for Institutional Review Board (IRB) approval of research

Criterion	Y/N	Comment/Page Reference
(a)(1)(i) Risks to subjects are minimized by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk.	Y	
(a)(1)(ii) Risks to subjects are minimized, whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.	N/A	
(a)(2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (for example, the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.	Y	
(a)(3) Selection of subjects is equitable, taking into account the purposes of the research and the setting in which it will be conducted, and being particularly cognizant of the special problems of research involving vulnerable populations, such as prisoners, mentally disabled persons, or economically or educationally disadvantaged persons.	Y	
(a)(4) Informed consent will be sought from each prospective subject, in accordance with, and to the extent required by §26.1116.	Y	
(a)(5) Informed consent will be appropriately documented, in accordance with, and to the extent required by §26.1117.	Y	
(a)(6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.	Y	
(a)(7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.	Y	
(b) When some or all of the subjects are likely to be vulnerable to coercion or undue influence, additional safeguards have been included in the study to protect the rights and welfare of these subjects.	N/A	EPA suggests that students, employees and their spouses of PI, lab, or study sponsor be excluded

40 CFR §26.1116
General requirements for informed consent of human subjects

Criterion		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 or the subject's legally authorized representative		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	
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	
(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	
	(2) A description of any reasonably foreseeable risks or discomforts to the subject	Y	
	(3) A description of any benefits to the subject or to others which may reasonably be expected from the research	Y	
	(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject	N/A	No alternative procedures; alternative is not to participate
	(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained	Y	
	(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	
	(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject	Y	
	(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	
(b) When appropriate, one 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	
	(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent	Y	
	(3) Any additional costs to the subject that may result from participation in the research	N/A	No anticipated costs to the subject
	(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject	Y	EPA recommends adding additional information to protocol and consent form about how withdrawal during field trial will occur
	(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	
	(6) The approximate number of subjects involved in the study	Y	
(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	

40 CFR §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 or the subject's legally authorized representative. A copy shall be given to the person signing the form.	Y	
(b)(1) **The consent form may be a written consent document that embodies the elements of informed consent required by 40 CFR §26.1116.** This form may be read to the subject or the subject's legally authorized representative, but in any event, the investigator shall give either the subject or the representative adequate opportunity to read it before it is signed; or	Y	
(b)(2) The consent form may be a short form written consent document stating that the elements of informed consent required by 40 CFR §26.1116 have been presented orally to the subject or the subject's legally authorized representative. 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 or the representative. Only the short form itself is to be signed by the subject or the representative. However, the witness shall sign both the short form and a copy of the summary, and the person actually obtaining consent shall sign a copy of the summary. A copy of the summary shall be given to the subject or the representative, in addition to a copy of the short form.		

40 CFR §26.1125
Submission of proposed human research for EPA review

Any person or institution who intends to conduct or sponsor human research covered by §26.1101(a) shall, **after receiving approval from all appropriate IRBs,** submit to EPA prior to initiating such research **all information relevant to the proposed research specified by 40 CFR §26.1115(a), and the following additional information, to the extent not already included:**

	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	
		(2) The measures proposed to minimize risks to the human subjects;	Y	
		(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	
		(5) The balance of risks and benefits of the proposed research.	Y	
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.		Y	
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		Y	Arctec must provide EPA with all recruitment materials, including scripts and email templates prior to initiating the study
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.		Y	
	§1125(e): All correspondence between the IRB and the investigators or sponsors.		Y	Separate file "arctec OLE IRB correspondence and approval"
	§1125(f): Official notification to the sponsor or investigator. . . that research involving human subjects has been reviewed and approved by an IRB.		Y	Separate file "arctec OLE IRB correspondence and approval"
all information relevant to the proposed research specified by § 26.1115(a)	(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	
	(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; • a written summary of the discussion of controverted issues and their resolution.		Y	Separate file "IRB minutes"
	(3) Records of continuing review activities.		N/A	
	(4) Copies of all correspondence between the IRB and the investigators.		Y	Separate file "arctec OLE IRB correspondence and approval"

	(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; any employment or other relationship between each member and the institution, for example, full-time employee, a member of governing panel or board, stockholder, paid or unpaid consultant.	Y	Separate file "arctec OLE IRB correspondence and approval"
	(6) Written procedures for the IRB in the same detail as described in §26.1108(a) and §26.1108(b).	Y	Provided to EPA previously
	(7) Statements of significant new findings provided to subjects, as required by §26.1116(b)(5).	N/A	

Attachment 3: EPA's Power vs. Sample Size Calculation for Tick Repellency Studies

Objective

To determine the sample size of N subjects such that tick repellency studies have sufficient power to obtain a given degree of **precision** in the estimate of median Complete Protection Time (mCPT). This precision – designated as "K" -- will be expressed as the ratio: 95% LCL_{mCPT}/estimated mCPT.

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

To develop estimates of a required sample size for a tick repellency study to achieve certain stated efficacy criteria and estimate a complete protection time (CPT)[1], it is necessary to determine the distribution of tick repellent crossing times (generally considered to be time of first confirmed crossing). However, the underlying distribution of the CPT of a product being tested in a tick repellency study is not known prior to the testing phase. What is known about the distribution is that CPT values are (necessarily) non-negative and are (generally) right-censored after 10 (or 12 hours) in most tick repellency studies. From an EPA analysis using the CPT data of a tick repellency study[2], it is reasonable to assume that the CPT data follow Weibull distributions and the estimated P5MRs of the CPT distributions in this study range from 0.27 – 0.54. (Appendix 14.2B)

On this basis, EPA assumed for this sample size determination exercise that a distribution of tick repellent crossing times follows a Weibull distribution. A Weibull distribution is commonly used in reliability analysis, in survival analysis, in predicting delivery times, in weather forecasting and hydrology, and in extreme value prediction. Its utility in a wide variety of applications is due in part to its flexibility to take on a variety of shapes depending on the parameters selected to describe the distribution. Oftentimes, the Weibull plot is described by two parameters: $\boldsymbol{\kappa}$ (the "shape" parameter and sometimes referred to in some parameterizations as "a") and λ (the scale parameter and sometimes referred to as "b").[3] The PDF (probability density function) and CDF (cumulative distribution function) of the aforementioned two-parameter Weibull distribution are defined, respectively, as follows:

$$f(x, \boldsymbol{\kappa}, \lambda) = \begin{cases} \frac{\boldsymbol{\kappa}}{\lambda}\left(\frac{x}{\lambda}\right)^{\boldsymbol{\kappa}-1} e^{-(x/\lambda)^{\boldsymbol{\kappa}}} & x \geq 0, \\ 0 & x < 0 \end{cases}$$

$$F(x, \boldsymbol{\kappa}, \lambda) = \begin{cases} 1 - e^{-(x/\lambda)^{\boldsymbol{\kappa}}} & x \geq 0, \\ 0 & x < 0 \end{cases}$$

and are illustrated in the associated plots in Figures A1 and A2 for some illustrative $\boldsymbol{\kappa}$ and λ values.

Parameterizing the Weibull distribution in terms of $\boldsymbol{\kappa}$ and λ is, however, not necessarily intuitive with respect to studying – and judging -- the efficacy of skin-applied tick repellents as measured by CPT for individuals using the repellent. Instead, it is more natural and desirable to be able to express the efficacy of the repellent in terms of both the expected precision of the estimated median CPT (mCPT) and in terms of the estimated variability of mCPT in (or across) the population. More specifically: the testing of a given repellent should be able to generate a reasonably precise estimate of the mCPT that is expected to be generally close to what a sizable fraction of the population would be expected to experience (or, more accurately, a mCPT that only a small fraction of the population would ideally experience to be much shorter).

Following the above logic, we define the *precision of the CPT estimate* -- designated as "K" -- as follows:

K = 95% LCL_{mCPT}/estimated mCPT

where:

---

[1] The Complete Protection Time (CPT) is defined as the time from initial application of the repellent by the test subject to the time of first confirmed crossing. A crossing (i.e. "not-repelled") is considered to be when a tick crosses the ring mark within 3 min and stays in the treated area for a minimum of 60s. Ticks that do not crawl onto the treated skin as well as those that walk down to the wrist or dropped off are regarded as success (i.e. 'repelled'). A crossing is a confirmed crossing if it is followed by another crossing within 30 minutes.

