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

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

March 25, 2021

MEMORANDUM

SUBJECT: Science and Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing Oil of Lemon Eucalyptus (OLE or Citriodiol) and 2-undecanone (Methyl Nonyl Ketone or MNK)

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REF: Dr. Scott P. Carroll, Study Director. (2020) Protocol for "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions." Protocol No. MIM-007. Unpublished document sponsored by Mimikai, 1564 Green Valley Road, Danville, CA 94526. February 17, 2020; Amended December 23, 2020. 95p. MRID 510641-10.

We have reviewed the referenced protocol for laboratory testing for a skin-applied repellent product containing Oil of Lemon Eucalyptus (OLE or Citriodiol) and 2-undecanone (methyl nonyl ketone or MNK) against three species of ticks in a laboratory setting from both scientific and ethical perspectives. The product is Mimikai Lilly Pilly Repellent, applied by bag-on-valve spray. This protocol was submitted by Carroll-Loye Biological Research. The study is sponsored by Mimikai. This review assesses the scientific aspects of the proposed research for a product performance study to evaluate the efficacy of skin applied insect repellent product according to guidelines from the U.S. Environmental Protection Agency (EPA) OPPTS 810.3700 Guideline, *Insect Repellents to be Applied to Human Skin*, as well as the recommendations from the EPA and

the Human Studies Review Board (HSRB) related to testing skin-applied repellent efficacy using human subjects. Ethical aspects of the proposed research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L.

A. History of Submission

In 2018, Mimikai Inc. submitted a study protocol, MIM-005, for joint review of EPA and HSRB. The proposed product, Mimikai Fragrance Free, was a mosquito and tick repellent, containing a combination of a conventional active ingredient, MNK, and a biopesticide, OLE, at respective concentrations of 15% and 10% by weight. Mimikai Inc. sought to bridge toxicity data from a 21-day dermal toxicity study (MRID 43110301) used in the registration of a similar product (EPA Reg. No. 82669-2), containing a lower concentration of MNK (7.75%). This bridging request was deemed unacceptable for fulfilling toxicity data requirements. Consequently, the protocol application (93616PA1 Mosquito and Tick HSRB Efficacy Study Protocols; Decision Number: 543565) was withdrawn on March 22, 2019.

Mimikai Inc. reformulated the product to contain 7.75% of MNK and the same amount of OLE, and request bridging to 21-day dermal toxicity study (MRID 43110301). Mimikai submitted protocol MIM-007, dated February 17, 2020, to the EPA for review. The EPA identified deficiencies with the February 17, 2020 version of the protocol. In response, the sponsor revised the protocol and submitted the revised protocol dated December 23, 2020 to the overseeing institutional review board (IRB) for review and approval. Following approval by the IRB, the sponsor submitted the revised protocol and updated IRB review documents to the EPA.

This review is of the amended protocol dated December 23, 2020.

B. Completeness of Protocol Submission

The protocol and related documents submitted to EPA were reviewed for completeness against the required elements listed in 40 CFR §26.1125. EPA checklist is appended to this review. The submission of the protocol dated December 23, 2020 did not include the original IRB correspondence volume provided with the February 17, 2020 submission; rather, it included only the information related to the approval of the amended protocol. The EPA reviewed the IRB correspondence associated with both of the protocol packages (February 17 and December 23) in determining whether a complete package was submitted. All elements of required documentation have been provided.

C. Summary Assessment of Scientific Aspects of the Proposed Research

Objectives

The objective of the study is stated in §1.1 (p. 5) as "*The research objective is to determine the duration and efficacy of the Test Material, when applied at a typical consumer dose, in repelling the following tick species: Deer tick (blacklegged tick) - Ixodes scapularis; American dog tick - Dermacentor variabilis, and Lone star tick - Amblyomma americanum.*"

Endpoints and Definitions

The efficacy endpoint is the First Confirmed Crossing (FCC), defined as a crossing followed by another crossing within 30 minutes (§1.1; p. 5). Complete Protection Time (CPT) is the measurement for residual repellency or time from product application to product failure. Time to product failure is measured by FCC per tick species per subject. CPT is defined as "*the time between application of Test Material and the First Confirmed Crossing of an actively foraging tick from the untreated skin surface of a subject's hand 3 cm or more into the treated forearm skin area"* (§1.1; p. 5).

Study Plan

This proposed laboratory study using human subjects for the testing duration of efficacy of a repellent product, Mimikai Lilly Pilly, at preventing tick species from crawling on human hosts. The proposed test is designed to determine the CPT of the product at a standard application rate of 0.5g/600 cm^2. The CPT (signaling product failure) is measured for each of the three tick species by two ticks of the same species crossing on treated skin within 30 minutes.

The study plan includes a minimum of four days. The first day visit will take place within 30 days prior to repellency test day and it will last from 2 to 2.5 hours (§4.8.1, p. 28). The first day visit includes orientation, obtaining participants' consent, and taking measurements of participants' forearm length and circumferences for calculation of the skin surface area. [Informed Consent Form (ICF) in Protocol Appendix 1] During this visit, subjects' attractiveness to ticks is confirmed. If during pre-test training a subject misses five exposures due to ticks failing to crawl on the subject, the subject will be asked to withdraw (§1.3.2; pg. 7-8 and §4.7.6; pg. 27). Participants will also spend 30 minutes in pre-training activities, including practice handling ticks and becoming familiar with tick behavior (§1.3.2; pg. 7). Subjects will learn how to manipulate ticks with fine paintbrushes, place them on their own forearms, observe and quantify tick movement on their arms, and dispose of used ticks (Protocol Appendix 3).

Repellency testing will begin during the second day visit and may extend to three or more days. Each test day will take from four to 14 hours, depending on residual activity of test substance (ICF in Protocol Appendix 1). Repellency evaluation will require 33 subjects, 25 test subjects and 8 alternates, and a minimum of three days to complete testing using all three tick species per subject (§4.7, pg. 25). This proposed laboratory study using human subjects for the testing lasting efficacy of a repellent product, Mimikai Lilly Pilly, at preventing tick species from crawling on human hosts. The proposed test is designed to determine the CPT of the product at a standard application rate of 0.5g/600 cm^2. The CPT (signaling product failure) is measured for each of the three tick species by two ticks of the same species crossing on treated skin within 30 minutes.

The pattern in the proposed research consists of exposing a new tick to repellent treated skin for three minutes at 15 minute intervals until either CPT is reached per tick species per subject or subject reaches end of test day without experiencing CPT for the tick species tested. Each subject will act as his/her own control for screening actively questing ticks for testing repellency.

Both forearms of each subject, control and treated forearm, will be arranged in an identical fashion, washed with unscented detergent, rinsed with 70% ethanol solution and towel-

dried. The ventral surface of both forearms will be marked with three lines. The first will be the reference line, placed on the secondary crease of the wrist. The line for tick placement will be a longitudinal line placed on the palm of the hand three cm from the reference line. The boundary line will be three cm distal to reference line toward the elbow on the treated area. These lines are used to define the criteria for actively questing ticks on untreated arms and for repellency on treated arms. The arm is positioned at a 30° angle with the underside of the hand resting horizontally on the bench. An illustration is provided in Figure 1 (§4.8.3; p. 29).

Every 15 minutes a researcher will announce the beginning of a new testing period. At each testing period, each subject selects an unused tick and screens it on the untreated arm for active questing behavior. A qualifying tick begins walking on the hand of the subject's untreated arm within approximately 15 seconds of being released. Next, a tick is scored as actively questing if it crosses the reference line toward the elbow (§4.7.3; p. 27). Only actively questing ticks as defined in §4.8.3.1 will be selected for repellency testing and those that fail will be discarded. The screening process will be repeated every 15 minutes for new ticks until an actively questing tick is identified or the stopping rule is invoked (§4.7.6). Once an actively questing tick is identified, it is transferred to the treated arm for up to three minutes from the moment the tick begins moving. A crossing occurs when a tick travels upward toward the elbow three cm or more within three minutes of beginning to move. A tick is repelled when it changes direction away from boundary line at the margin of treated skin, or upon approach to treated skin, or does not cross more than three cm within three minutes of entering treated area (§4.8.3.1; p. 30). The number of crossings on each subject's treated arm will be recorded. Data sheets for data collection are provided in Protocol Appendix 4.