[2] Buchel K., Bendin, J., Gharbi, A., Rahlenbeck, S., Dautel, H. *Repellent efficacy of DEET, Icaridin, and EBAAP against Ixodes ricinus and Ixodes scapularis nymphs (Acari, Ixodidae).* Ticks and Tick-borne Diseases, 6 (2015) 494-498

[3] A Weibull distribution can sometimes be described by 3 parameters, with a "location" parameter added as a third parameter to the "scale" and "shape" parameter of the 2-parameter Weibull distribution.

mCPT= estimated median complete protection time

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

Similarly, the degree of variation of the CPT distribution in the population will be defined as the P5MR which we define here as the ratio between the mCPT of the 5th percentile of the population to the mCPT of the population:

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

where:

mCPT= median complete protection time

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

Re-parameterization of Standard Weibull Equation

While the above mCPT and P5MR parameterizations of the Weibull distribution are intuitively appealing for judging and evaluating repellent efficacy, they are non-standard parameterizations and it is necessary -- for comparison and simulation purposes -- to convert these to the more standard **κ** (shape) and λ (scale) values. To do this, EPA developed an equation such that interconversion between the standard (**κ** (shape) and λ (scale)) parameterization of the Weibull to this alternate version (with the Weibull distribution instead expressed in terms of P5MR and mCPT). Briefly, the cumulative probability function of CPT is assumed to be a 2- parameter Weibull distribution:

$$P(CPT, \boldsymbol{\kappa}, \lambda) = 1 - e^{-(CPT/\lambda)^{\boldsymbol{\kappa}}}$$

Given that a value of the mCPT represents the median or 50th percentile of the CPT and the value of P5MR represents the ratio of the 5%-tile of the CPT distribution to the mCPT, we can develop the following two equations to represent the cumulative distribution functions at the median CPT and the 5th percentile CPT:

$$P(mCPT, \kappa, \lambda) = 1 - e^{-\left(\frac{mCPT}{\lambda}\right)^{\kappa}} = 0.5 \quad (median)$$

$$P(P5MR \times mCPT, \kappa, \lambda) = 1 - e^{-\left(\frac{P5MR \times mCPT}{\lambda}\right)^{\kappa}} = 0.05 \quad (5th\ percentile)$$

Algebraically solving the equations above (see Appendix 14.2A for full derivation), we develop expressions for **κ** and λ:

$$\boldsymbol{\kappa} = \ln\left[\frac{\ln(0.95)}{\ln(0.5)}\right] / \ln(P5MR)$$

$$\lambda = e^{\frac{1}{\kappa} \times \ln\left[-\frac{mCPT^{\kappa}}{\ln(0.5)}\right]}$$

Table A1 below compares these two parameterizations for the example PDF and CDF distributions shown in Figures A1 and A2, respectively, for the **κ** and λ parameterizations shown there, illustrating the conversion to this new parameterization:

Table A1. Re-parameterization of Weibull Distribution Parameters from Traditional (**κ**, λ) to Revised (P5MR, mCPT) for Example Weibull Distributions Appearing in Figures A1 and A2.

Parameterization Scheme				Description/Comments
Traditional		Revised		
Scale (λ) [a]	Shape (κ)	mCPT[b]	P5MR[c,d]	
1	0.5	0.480453	0.005476	- κ values of less than 1 indicate a crossing rate decreases over time, and defective items fail early or are otherwise removed from the population.
1	1	0.693147	0.074001	- κ values equal to 1 indicate a constant crossing rate over time possibly suggesting crossing is due to random external events. - Here, the Weibull distribution reduces to the "exponential" distribution; - Note that mCPT here = 0.693 = ln(2)
1	1.5	0.78322	0.176261	- κ values greater than 1 suggests that the crossing rate increases over time, as when there is an "aging" process or components are more likely to fail over time.
1	5	0.92932	0.594083	

[a] The Weibull scale parameter is the 63.2 percentile of the distribution. If the scale parameter is 1, then this means that 63.2% of the observed values will be smaller than 1. Note in the CDF in Figure A2, as a consequence, that all λ=1 distributions intersect at the 63.2 percentile.

[b] $mCPT = [\ln(2)*\exp(\boldsymbol{\kappa} *\ln(\lambda))]^{(1/\boldsymbol{\kappa})}$

[c] $P5MR = \exp(\ln(\ln(0.95)/\ln(0.5))/\boldsymbol{\kappa})$

[d] Note that as κ increases, the P5MR value becomes larger, indicating that the values at the 5th percentile approach the values present at the 50th percentile, and the PDF becomes tighter and more peaked. K values of between 3 and 4 often lead to distributions that appear normal.

Figure A1. Probability Density Function (PDF) for Weibull Plot with λ (scale) =1 and κ (shape) ranging from 0.5 to 5

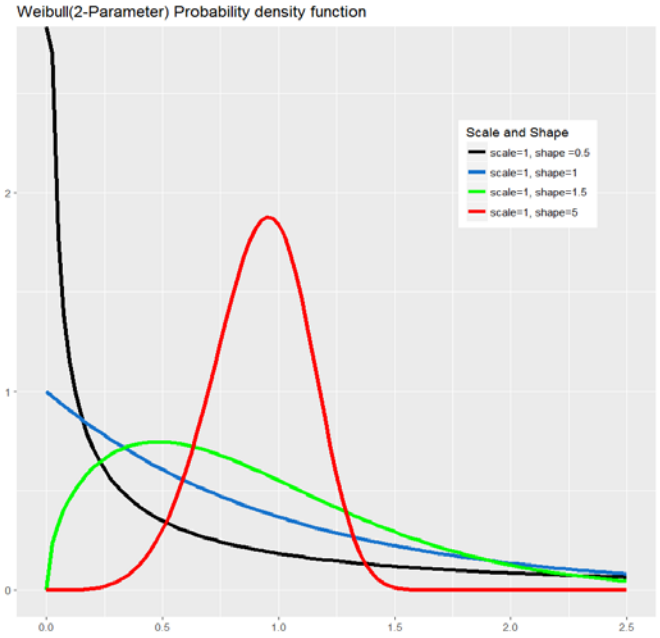


Figure A2. Cumulative Distribution Function (CDF) for Above Weibull PDF

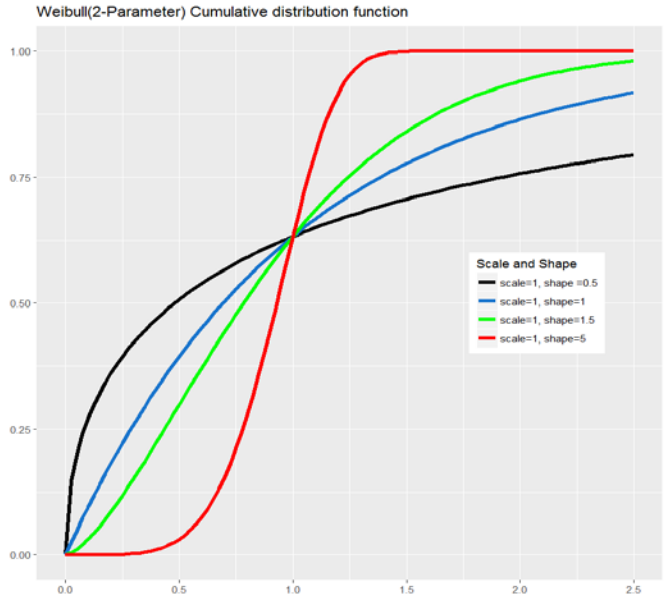
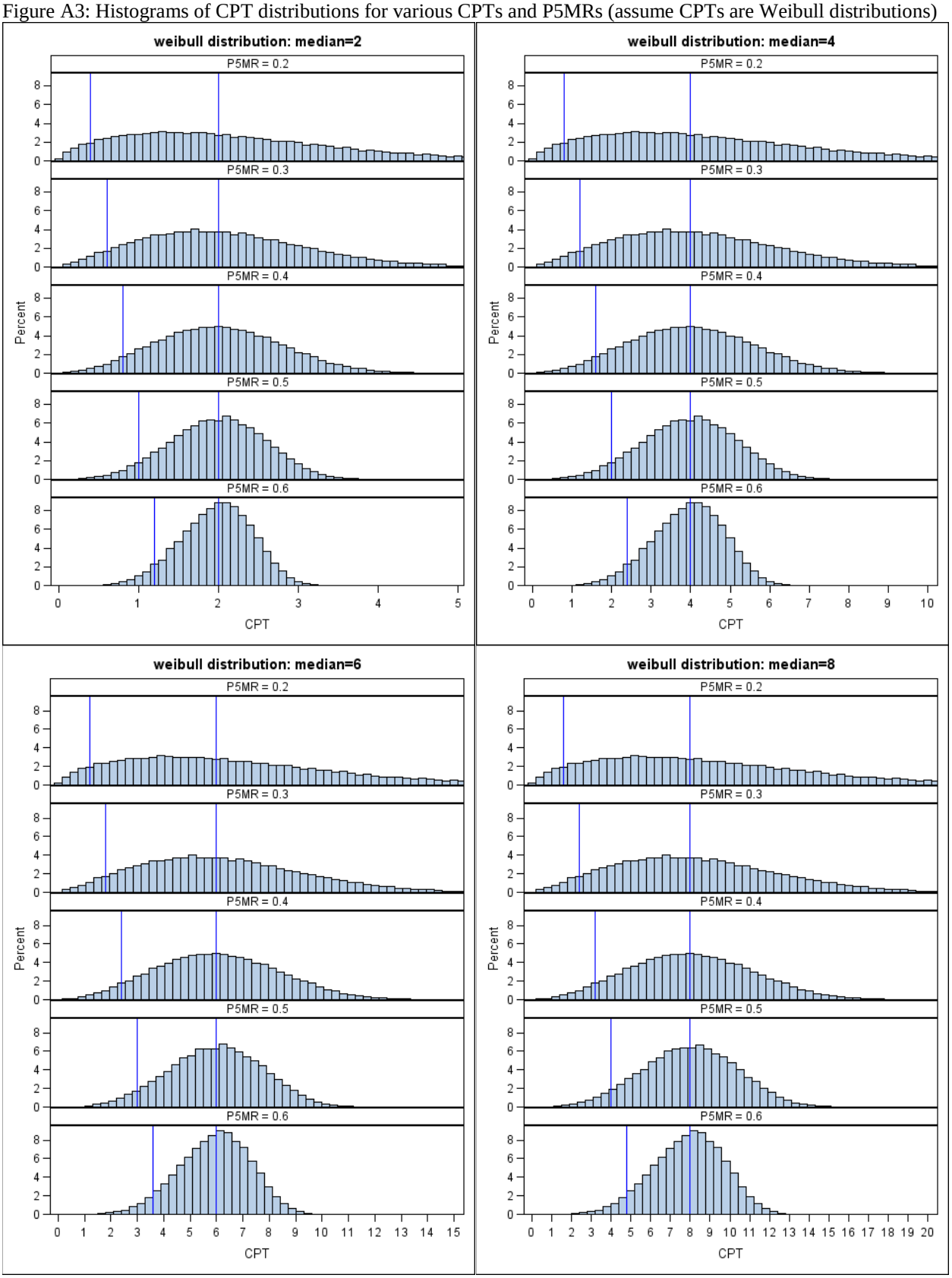