All subjects are asked to contact the Study Director and a physician of their own choice at any time should they develop a skin rash (a delayed hypersensitivity reaction) or any other adverse effects within seven days of the conclusion of the test day (§1.3.6; pg. 8).

Sample size

The study protocol proposes to test the product on a sample size of 25 subjects per tick species according to the EPA Power Analysis for Determination of Sample Size (Protocol Appendix 8). 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 needed. Enrollment will include eight additional subjects as alternates. "*Alternate subjects may return later to replace subjects that initiate testing but withdraw before useful data are generated. They also serve as insurance against any enrolled subjects who fail to appear"* (§3.5; p.19). Total number of subjects participating in testing each species of tick will be up to 33 participants (§4.1; p. 19).

Randomization

At the first visit, subjects will be assigned unique sequential numbers in order of arrival and the numbers will be randomized for assigning subjects to repellency tests. The randomization procedure is described in §3.2 (p. 15). Recruitment will remain open until at least 44 candidates (22 males and 22 females) are enrolled. Subjects will be randomly designated as treated or alternate subjects. In a sample of 25 subjects, an approximately even sex ratio of 50:50

males to females will be achieved by randomly choosing subjects from either of the two genders, 12 of one gender and 13 of the other (§3.2, p. 15). The remaining eight subjects will serve as alternates (§4.7, p. 25). In addition, the treatment will not be randomly applied to either right or left arm. The treatment will be applied to the non-dominant arm to facilitate handling ticks (§4.7; p. 26).

Test Substance Application

The product, Mimikai Lilly Pilly Repellent, will be applied at the EPA standard rate of 0.5 g / 600 cm^2 for testing repellency.[1] Prior to application, forearms will be cleansed using unscented soap, rinsed with 70% alcohol solution and towel dried. Doses will be converted to volume using test substance specific gravity, then individual doses calculated based on the subject's skin surface area will be dispensed from tuberculin (1 ml) syringes and evenly applied to the non-dominant forearm in a light rubbing motion by researchers wearing surgical gloves. Multiple research personnel will make the applications, and each application will take approximately two minutes. Treatment will not be applied randomly to either right or left arm. The treated arm will be the non-dominant arm to facilitate handling ticks (§4.7; p. 26).

Estimation of Skin Surface Area

Forearm surface area will be calculated from length of forearm by its average circumference. Average circumference will be measured at four points: upper forearm, lower forearm, and two equally spaced points in between (§4.5; p. 20).

Margin of Exposure

The product, Mimikai Lilly Pilly Repellent, will be tested using three tick species of public health significance: *Ixodes scapularis; Dermacentor variabilis,* and *Amblyomma americanum,* at the EPA standard dose of 0.5g/ 600 cm^2.[2] Mimikai Lilly Pilly Repellent is classified as toxicity category IV for all routes of exposure, Acute Oral Toxicity; Acute Dermal Toxicity; Acute Inhalation; Acute Eye Irritation, and Primary Dermal Irritation (Protocol Appendixix 6). The product contains the active ingredients OLE at a concentration of 11.0% w/w and MNK at a concentration of 7.75% by weight of product (w/w).

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. OLE contains 65% PMD according to EPA's risk assessment [EPA Memorandum Feb. 4, 1999; Biopesticide Registration of Citriodiol (100% pure, containing 65 % PMD)]. OLE is classified as toxicity category II for Eye Irritation (MRID 446242-05); toxicity category III for Acute Oral (LD_{50} > 2,408 mg/kg (MRID 446242-03)), Acute Dermal (LD_{50} > 2,000 mg/kg (MRID 446242-04)), and Dermal Irritation (MRID

[1] Dawson, Liza. April 22-23, 2015 EPA Human Studies Review Board Meeting Report. https://www.epa.gov/sites/production/files/2015-06/documents/hsrb_april_2015_meeting_final_report.pdf. p. 12.

[2] Dawson, Liza. April 22-23, 2015 EPA Human Studies Review Board Meeting Report. https://www.epa.gov/sites/production/files/2015-06/documents/hsrb_april_2015_meeting_final_report.pdf. p. 12.

446242-06); and toxicity category IV for Acute Inhalation (LC_{50} > 2.06 mg/L (MRID 446241-04)). It is not a dermal sensitizer (MRID 446242-07) and not mutagenic (MRID 446242-08). A 90-Day dermal study in rats (MRID 444387-10) tested PMD (98.3 % pure) at increasing doses of 0, 1,000 and 3,000 mg/kg/day. The No Adverse Effect Level (NOAEL) = 1,000 mg/kg/day, and the Lowest Adverse Effects Level (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. Risk characterization for infants and children is based on data from a developmental study using female rats (MRID 444387-11) in which the NOAEL = 3,000 mg/kg/day. No LOAEL was established, 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.

The active ingredient MNK is classified as toxicity category III for acute dermal (LD_{50} > 2,000 mg/kg (MRIDs 419041-02 and 431638-01), acute eye irritation (MRIDs 419041-04), and acute dermal irritation (MRID 419041-05); and as toxicity category IV for acute oral (LD_{50} >5,000 mg/kg (MRID 419041-01)) and acute inhalation (LC_{50} > 5.43 mg/L (MRID 419041-03). MNK is a weak sensitizer (MRID 419041-06). The reported NOAEL for systemic toxicity is 300 mg/kg/day and 100 mg/kg/day for dermal irritation in New Zealand white rabbits, based on 21-day sub-chronic dermal exposure (MRID 431103-01). The study limited testing to 300 mg/kg/day. A 90-day inhalation study was not conducted because chronic inhalation effects are not expected based on low vapor pressure (4.49×10^{-2} Torr). For maternal and developmental toxicity, the reported NOAEL > 1,000 mg/kg/day at the highest dose tested. MNK is not mutagenic (U.S. EPA Reregistration Eligibility Decision (RED) for Methyl Nonyl Ketone. July 1995. 738-R-95-038, and EPA Preliminary Work Plan and Summary Document. MNK. Registration Review. March 28, 2012, in Protocol Appendix 6).

Mimikai Lilly Pilly is categorized as Toxicity Category IV for all route of exposures (MRIDs 510641-03 thru 510641-08 in Protocol Appendix 6). The risk for the product Mimikai Lilly Pilly Repellent was estimated based on the dermal loading rate instead of body burden because the endpoint selected for dermal exposure is based on skin irritation, which is a superficial effect in a localized area rather than a systemic effect that occurs after absorption (*see* Attachment 4). Therefore, this method of risk estimation is more biologically relevant. Risk was estimated using the dermal loading rate in the 21-day dermal toxicity study (3.3 mg ai/cm^2) divided by the loading rate of the active ingredient on the skin provided by the applicant (0.064 mg ai/cm^2). The resulting risk estimate, margin of exposure, (MOE) is 52. Since 52 exceeds the LOC of 10, there is no risk of concern to the participants in this study (*see* Attachment 4).

In order to calculate the dermal loading rate in the 21-day dermal toxicity study, the dose of 100 mg/kg/day is multiplied by the average weight of the rabbit in the study, which was 3.3 kg. The resulting dose to the rabbit is 330 mg MNK/rabbit. This is then divided by the surface area of the exposed patch of skin of the rabbit which was 100 cm^2. This results in a dermal loading rate of 3.3 mg MNK/cm^2. This rate is then compared to the loading rate in the protocol which was 0.833 mg product/cm^2. Since the protocol is using the actual product, the active ingredient percentage (7.75% MNK) needs to be taken into consideration, 0.833 is multiplied by 0.0775, resulting in a loading of 0.064 mg/cm^2 on the human subject. The loading rates are then compared, 3.3/0.064 to result in an MOE of 52.

Stopping Rule, Replacing Subjects, and Subject Withdrawal

Stopping rules (§4.7.6) will be applied:

a) when consented duration is reached (i.e., 14 hours of testing),
b) for safety reasons,
c) when a subject receives confirmed crossing for all three species tested that day, and
d) when a subject is unattractive to ticks during pre-test training and during repellency testing.