Figure A3: Histograms of CPT distributions for various CPTs and P5MRs (assume CPTs are Weibull distributions)



An example of the (varied) kinds of distributional "shapes" associated with various parameterizations is shown in Figure A3 as histograms of the CPT. More specifically, Figure A3 presents the CPT distributions with different medians and values of P5MR (ratio 5%-tile/mCPT). These present the CPT distributions with different mCPTs (2-, 4-, 6-, and 8- hrs) and values of the P5MR ratio (P5MR= 0.2, 0.3, 0.4, 0.5, and 0.6) for the (assumed) Weibull Distributions. As seen in Figure A3, larger mCPTs are associated with a shift in the distribution toward the right. In addition -- and importantly -- smaller P5MR values in this range are associated with "flatter" distributions and larger P5MRs are associated with more "peaked" distributions, with these more peaked distributions showing a greater percentage of the distribution centered around the median. From a regulatory perspective, a CPT distribution with a larger P5MR is more desirable than a CPT distribution with smaller P5MR since this means that a greater percentage of the user population experiences an actual CPT closer to the (advertised) mCPT. Further, it could be argued from a public policy perspective that a large variability in CPT in the population for a given repellent is not a desirable characteristic, and does not accurately portray or indicate any "expected" mCPT on the part of the consumer.

OPP staff have judged what might be considered reasonable values for input parameters (***precision*** of the estimated mCPT and ***variability*** in CPT in (or among) users of the tested product) in order to estimate required number of test subjects to achieve a desired set of aims regarding precision around the estimate of the mCPT. These judgments are based in part on the data of a study[4] and in part on general thoughts regarding consumer and other expectations with respect to product efficacy. Specifically, EPA has estimated the power associated with various sample sizes where power -- as defined here – is the probability that the ratio of the (95% LCL_{mCPT})/(estimated mCPT) is equal or greater than a given acceptable K (a scalar which measures the precision of the estimates in estimating the mCPT). Such tick repellency study design power depends on:

- Number of test subjects
  - The larger the number of test subjects, the greater the power

- (The required) precision (K) for estimated mCPT
  - The precision of an estimated mCPT from a study is expressed by the value of the ratio 95% LCL_{mCPT}/estimated mCPT. The value of ratio is in the interval (0, 1).
  - K is the smallest acceptable value of the ratio 95% LCL_{mCPT}/estimated mCPT for a given trial to be considered a "success", and conceptually represents an inverse of precision ("tightness") in the estimate of the mCPT: a larger K represents a greater "tightness" around the estimated mCPT. As K is chosen to be smaller, there is a greater probability that ratio 95% LCL_{mCPT}/estimated mCPT $\geq$ K (and the trial is considered to be a "success" in the power calculation)

- P5MR
  - P5MR = ratio of the 5^{th} percentile/mCPT
  - As the variation (dispersion or spread) of the distribution of CPT in the population becomes smaller, the 95% confidence interval of the estimated mCPT also becomes narrower (i.e. the 95% LCL_{mCPT} is closer to the estimated mCPT and the mCPT is better estimated, *certeris paribus*). Therefore, a smaller variation in the distribution of CPT will result in a larger P5MR and a higher probability that the ratio 95% LCL_{mCPT}/estimated mCPT $\geq$ K. A CPT distribution with greater P5MR is generally more desirable than a CPT distribution with smaller P5MR.

Ideally, a tick repellency study will be designed to have a sufficient number of test subjects such that one can have reasonable assurance that there is adequate power (defined here as a high probability that the ratio 95% LCL/estimated mCPT > K) given a shape/spread of the CPT distribution in the population. This shape/spread of the CPT in the population is defined by the P5MR.

Brief Description of the Conduct of a Ticks Repellent Study

In the tick repellency studies, each test subject has 3 lines drawn on the testing arm, with a distance of 3 cm between any two adjacent lines as shown in the Figure 2. Product will be applied from the boundary line (at the

[4] See Appendix 14.2B for Weibull parameters fit to CPT data of a tick study

wrist) to the elbow. One tick at a time will be released at the release line. A "crossing" is recorded if the test organism crosses the boundary line at least 3 cm into the treated area within 3 minutes, and remains in the treated area for at least one minute.

A crossing is a confirmed crossing if it is followed by another crossing within 30 minutes. For subjects who receive confirmed crossings, the CPTs are set as 0 if the first confirmed crossing occurs during the test of 1 tick immediately following product application; otherwise, the CPTs are rounded down to the nearest quarter hour (i.e., the starting time of the testing interval in which the first confirmed crossing occurs). We approximated that it would take 3 minutes to test 1 tick in each 15-minute interval. For those subjects for which there are no confirmed crossings through the end of the testing day, CPTs are considered to be right censored at a time that is rounded down to the nearest half hour.

Description of (Computer) Simulation Procedure:

To start the simulated study trials, 4000 datasets were created with each dataset consisting of 10 data points (representing CPTs of 10 subjects) that were generated randomly from a Weibull distribution with a median CPT=2 and ratio of the 5%-tile/median P5MR= 0.2. If the randomly generated CPTs for the 10 subjects are ≤ 3, 4-18, 19-33, 34-48, 49-63, … -minutes, the CPTs are set to be 0-, 0.25-, 0.5-, 0.75-, 1-hours…, respectively, to simulate the study design in which each study participant would take about 3 minutes to test 1 tick for every 15 minutes until the first confirmed crossing. If the randomly generated CPTs are greater than 723 minutes, they are considered in the calculation to be (right) censored at 12 hours.

After generating the CPTs as described in the previous paragraph, the Kaplan Meier Estimator is used to estimate the mCPT and its 95% CI for each of the 4000 (10-person) datasets. The proportion of datasets in which the ratio of 95% LCL_{mCPT}/mCPT ≥ K as 0.6 is considered to be the "power" of the study design. More specifically: if the value of 95% LCL/mCPT ≥ 0.6 is considered a "success", the power is calculated as the proportion of successes in the 4000 datasets consisting of 10 data points each.