Subjects are stopped from testing when any qualifying tick per five exposures of each species fails to cross onto untreated skin (§4.7.6; p. 27, Protocol Appendix 3).

Withdrawal and Criteria on Use of Data and Subject Replacement

Participants are free to withdraw at any time without penalty or loss of compensation or benefits. Data collected to the point of withdrawal will be used in the statistical analysis of the data unless the participant requests that their data are not used (ICF in Protocol Appendix 1). If more than eight of 25 subjects withdraw prematurely, those with the briefest participation will be replaced first. If a subject withdraws before completing a test day**,** the withdrawn subject is replaced on a subsequent day if his/her total exposure time is less than 90% of the average exposure time of subjects that do not withdraw, and no more than eight of 25 subjects have withdrawn (§4.8.4; p. 31). If the subject withdraws after a full day of testing one tick species, his/her data will be used for that day, and the subject will be replaced for testing next tick species on the next day (§4.7.6, p. 28).

Data Collection

Data collected from each subject include the following measurements: crossings and repulsions per tick species at each time point; exposure delay; time (in minutes) between application and first exposure; time (in minutes) to FCC; and cumulative time period between time of application and time to FCC (in minutes) (§4.9, p. 31). Examples of raw data collection sheets are provided in Protocol Appendix 4.

Statistical Analysis

The median CPT with 95% confidence intervals will be estimated per tick species across 25 subjects, using Kaplan-Meier survival analysis. The estimated CPT and Kaplan-Meier Survival curves will be reported.

How and to What Will Human Subjects be Exposed/Product Description

Subjects will be exposed to ticks and the repellent product, Mimikai Lilly Pilly. Each day of testing will take a minimum of 4 hours and a maximum of 14 hours from time of product application. Repellency evaluation will take three days of testing per subject to complete testing using all three tick species. Ticks employed for testing repellency are pathogen-free and sourced

from laboratory-reared colonies. Proposed exposure periods consist of exposing ticks to untreated human skin for screening and to treated forearm skin for three minutes at 15 minute intervals for a maximum of 14 hours of testing per species, or until the time point when repellent breakdown, or CPT is reached by subject, whatever occurs first.

The product, Mimikai Lilly Pilly, is a pressurized bag-on-valve (BOV) formulation, containing 11.0% by weight of product (w/w) of the active ingredients OLE and 7.75% w/w of MNK. Mimikai Lilly Pilly is categorized as Toxicity Category IV for all route of exposures (Protocol Appendix 6).

Good Laboratory Practice (GLP) Compliance and Quality Assurance

Good Laboratory Practices, as defined by 40 CFR part 160, will be followed throughout this study. *"A separate, professional Quality Assurance Unit (QAU) will inspect the study at critical phases and maintain written, signed records of each inspection. The QAU will report to management as defined in the organizational chart for Carroll-Loye Biological Research. Protocol Review and Comments must take place before data collection commences. In-Life Inspection must include observing the measurement and recording of key variables by subjects and researchers. In addition, the Final Report will be audited for completeness and accuracy. A QAU Statement will address compliance and noncompliance or any omissions in auditing. Findings from the In-Life Inspection and the Final Report, as well as the. QAU Statement will be transmitted to both the Study Director and to the Sponsor Monitor"*(§5; p. 32).

Compliance with FIFRA and EPA Regulations

Data resulting from execution of this protocol as well as study conduct will be reviewed by the US EPA and its HSRB for compliance with FIFRA 12(a)(2)(P) and 40 CFR 26 subparts K, L and M, and will be independently audited by a QAU for compliance with Good Laboratory Practice Regulations (40 CFR 160).The QA representative will conduct critical phase inspections to ensure study integrity and maintain written and signed records of each inspection.