The process described in previous paragraph is then repeated for each combination of different mCPT = 2, 4, 6, 8, and 10 hours; P5MR = 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8; sample size per dataset = 10, 11, 12 … 30; and the lowest acceptable K = 0.6, 0.7, and 0.8; all assuming that CPT follows Weibull distributions.

Results of Simulation

Tables A2, A3, … A7 present the power estimates from simulations in which the data were randomly generated from Weibull distributions for K = 0.6, 0.7, and 0.8. These are shown for various values of mCPT (ranging from 2 to 10 hours), P5MR (ranging from 0.2 to 0.8), and Sample Size (ranging from 10 to 30). As described earlier, K reflects a measure the precision of the estimate of mCPT with larger K values representing tighter estimates. For example, the K value of 0.6 requires that the 95% LCL on an estimated median protection of 10 hours be no less than 6 hours (for a "success") while a K value of 0.8 requires that the 95% LCL on that same median protection time be no less than 8 hours. A required precision of a K of 0.8, then, requires a more precise estimate of the mCPT than a K of 0.6 for this trial to be considered a "success" in the power calculation.

As can be seen within each Table, the power of a study to achieve a given acceptable ratio K value (e.g., 0.6, 0.7, or 0.8 representing 95% LCLmCPT/mCPT) value increases as the assumed P5MR value of the distribution increases (for example, from 0.2 to 0.8) or as the sample size increases (from 10 to 20 or from 21 to 30). This is expected since a tighter (or more "peaked") distributions (as evidenced by a larger P5MR value) will require fewer random "draws" to accurately estimate the mCPT. Across the Tables, we also see that as the acceptable K value increases from 0.6 to 0.8, the power of a study to achieve "95% LCLmCPT/mCPT ≥ K" decreases since stricter requirements for a "success" are being levied.

Table A2: Power when the lowest acceptable ratio 95% LCLmCPT/mCPT = 0.6 (Weibull distribution)												
median	P5MR	Sample Size										
		_10	_11	_12	_13	_14	_15	_16	_17	_18	_19	_20
2	0.2	0.045	0.198	0.150	0.356	0.280	0.245	0.426	0.387	0.548	0.512	0.435
	0.3	0.109	0.361	0.313	0.577	0.496	0.467	0.664	0.642	0.800	0.747	0.693
	0.4	0.231	0.572	0.514	0.786	0.719	0.708	0.860	0.845	0.929	0.913	0.886
	0.5	0.410	0.780	0.738	0.924	0.890	0.883	0.960	0.959	0.986	0.982	0.977
	0.6	0.638	0.932	0.914	0.986	0.975	0.979	0.993	0.996	0.995	0.997	0.997
	0.7	0.871	0.993	0.988	0.993	0.994	0.995	0.995	0.996	0.979	0.990	0.994
	0.8	0.979	0.973	0.988	0.946	0.963	0.941	0.924	0.947	0.874	0.907	0.941
4	0.2	0.037	0.175	0.130	0.328	0.257	0.222	0.405	0.360	0.523	0.474	0.399
	0.3	0.097	0.340	0.290	0.560	0.477	0.437	0.655	0.621	0.789	0.735	0.687
	0.4	0.213	0.542	0.505	0.769	0.712	0.685	0.855	0.827	0.934	0.902	0.893
	0.5	0.402	0.757	0.734	0.918	0.895	0.873	0.962	0.955	0.989	0.979	0.980
	0.6	0.648	0.924	0.918	0.979	0.980	0.973	0.996	0.995	0.999	0.999	0.998
	0.7	0.871	0.992	0.992	0.999	0.999	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	0.987	0.999	1.000	0.998	0.999	1.000	0.998	0.999	0.993	0.998	0.998
6	0.2	0.038	0.162	0.129	0.316	0.252	0.213	0.387	0.343	0.512	0.463	0.389
	0.3	0.093	0.325	0.273	0.540	0.463	0.421	0.642	0.601	0.775	0.723	0.680
	0.4	0.203	0.529	0.499	0.762	0.703	0.677	0.844	0.826	0.931	0.899	0.890
	0.5	0.398	0.749	0.729	0.914	0.894	0.868	0.962	0.950	0.989	0.980	0.977
	0.6	0.637	0.925	0.916	0.982	0.982	0.976	0.997	0.996	1.000	0.999	0.999
	0.7	0.870	0.992	0.990	0.999	0.999	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.987	0.999	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000
8	0.2	0.120	0.200	0.182	0.337	0.291	0.229	0.407	0.353	0.526	0.466	0.405
	0.3	0.116	0.327	0.290	0.538	0.471	0.417	0.640	0.598	0.773	0.723	0.676
	0.4	0.202	0.523	0.491	0.754	0.700	0.672	0.845	0.816	0.930	0.897	0.885
	0.5	0.390	0.745	0.724	0.913	0.890	0.865	0.960	0.950	0.989	0.980	0.978
	0.6	0.629	0.923	0.915	0.981	0.981	0.974	0.996	0.995	1.000	0.999	0.999
	0.7	0.865	0.992	0.990	0.998	0.999	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10	0.2	0.371	0.374	0.407	0.467	0.481	0.369	0.558	0.477	0.652	0.548	0.532
	0.3	0.330	0.450	0.446	0.614	0.585	0.491	0.712	0.649	0.817	0.753	0.725
	0.4	0.338	0.576	0.566	0.779	0.746	0.690	0.866	0.831	0.937	0.899	0.894
	0.5	0.442	0.754	0.739	0.918	0.896	0.867	0.961	0.953	0.988	0.980	0.978
	0.6	0.637	0.920	0.914	0.980	0.981	0.974	0.997	0.996	1.000	0.999	0.999
	0.7	0.865	0.992	0.991	0.999	0.999	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

NOTE: Yellow indicates power > 0.8; orange indicates power > 0.9

Table A3: Power when the lowest acceptable ratio 95% LCLmCPT/mCPT = 0.6 (Weibull distribution)											
median	P5MR	Sample Size									
		_21	_22	_23	_24	_25	_26	_27	_28	_29	_30
2	0.2	0.610	0.556	0.727	0.657	0.806	0.736	0.720	0.815	0.777	0.855
	0.3	0.840	0.808	0.906	0.867	0.950	0.915	0.919	0.952	0.947	0.970
	0.4	0.959	0.948	0.982	0.970	0.991	0.985	0.989	0.995	0.993	0.997
	0.5	0.996	0.992	0.998	0.996	0.999	0.999	0.998	0.998	1.000	0.999
	0.6	0.997	0.998	0.995	0.999	0.996	0.996	0.998	0.992	0.997	0.998
	0.7	0.983	0.987	0.967	0.979	0.979	0.964	0.980	0.947	0.968	0.971
	0.8	0.865	0.901	0.819	0.850	0.869	0.810	0.859	0.760	0.806	0.837
4	0.2	0.586	0.528	0.705	0.638	0.789	0.721	0.694	0.808	0.763	0.857
	0.3	0.824	0.807	0.902	0.865	0.942	0.918	0.907	0.957	0.939	0.974
	0.4	0.958	0.952	0.978	0.973	0.989	0.985	0.987	0.996	0.993	0.998
	0.5	0.996	0.994	0.998	0.998	0.999	1.000	0.999	1.000	1.000	1.000
	0.6	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	0.999
	0.8	0.994	0.997	0.989	0.996	0.993	0.991	0.997	0.986	0.992	0.992
6	0.2	0.575	0.517	0.689	0.626	0.782	0.715	0.683	0.804	0.750	0.852
	0.3	0.819	0.794	0.891	0.861	0.942	0.917	0.905	0.955	0.939	0.974
	0.4	0.955	0.949	0.977	0.969	0.990	0.985	0.986	0.996	0.994	0.997
	0.5	0.997	0.992	0.999	0.998	0.999	1.000	0.999	1.000	1.000	1.000
	0.6	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	0.999	1.000	1.000	1.000	1.000	0.998	0.999	0.998
8	0.2	0.573	0.519	0.690	0.627	0.783	0.713	0.681	0.801	0.747	0.850
	0.3	0.810	0.802	0.889	0.859	0.938	0.913	0.900	0.954	0.935	0.972
	0.4	0.953	0.948	0.976	0.973	0.991	0.986	0.987	0.996	0.994	0.997
	0.5	0.997	0.993	0.998	0.997	0.999	1.000	1.000	1.000	1.000	1.000
	0.6	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10	0.2	0.652	0.633	0.743	0.716	0.819	0.773	0.721	0.840	0.784	0.883
	0.3	0.836	0.827	0.907	0.881	0.946	0.922	0.908	0.965	0.944	0.978
	0.4	0.956	0.950	0.978	0.975	0.990	0.986	0.988	0.995	0.994	0.998
	0.5	0.997	0.993	0.999	0.998	0.999	1.000	1.000	1.000	1.000	1.000
	0.6	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table A4: Power when the lowest acceptable ratio 95% LCLmCPT/mCPT = 0.7 (Weibull distribution)												
median	P5MR	Sample Size										
		_10	_11	_12	_13	_14	_15	_16	_17	_18	_19	_20
2	0.2	0.013	0.077	0.048	0.165	0.116	0.089	0.198	0.169	0.287	0.246	0.184
	0.3	0.036	0.172	0.132	0.320	0.262	0.216	0.393	0.346	0.520	0.452	0.401
	0.4	0.096	0.314	0.275	0.516	0.454	0.402	0.624	0.573	0.741	0.684	0.652
	0.5	0.198	0.504	0.484	0.717	0.681	0.622	0.826	0.776	0.909	0.850	0.862
	0.6	0.403	0.717	0.726	0.882	0.872	0.833	0.949	0.921	0.975	0.958	0.968
	0.7	0.671	0.908	0.926	0.970	0.975	0.964	0.989	0.988	0.978	0.985	0.993
	0.8	0.922	0.969	0.983	0.944	0.963	0.940	0.924	0.946	0.874	0.907	0.940
4	0.2	0.009	0.061	0.038	0.134	0.090	0.065	0.167	0.129	0.240	0.206	0.149
	0.3	0.027	0.139	0.105	0.269	0.213	0.174	0.339	0.293	0.456	0.404	0.343
	0.4	0.071	0.271	0.229	0.469	0.403	0.353	0.581	0.520	0.708	0.654	0.609
	0.5	0.169	0.466	0.432	0.709	0.646	0.604	0.796	0.761	0.899	0.856	0.838
	0.6	0.357	0.708	0.689	0.888	0.865	0.833	0.951	0.932	0.983	0.972	0.972
	0.7	0.635	0.922	0.913	0.980	0.983	0.972	0.996	0.995	0.999	1.000	0.999
	0.8	0.910	0.995	0.996	0.998	0.998	1.000	0.998	0.999	0.993	0.998	0.998
6	0.2	0.013	0.060	0.038	0.130	0.089	0.066	0.163	0.121	0.234	0.205	0.147
	0.3	0.026	0.139	0.098	0.263	0.212	0.169	0.333	0.283	0.446	0.396	0.326
	0.4	0.069	0.265	0.224	0.466	0.395	0.341	0.564	0.512	0.697	0.642	0.589
	0.5	0.158	0.458	0.419	0.697	0.631	0.592	0.788	0.755	0.897	0.851	0.831
	0.6	0.347	0.697	0.678	0.885	0.855	0.820	0.943	0.928	0.983	0.966	0.967
	0.7	0.628	0.912	0.906	0.979	0.979	0.970	0.996	0.995	1.000	0.999	0.998
	0.8	0.906	0.996	0.996	0.999	0.999	1.000	1.000	1.000	0.999	1.000	1.000
8	0.2	0.098	0.104	0.101	0.155	0.131	0.086	0.193	0.140	0.249	0.201	0.161
	0.3	0.052	0.141	0.116	0.267	0.212	0.173	0.334	0.284	0.443	0.392	0.329
	0.4	0.072	0.261	0.221	0.463	0.388	0.341	0.559	0.510	0.698	0.642	0.587
	0.5	0.161	0.457	0.420	0.689	0.626	0.587	0.784	0.754	0.896	0.848	0.827
	0.6	0.350	0.699	0.674	0.888	0.858	0.823	0.945	0.929	0.982	0.968	0.964
	0.7	0.625	0.914	0.906	0.980	0.979	0.972	0.996	0.996	1.000	0.999	0.999
	0.8	0.905	0.995	0.996	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10	0.2	0.352	0.291	0.346	0.315	0.358	0.248	0.387	0.289	0.436	0.329	0.327
	0.3	0.277	0.284	0.308	0.371	0.373	0.272	0.453	0.367	0.544	0.449	0.419
	0.4	0.225	0.338	0.335	0.502	0.469	0.379	0.613	0.539	0.723	0.658	0.617
	0.5	0.226	0.475	0.453	0.705	0.644	0.595	0.795	0.761	0.902	0.850	0.839
	0.6	0.360	0.696	0.685	0.890	0.862	0.828	0.947	0.930	0.982	0.969	0.965
	0.7	0.626	0.921	0.913	0.978	0.980	0.973	0.995	0.995	1.000	0.999	0.998
	0.8	0.911	0.997	0.996	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table A5: Power when the lowest acceptable ratio 95% LCLmCPT/mCPT = 0.7 (Weibull distribution)											
median	P5MR	Sample Size									
		_21	_22	_23	_24	_25	_26	_27	_28	_29	_30
2	0.2	0.327	0.267	0.420	0.353	0.532	0.435	0.400	0.516	0.462	0.585
	0.3	0.569	0.514	0.666	0.609	0.753	0.700	0.663	0.784	0.713	0.842
	0.4	0.763	0.759	0.855	0.832	0.904	0.886	0.857	0.934	0.898	0.951
	0.5	0.917	0.924	0.952	0.948	0.976	0.972	0.960	0.986	0.975	0.989
	0.6	0.983	0.988	0.986	0.991	0.993	0.993	0.994	0.990	0.995	0.998
	0.7	0.983	0.986	0.967	0.979	0.979	0.964	0.980	0.947	0.968	0.971
	0.8	0.865	0.901	0.819	0.850	0.869	0.810	0.859	0.760	0.806	0.837
4	0.2	0.279	0.225	0.371	0.299	0.467	0.376	0.340	0.462	0.400	0.533
	0.3	0.513	0.455	0.624	0.558	0.727	0.652	0.619	0.746	0.687	0.808
	0.4	0.752	0.722	0.846	0.804	0.907	0.873	0.853	0.928	0.893	0.953
	0.5	0.925	0.923	0.960	0.951	0.980	0.973	0.972	0.990	0.986	0.995
	0.6	0.992	0.990	0.997	0.994	0.998	1.000	0.999	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	0.999
	0.8	0.994	0.997	0.989	0.996	0.993	0.991	0.997	0.986	0.992	0.992
6	0.2	0.279	0.222	0.369	0.285	0.461	0.369	0.328	0.450	0.392	0.525
	0.3	0.501	0.447	0.620	0.547	0.720	0.638	0.610	0.736	0.673	0.800
	0.4	0.737	0.714	0.836	0.795	0.901	0.863	0.839	0.922	0.888	0.948
	0.5	0.922	0.913	0.958	0.947	0.978	0.972	0.971	0.989	0.984	0.993
	0.6	0.990	0.990	0.997	0.995	0.998	0.999	0.998	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	0.999	1.000	1.000	1.000	1.000	0.998	0.999	0.998
8	0.2	0.276	0.226	0.370	0.296	0.456	0.369	0.329	0.455	0.391	0.528
	0.3	0.495	0.442	0.614	0.545	0.719	0.636	0.607	0.731	0.676	0.799
	0.4	0.745	0.712	0.840	0.794	0.898	0.867	0.844	0.920	0.889	0.948
	0.5	0.921	0.916	0.959	0.946	0.979	0.971	0.969	0.990	0.985	0.993
	0.6	0.992	0.988	0.997	0.995	0.998	0.999	0.998	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10	0.2	0.402	0.385	0.461	0.434	0.537	0.484	0.413	0.551	0.477	0.618
	0.3	0.550	0.519	0.651	0.608	0.740	0.672	0.630	0.758	0.698	0.825
	0.4	0.753	0.733	0.847	0.813	0.902	0.869	0.848	0.926	0.891	0.952
	0.5	0.925	0.914	0.961	0.947	0.980	0.971	0.972	0.989	0.985	0.994
	0.6	0.993	0.988	0.996	0.996	0.998	0.999	0.999	1.000	1.000	1.000
	0.7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table A6: Power when the lowest acceptable ratio 95% LCLmCPT/mCPT = 0.8 (Weibull distribution)												
median	P5MR	Sample Size										
		_10	_11	_12	_13	_14	_15	_16	_17	_18	_19	_20
2	0.2	0.003	0.026	0.008	0.051	0.030	0.020	0.056	0.049	0.082	0.070	0.044
	0.3	0.009	0.050	0.031	0.099	0.078	0.051	0.131	0.087	0.192	0.137	0.114
	0.4	0.022	0.098	0.078	0.175	0.160	0.112	0.257	0.176	0.335	0.254	0.249
	0.5	0.054	0.173	0.171	0.303	0.309	0.208	0.435	0.308	0.544	0.419	0.434
	0.6	0.129	0.296	0.335	0.492	0.505	0.387	0.649	0.526	0.745	0.632	0.668
	0.7	0.307	0.532	0.585	0.729	0.758	0.657	0.857	0.793	0.905	0.858	0.893
	0.8	0.618	0.818	0.867	0.894	0.922	0.870	0.908	0.912	0.868	0.891	0.932
4	0.2	0.002	0.019	0.006	0.039	0.023	0.014	0.039	0.032	0.069	0.050	0.030
	0.3	0.005	0.042	0.023	0.088	0.058	0.039	0.102	0.081	0.162	0.131	0.090
	0.4	0.014	0.090	0.060	0.182	0.136	0.103	0.231	0.190	0.328	0.286	0.212
	0.5	0.047	0.190	0.148	0.353	0.284	0.248	0.435	0.393	0.558	0.519	0.443
	0.6	0.114	0.381	0.323	0.606	0.521	0.496	0.692	0.668	0.819	0.782	0.725
	0.7	0.302	0.668	0.620	0.860	0.809	0.795	0.914	0.906	0.964	0.953	0.944
	0.8	0.642	0.934	0.921	0.985	0.978	0.983	0.992	0.995	0.992	0.997	0.996
6	0.2	0.006	0.015	0.006	0.037	0.020	0.012	0.035	0.028	0.062	0.044	0.024
	0.3	0.005	0.040	0.017	0.081	0.051	0.036	0.098	0.071	0.148	0.121	0.080
	0.4	0.013	0.083	0.058	0.170	0.127	0.095	0.222	0.172	0.310	0.262	0.208
	0.5	0.039	0.173	0.139	0.325	0.270	0.222	0.411	0.365	0.539	0.490	0.419
	0.6	0.105	0.349	0.307	0.573	0.505	0.451	0.680	0.630	0.810	0.748	0.712
	0.7	0.288	0.624	0.600	0.835	0.800	0.763	0.914	0.889	0.964	0.944	0.940
	0.8	0.640	0.924	0.914	0.981	0.984	0.971	0.996	0.994	0.999	1.000	0.998
8	0.2	0.092	0.059	0.070	0.065	0.067	0.037	0.067	0.047	0.083	0.049	0.040
	0.3	0.031	0.045	0.035	0.087	0.061	0.038	0.098	0.072	0.149	0.115	0.078
	0.4	0.015	0.082	0.054	0.169	0.124	0.090	0.215	0.169	0.300	0.254	0.201
	0.5	0.036	0.170	0.136	0.319	0.265	0.214	0.404	0.361	0.539	0.475	0.411
	0.6	0.104	0.344	0.301	0.569	0.496	0.445	0.675	0.628	0.803	0.748	0.710
	0.7	0.277	0.620	0.598	0.833	0.798	0.752	0.909	0.882	0.964	0.939	0.940
	0.8	0.636	0.912	0.915	0.978	0.982	0.973	0.996	0.996	0.999	0.999	0.999
10	0.2	0.348	0.254	0.319	0.235	0.301	0.203	0.285	0.206	0.302	0.199	0.229
	0.3	0.260	0.200	0.240	0.207	0.242	0.146	0.246	0.168	0.283	0.194	0.190
	0.4	0.176	0.165	0.182	0.224	0.220	0.146	0.291	0.213	0.347	0.284	0.242
	0.5	0.109	0.202	0.184	0.339	0.298	0.233	0.424	0.361	0.543	0.472	0.421
	0.6	0.116	0.342	0.309	0.567	0.494	0.443	0.667	0.622	0.802	0.743	0.707
	0.7	0.276	0.618	0.595	0.826	0.793	0.760	0.908	0.881	0.962	0.945	0.938
	0.8	0.637	0.926	0.914	0.982	0.981	0.977	0.996	0.996	1.000	0.999	0.999