Study Site Location and Testing Facility:

Testing Facility: Carroll-Loye Biological Research; 711 Oak Avenue, Davis, CA 95616

Study Director: Dr. Scott P. Carroll

Study Sponsor: Mimikai;1564 Green Valley Road, Danville, CA 94526

D. Compliance with Applicable Scientific Standards

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

- Experimental design
- Data analysis
- Risk minimization

E. EPA Science Comments

The study protocol should be revised according to the following recommendations before the research goes forward:

1. Revise the statement, "*one tick species will be used on each test day for a total of three test days*" (§4.7, pg. 26), which seems to indicate testing of one single tick species per day. This is inconsistent with the statement, "*multiple species are being tested on a single day*" (4.8.3; pg. 29).
2. Expand the list of proposed tick species for testing as follows: *Ixodes scapularis*, *Amblyomma americanum,* and either *Dermacentor variabilis*, or *Dermacentor andersoni*, or *Rhipicephalus sanguineus.*
3. Explain how data from subjects withdrawing before completing one day of testing, who will not be replaced, will be treated for statistical analysis.
4. Confirm that that the same group of 25 subjects will test all three tick species on more than one day of testing.
5. Clarify whether a subject will be stopped from testing all together when one qualifying tick fails to cross on 5 exposure during screening on the control arm, or whether the subject will be stopped from testing with that species only.
6. Establish a criterion that a subject will be stopped from testing when they fail the screen on two species of ticks.
7. Revise the protocol to note that testing on a specific tick species will be stopped when more than five exposures are missed due to qualifying ticks of that species failing to cross on untreated arm of subjects.

F. Summary Assessment of Ethical Aspects of the Proposed Research

Here is a summary of the EPA's observations about the ethical aspects of the proposed protocol. 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 repellent product, Mimikai Lilly Pilly, against ticks. Efficacy will be expressed as CPT. 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 efficacy of this product. Research with human subjects is necessary because there are no reliable non-human methods for generating the necessary data. As intended, the data resulting from this proposed study will be used to support registration of this product, Mimikai Lilly Pilly Repellent.

2. **Subject Selection:** The protocol calls for testing each product with 25 subjects, with an approximately equal number of males and females. In addition, eight subjects will be enrolled as alternates, to take the place of any test subjects who withdraw before or on the day of testing (at least two subjects of each gender). A total of 33 individuals (25 test subjects, 8 alternates) will be selected to test each product. Therefore, a total of 40

subjects would be needed assuming each individual participates only in a single test day.

Subjects will be recruited through print and digital advertising conducted either by Carroll-Loye or a local subject recruitment service. Recruitment will occur in the greater Sacramento area. Recruitment materials, including advertisements and phone scripts, have been reviewed and approved by Advarra IRB. Advertisements will provide basic information about the study, and a phone number for research staff tasked with screening callers. The Carroll-Loye researcher who conducts the phone screen will follow an IRB-approved script and will ask interested individuals to screen themselves against the most common exclusion criteria.

The results of testing this product should be as generalizable as possible to the target population of skin-applied insect repellent users. Every effort will be made to achieve an appropriate demographic composition of the pool of recruited and enrolled subjects. The final study report will include demographic information about the subjects who participated, based on gender, age, and ethnic background, due to availability of test subjects on each test day. Recruitment will be open until at least 44 individuals agree to attend a consent meeting, with a target of at least 22 males and 22 females.

Prior to participating in the efficacy testing, subjects will participate in a session to confirm subjects' attractiveness to ticks and a training on how to observe and handle ticks during the study (tick placement, removing ticks before they bite). Subjects who are not deemed attractive to ticks or able to place them properly based on this assessment will be withdrawn from further study participation.

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

3. **Informed Consent:** During the recruitment period, interested candidates will contact study staff via phone or email to learn more about the study and to self-evaluate whether they meet the eligibility criteria. Those who are interested in continuing with enrollment will be invited to meet one on one with the study staff. The study staff member will begin by reviewing the eligibility criteria and informing female subjects about the requirement for pregnancy testing. Individuals who are qualified proceed to the consent process, where the researcher provides information about the study orally and to describe the elements of study participation step by step. Subjects will be reminded that they can ask questions and meet privately with the Study Director at any time, and that they are free to withdraw from the study at any time without forfeiting any benefits to which they are entitled. Those who wish to continue will be provided the with the consent form, the Experimental Subjects' Bill of Rights, a copy of the protocol, and any supporting documents. The researcher will read aloud the consent form and Bill of Rights, and answer any questions from the participating individual. Again, candidates will be reminded that they are not obligated to consent to enroll and that they are free to withdraw from participation at any time without penalty. All individuals will be provided a copy of their signed consent form and Bill of Rights.