Table A7: Power when the lowest acceptable ratio 95% LCLmCPT/mCPT = 0.8 (Weibull distribution)											
median	P5MR	Sample Size									
		_21	_22	_23	_24	_25	_26	_27	_28	_29	_30
2	0.2	0.097	0.070	0.135	0.097	0.180	0.137	0.108	0.181	0.135	0.223
	0.3	0.185	0.168	0.243	0.231	0.318	0.294	0.215	0.357	0.253	0.406
	0.4	0.332	0.335	0.413	0.405	0.490	0.483	0.374	0.555	0.436	0.617
	0.5	0.505	0.542	0.605	0.623	0.686	0.693	0.580	0.765	0.646	0.801
	0.6	0.720	0.757	0.801	0.823	0.860	0.860	0.815	0.909	0.851	0.934
	0.7	0.898	0.925	0.920	0.937	0.950	0.939	0.940	0.934	0.940	0.960
	0.8	0.858	0.898	0.816	0.848	0.867	0.810	0.857	0.760	0.805	0.836
4	0.2	0.077	0.047	0.117	0.069	0.159	0.112	0.084	0.141	0.110	0.174
	0.3	0.190	0.145	0.261	0.192	0.337	0.260	0.224	0.333	0.280	0.393
	0.4	0.373	0.319	0.477	0.397	0.578	0.480	0.462	0.581	0.517	0.654
	0.5	0.621	0.566	0.735	0.667	0.816	0.747	0.736	0.825	0.788	0.870
	0.6	0.856	0.838	0.919	0.890	0.958	0.931	0.935	0.966	0.956	0.977
	0.7	0.983	0.978	0.993	0.987	0.997	0.994	0.996	0.998	1.000	0.999
	0.8	0.994	0.996	0.989	0.996	0.993	0.991	0.997	0.986	0.992	0.992
6	0.2	0.070	0.043	0.104	0.065	0.151	0.100	0.075	0.130	0.100	0.167
	0.3	0.176	0.131	0.245	0.178	0.323	0.243	0.204	0.316	0.254	0.376
	0.4	0.347	0.297	0.457	0.387	0.554	0.468	0.426	0.561	0.493	0.639
	0.5	0.592	0.544	0.701	0.652	0.796	0.733	0.697	0.821	0.757	0.868
	0.6	0.837	0.824	0.904	0.879	0.948	0.934	0.916	0.964	0.947	0.979
	0.7	0.981	0.977	0.993	0.988	0.996	0.996	0.995	0.999	0.999	0.999
	0.8	1.000	0.999	0.999	1.000	1.000	1.000	1.000	0.998	0.999	0.998
8	0.2	0.073	0.054	0.110	0.076	0.144	0.107	0.075	0.134	0.095	0.166
	0.3	0.169	0.129	0.239	0.177	0.316	0.238	0.196	0.309	0.252	0.367
	0.4	0.342	0.292	0.447	0.375	0.547	0.458	0.424	0.554	0.491	0.634
	0.5	0.582	0.535	0.703	0.646	0.790	0.731	0.691	0.820	0.758	0.867
	0.6	0.833	0.823	0.908	0.881	0.947	0.928	0.914	0.965	0.944	0.978
	0.7	0.974	0.975	0.990	0.988	0.995	0.995	0.994	0.999	0.999	0.999
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10	0.2	0.222	0.235	0.225	0.245	0.266	0.251	0.178	0.276	0.208	0.297
	0.3	0.243	0.231	0.298	0.262	0.364	0.305	0.242	0.372	0.294	0.415
	0.4	0.370	0.329	0.462	0.401	0.557	0.474	0.423	0.566	0.491	0.636
	0.5	0.585	0.541	0.700	0.643	0.790	0.726	0.686	0.813	0.751	0.862
	0.6	0.827	0.825	0.903	0.875	0.947	0.927	0.918	0.965	0.947	0.978
	0.7	0.978	0.977	0.991	0.986	0.995	0.995	0.995	1.000	0.999	1.000
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

The SAS Code used to generate the simulated data and the associated tables are presented in Appendix 14.2C.

14.2A Re-parameterization of Standard Weibull Equation

Given the definition of PDF and CDF from first principles:

$$P(mCPT, \kappa, \lambda) = 1 - e^{-\left(\frac{mCPT}{\lambda}\right)^{\kappa}} = 0.5 \quad (median)$$

$$P(P5MR \times mCPT, \kappa, \lambda) = 1 - e^{-\left(\frac{P5MR \times mCPT}{\lambda}\right)^{\kappa}} = 0.05 \quad (5th\ percentile)$$

Then:

$$e^{-\left(\frac{mCPT}{\lambda}\right)^{\kappa}} = 0.5 \quad (median)$$

$$e^{-\left(\frac{P5MR \times mCPT}{\lambda}\right)^{\kappa}} = 0.95 \quad (5th\ percentile)$$

and

$$-\left(\frac{\mathrm{mCPT}}{\lambda}\right)^{\kappa} = \ln(0.5) \qquad (1)$$

$$-\left(\frac{\mathrm{P5MR \times mCPT}}{\lambda}\right)^{\kappa} = \ln(0.95) \qquad (2)$$

Divide (2) by (1), we have:

$$\left[\frac{\frac{P5MR \times mCPT}{\lambda}}{\frac{mCPT}{\lambda}}\right]^{\kappa} = \frac{\ln(0.95)}{\ln(0.5)}$$

$$\kappa = \ln\left[\frac{\ln(0.95)}{\ln(0.5)}\right] / \ln(P5MR) \qquad (3)$$

From (1):

$$\left(\frac{mCPT}{\lambda}\right)^{\kappa} = -\ln(0.5)$$

$$\kappa \times ln\left(\frac{mCPT}{\lambda}\right) = \ln[-\ln(0.5)]$$

$$\ln\left(\frac{mCPT}{\lambda}\right) = \frac{1}{\kappa}\ln[-\ln(0.5)]$$

$$\ln(mCPT) - \ln(\lambda) = \frac{1}{\kappa}\ln[-\ln(0.5)]$$

$$\ln(\lambda) = \ln(mCPT) - \frac{1}{\kappa}\ln[-\ln(0.5)]$$

$$= \frac{1}{\kappa}[\kappa \ln(mCPT) - \ln[-\ln(0.5)]]$$

$$= \frac{1}{\kappa}[\ln(mCPT^{\kappa}) - \ln[-\ln(0.5)]]$$

$$= \frac{1}{\kappa}\ln\left[-\frac{mCPT^{\kappa}}{\ln(0.5)}\right]$$

$$\lambda = e^{\frac{1}{\kappa}\ln\left[-\frac{mCPT^{\kappa}}{\ln(0.5)}\right]} \qquad (4)$$

So…

$$\kappa = \ln\left[\frac{\ln(0.95)}{\ln(0.5)}\right] / \ln(P5MR)$$

$$\lambda = e^{\frac{1}{\kappa} \times \ln\left[-\frac{mCPT^{\kappa}}{\ln(0.5)}\right]}$$

(As shown in the main text)

14.2B Estimated Weibull Parameters of CPT data a tick repellency study

Background

In 2015, Buchel. K et. al published the results of a tick repellency study title "*Repellent efficacy of DEET, Icaridin, and EBAAP against Ixodes ricinus and Ixodes scapularis nymphs (Acari, Ixodidae)*". In this study, there were 10 volunteers for each of 3 repellents × 2 tick species. Each volunteer tested 5 ticks every 30 minutes until CPT was reached, up to 12.5 hours. The authors of the study kindly provided the raw data to EPA to allow us to investigate the characteristics of tick CPT data (i.e. distributions and parameters of the distributions) to better develop a sample size simulation.

Methods

After obtaining the raw data from the authors of study, EPA staff reviewed and made some corrections in the CPT data per EPA definition of CPT.

Weibull distributions, normal distributions, and lognormal distributions were used to analyze the data to determine the best fit the CPT data. Weibull distributions were selected as the best fit distributions based on the lower AIC values (Table 5).

The P5MRs of the distributions were calculated using the estimated parameters of CPT distributions, assuming the data following Weibull distributions.

Conclusion

- It is reasonable to assume that the CPT data of the tick study follow Weibull distributions
- The estimated P5MR (5th percentile/median) of the tick CPT data ranges from 0.27 – 0.54

Table A8: compare the fitness of Weibull, normal, and lognormal distributions for the CPT data

Species	product	AIC (smaller is better)		
		WEIBULL	NORMAL	LNORMAL
I. ricinus	DEET	9.526	35.761	11.215
	EBAAP	22.620	43.660	25.601
	Icaridin	6.838	47.639	9.157
I. scapularis	DEET	11.119	40.365	11.250
	EBAAP	17.178	28.812	19.908
	Icaridin	8.623	36.826	10.103
Note: yellow-shaded cells indicate the selected distributions				

Table A9: Estimated Weibull Parameters using MLE

Species	product	Weibull_Scale	Weibull_Shape	p5	p50	P5MR
I. ricinus	DEET	4.276	3.773	1.946	3.880	0.502
	EBAAP	3.821	1.981	0.853	3.175	0.269
	Icaridin	8.792	4.219	4.348	8.060	0.539
I. scapularis	DEET	5.074	3.287	2.056	4.539	0.453
	EBAAP	2.250	2.586	0.713	1.952	0.365
	Icaridin	4.657	3.978	2.208	4.248	0.520

SAS code

```
*=======================================================*
```

```
* Programmer: James Nguyen, USEPA                          *
*                                                          *
* Project: Tick Repellency Studies                         *
*                                                          *
* Study: CPT data in Kerstin Buchel 2015 article           *
*                                                          *
* Purpose: estimate parameters of the CPT data assume      *
*          the data follow Weibull distribution            *
*                                                          *
* Date: 12/14/2017                                         *
*==========================================================*;
options Formdlim="=" nodate nonumber ls=100 ps=100;

Proc import datafile="F:\Insect Repellency\Tick Repellent Studies\PCT Kerstin Buchel 2015
data.xlsx"
        dbms=xlsx out=Buchel replace;
run;
Proc sort data = Buchel; by species product; run;

ods graphics on;
ods rtf file="C:\Users\JNguyen\Desktop\Junks\Kaplan-Meier Survival Curves.rtf"
startpage=no;
proc lifetest data = Buchel method=km plots=(survival(atrisk=0 to 12 by 0.5));
        time CPT*status(0);
        strata Product;
        by species;
run;
ods rtf close;

*===> testing distributions;
ods output  FitStatistics=WEIBULL(rename=(Value=WEIBULL));
Proc lifereg data = Buchel;
        by species product ;
        model CPT*status(0)=/distribution=WEIBULL;
run;
ods output  FitStatistics=NORMAL(rename=(Value=NORMAL));
Proc lifereg data = Buchel;
        by species product ;
        model CPT*status(0)=/distribution= NORMAL;
run;
ods output  FitStatistics=LNORMAL(rename=(Value=LNORMAL));
Proc lifereg data = Buchel;
        by species product ;
        model CPT*status(0)=/distribution= LNORMAL;
run;

Data Distributions;
        merge WEIBULL NORMAL LNORMAL;
        by species product ;
        if criterion = "AIC (smaller is better)";
run;

ods rtf file="C:\Users\JNguyen\Desktop\Junks\Kaplan-Meier Survival Curves.rtf"
startpage=no;
Proc print data = distributions noobs; run;
ods rtf close;



*===> Estimate Weibull parameters;

ods output  ParameterEstimates=ParameterEstimates;
Proc lifereg data = Buchel;
        by species product ;
        model CPT*status(0)=;
run;

Proc transpose data = ParameterEstimates out=ParameterEstimates(drop=_NAME_);
        where Parameter in ("Weibull Scale","Weibull Shape");
        by species product ;
        var Estimate;
```

```
        ID Parameter;
run;

data ParameterEstimates;
        set ParameterEstimates;
        p5 = quantile('WEIBULL',0.05,Weibull_Shape,Weibull_Scale);
        p50 = quantile('WEIBULL',0.5,Weibull_Shape,Weibull_Scale);
        P5MR=p5/p50;
run;

ods rtf file="C:\Users\JNguyen\Desktop\Junks\Weibull Parameters.rtf" startpage=no;
Proc print data = ParameterEstimates noobs;
        format Weibull_Shape Weibull_Scale p5 p50 p5mr 6.3;
run;
ods rtf close;
```

14.2C SAS Codes for Simulations

```
*============================================================*
* Programmer: James Nguyen, USEPA                            *
*                                                            *
* Project: Ticks Repellency Studies                          *
*                                                            *
* Purpose: Power Analysis/sample size calculation for        *
*          study design of 1 tick/15 minuttes                *
*                                                            *
* Description:                                               *
*     - distributions: Weibull, Normal, Lognormal, Uniform   *
*     - create histograms of the distributions               *
*     - SAS Procedures: PROC LIFETEST and PROC ICLIFETEST    *
*                                                            *
* Date: 1/09/2018                                            *
*============================================================*;
options formdlim="=" ps=90 ls=90 nonumber nodate;

libname Ticks "C:\Users\JNguyen\Desktop\Ticks - 15 min interval";


%Macro distParam;
        if upcase(Distribution) = "WEIBULL" then do;
                * Weibull = f(x,a,b);
                a = log(log(0.95)/log(0.5))/log(P5MR);              b = exp((1/a)*log(-
(MED**a)/log(0.5)));
        end;
        if upcase(Distribution) = "UNIFORM" then do;
                * uniform = U[a, b];
                a = MED*(0.5*P5MR - 0.05)/0.45;             b = MED*2 - a;
        end;
        if upcase(Distribution) = "NORMAL" then do;
                *normal = N(a,b);
                a = MED;              b = MED*(1-P5MR)/1.645;
        end;
        if upcase(Distribution) = "LOGNORMAL" then do;
                * lognormal = exp(N(a,b));
                a = log(MED);         b = (log(MED)-log(MED*P5MR))/1.645;
        end;
%Mend;title;

%Macro generate;
        if upcase(Distribution) = "WEIBULL"   then CPT = rand("Weibull", a, b);
        if upcase(Distribution) = "LOGNORMAL" then CPT = exp(rand("Normal", a, b));
        if upcase(Distribution) = "NORMAL"           then CPT = rand("Normal", a, b);
        if upcase(Distribution) = "UNIFORM"          then CPT = a + (b-a)*rand("Uniform");
%Mend;


%Macro CPT;
```

```
        CPT=CPT*60;

        *==> the time to test 5 ticks is 20 minutes;

        if CPT <= 3                             then do; CPT = 0;
                censor = 0;    end;
        else if CPT > &maxT*60 + 3  then do; CPT = &maxT*60;                        censor
= 1;    end;
        else                                                    do; CPT = 15*ceil((CPT-
3)/15);         censor = 0;    end;

        CPT = CPT/60;
%Mend;title;


%Macro power;
        ods select none;
        ods output Quartiles=MPT;
        Proc lifetest data = Simmer(keep=MED P5MR N Sim CPT Censor);
                by MED P5MR N Sim;
                time CPT*Censor(1);
        run;

        ods select default;

        Proc datasets nolist; delete simmer; run;quit;

        Data MPT;
                set MPT;
                if percent = 50;
                power = (LowerLimit >= &K*Estimate);
        run;

        Proc SQL;
                create table &dist&MED as
                select MED, P5MR, N, avg(Power) as Power
                from MPT
                group by MED, P5MR, N;
        quit;

%Mend;title;

%Macro PowerCPT(med=, P5MRS=, nmin=,nmax=,maxT=,K=,dist=,NSim=, seed=);

        %let N=1;
        %let P5MR&N = %nrbquote(%scan(&P5MRS,&N, %str( )));
        %do %while (&&P5MR&N ^=);
                %let N=%eval(&N+1);
                %let P5MR&N = %nrbquote(%scan(&P5MRS,&N, %str( )));
        %end;
        %let N=%eval(&N-1);


        %do i = 1 %to &N;

                %if &i = 1 %then %do; data All_&dist&MED; set _NULL_; run; %end;

                Data Parameters;
                        MED = &MED;
                        P5MR = &&P5MR&i;
                        P5 = MED*P5MR;
                        label MED = "median" P5MR="5%-tile/median ratio";
                run;

                Data Parameters;
                        set Parameters;
                        Distribution = "&dist";
                        %distParam;
                run;

                data simmer;
```

```
                        call streaminit(&seed);
                        set Parameters;
                        do N = &Nmin to &Nmax;
                                do Sim = 1 to &NSim;
                                        do ID = 1 to N;
                                                %generate;
                                                output;
                                        end; *ID;
                                end; *Sim;
                        end; *N;
                        drop a b;
                run;

                Data Simmer;
                        set Simmer;
                        %CPT;
                run;

                %power;

                Data All_&dist&MED;
                        set All_&dist&MED &dist&MED;
                run;

                Proc datasets nolist; delete Parameters MPT &dist&MED; quit;

        %end;

        Data Ticks.&dist._T15_M&MED._K%sysevalf(100*&K)_N&nmin._&nmax._D&maxt;
                set All_&dist&MED;
        run;

        Proc datasets nolist; save sasmacr; run;quit;

%Mend;


dm log 'clear';%PowerCPT(med=2,  P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20,
maxT=12, K = 0.6, dist= weibull, NSim=4000, seed=56198);
dm log 'clear';%PowerCPT(med=4,  P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20,
maxT=12, K = 0.6, dist= weibull, NSim=4000, seed=56198);
dm log 'clear';%PowerCPT(med=6,  P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20,
maxT=12, K = 0.6, dist= weibull, NSim=4000, seed=56198);

dm log 'clear';%PowerCPT(med=8,  P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20,
maxT=12, K = 0.6, dist= weibull, NSim=4000, seed=56198);
dm log 'clear';%PowerCPT(med=10, P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20,
maxT=12, K = 0.6, dist= weibull, NSim=4000, seed=56198);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maxT=12, K = 0.8, dist= weibull, NSim=4000, seed=56198);



*=====> Create Figures and Print Results;


libname Ticks "C:\Users\JNguyen\Desktop\Ticks - 15 min interval";
%let folder=C:\Users\JNguyen\Desktop\Ticks - 15 min interval;

%Macro SGPLOT(dist=, K=, nmin=, nmax=, maxt=);
        title "&dist median = 2 hours, K = 0.&K";
        Proc SGPLOT data = Ticks.&dist._T15_M2_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt;
                scatter x = N y = Power/group = P5MR;
                series x = N y = Power/group = P5MR;
                refline 0.8 0.9/axis=y;
                yaxis min=0 max=1;
        run;
        title "&dist median = 4 hours, K = 0.&K";
        Proc SGPLOT data = Ticks.&dist._T15_M4_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt;
                scatter x = N y = Power/group = P5MR;
                series x = N y = Power/group = P5MR;
                refline 0.8 0.9/axis=y;
                yaxis min=0 max=1;
        run;
        title "&dist median = 6 hours, K = 0.&K";
        Proc SGPLOT data = Ticks.&dist._T15_M6_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt;
                scatter x = N y = Power/group = P5MR;
```

```
		series x = N y = Power/group = P5MR;
		refline 0.8 0.9/axis=y;
		yaxis min=0 max=1;
	run;
	title "&dist median = 8 hours, K = 0.&K";
	Proc SGPLOT data = Ticks.&dist._T15_M8_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt;
		scatter x = N y = Power/group = P5MR;
		series x = N y = Power/group = P5MR;
		refline 0.8 0.9/axis=y;
		yaxis min=0 max=1;
	run;
	title "&dist median = 10 hours, K = 0.&K";
	Proc SGPLOT data = Ticks.&dist._T15_M10_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt;
		scatter x = N y = Power/group = P5MR;
		series x = N y = Power/group = P5MR;
		refline 0.8 0.9/axis=y;
		yaxis min=0 max=1;
	run;
%Mend;

%Macro print(dist=, K=, nmin=, nmax=, maxt=);
	data &dist._K&K;
		set Ticks.&dist._T15_M2_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt
			Ticks.&dist._T15_M4_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt
			Ticks.&dist._T15_M6_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt
			Ticks.&dist._T15_M8_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt
			Ticks.&dist._T15_M10_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt;
	run;
	Proc transpose data = &dist._K&K out = &dist._K&K(drop=_NAME_);
		by MED P5MR;
		ID N;
		var Power;
	run;
	title "&dist K=0.&K.0";
	Proc print data = &dist._K&K noobs label; format _: 6.3; run;
%mend;



%SGPLOT(dist=Weibull, K=6, nmin=10, nmax=20, maxt=12);
%SGPLOT(dist=Weibull, K=6, nmin=21, nmax=30, maxt=12);

%SGPLOT(dist=Weibull, K=7, nmin=10, nmax=20, maxt=12);
%SGPLOT(dist=Weibull, K=7, nmin=21, nmax=30, maxt=12);

%SGPLOT(dist=Weibull, K=8, nmin=10, nmax=20, maxt=12);
%SGPLOT(dist=Weibull, K=8, nmin=21, nmax=30, maxt=12);



ods rtf file = "&folder\&dist 15 minutes.rtf" bodytitle;
%print(dist=Weibull, K=6, nmin=10, nmax=20, maxt=12);
%print(dist=Weibull, K=6, nmin=21, nmax=30, maxt=12);

%print(dist=Weibull, K=7, nmin=10, nmax=20, maxt=12);
%print(dist=Weibull, K=7, nmin=21, nmax=30, maxt=12);

%print(dist=Weibull, K=8, nmin=10, nmax=20, maxt=12);
%print(dist=Weibull, K=8, nmin=21, nmax=30, maxt=12);
ods rtf close;
```