4. **Risks to Subjects:** The protocol discusses five risks to subjects as a result of study participation: exposure to the test material, exposure to ticks and tick-borne illness, physical stress of test conditions, and psychological risks associated with disclosure of pregnancy testing results. The protocol notes that risks will be minimized as follows.

   Both active ingredients in the test product are registered with the EPA for use in skin-applied repellents at or above the concentrations used in this product. The EPA's science review concludes that the risks associated with exposure to the test substance during the study are low. To further minimize the risks, subjects will be enrolled only if they do not have a known sensitivity or allergy to insect repellents or common cosmetics. 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. Subjects will be trained to remove ticks before they begin to bite and attach to the subject. To eliminate the risk of transmission of tick-borne disease, ticks will be sourced from pathogen-free colonies. Ticks will only be used once with a single subject, and ticks will be destroyed at the end of the test day.

   Pregnancy testing will be conducted in private and only a single female member of the research team will discuss the results with the subject.

   Members of the research team will be qualified as first aid providers and available during any subject encounter. Additionally, a physician who is familiar with the protocol will be on-call during test days and available to answer any questions involving the safety and health of subjects. The consent form provides contact information for the Study Director and instructs subjects to contact the study team in the event of any adverse reaction during the study or if any adverse health condition arises within 7 days of their participation to account for delayed reactions such as hypersensitivity. The protocol describes the procedures that will be followed in the event a subject needs to be taken for immediate medical attention to ensure the remaining subjects' safety and to allow the test day to proceed.

   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.

5. **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 a new skin-applied insect repellent product. Use of this product could lead to fewer tick bites and reduced incidents of tick-borne illnesses.

6. **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.

7. **Independent Ethics Review:** Advarra IRB approved the protocol dated December 20, 2020, informed consent form, and recruitment materials (pp. 36-38). Advarra's IRB is registered with FDA and OHRP, and has a Federal-wide Assurance approved by OHRP (00023875). Advarra is fully accredited by the Association for the Accreditation of Human Research Protection Programs (AAHRPP). Satisfactory documentation of the IRB procedures and membership is on file with the Agency. Documentation regarding IRB approval of the protocol, consent and recruitment materials has been provided to the HSRB members with the background materials for this review.

8. **Respect for Subjects**: The subjects' identities will be protected as follows: each subject will be assigned a code number/identifier. The study records will be maintained in locked cabinets. Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing.

   Throughout the recruitment and consent processes, and again at the start of each test day, candidates and subjects will be informed that they are free to decline to participate or to withdraw at any time for any reason without forfeiting any benefits to which they are entitled.

   The protocol notes that subjects will be compensated for their time spent participating in the study as follows: $25 per hour for participation in consenting, screening, and pre-test training. Subjects will receive $200 for the first 8 hours of participation in a field testing day and $25 per hour for participation beyond 8 hours. Alternates will receive $75 if they are not chosen to replace a subject and enroll in the study. Breaks for subjects between exposures and provision of snacks and drinks have been incorporated into the study design.

   Any expenses for injury or illness incurred as a result of study participation will be paid by the study sponsor. Subjects will have access to first aid materials and a person qualified to administer first aid at any time during their study participation.

G. Compliance with Applicable Ethical Standard

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

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

The EPA will seek feedback on the protocol and its review from the HSRB under the Human Studies Rule at 40 CFR 26.1603.

H. EPA's Ethics Comments

The EPA's ethics comments are provided below. Minor comments on typographical errors have not been included here. After all necessary changes have been made, the revised protocol and supporting documents must be resubmitted for review and approval to the overseeing IRB prior to initiating the research.

1. Revise the protocol to include information about how adverse events will be evaluated and reported, if necessary to the IRB. Who on the staff will determine whether an adverse event is serious, and whether it is study-related? What criteria will be used to make this determination? What are the qualifications of the person making the determination?
2. The compensation discussed in the protocol refers to "a field study day." (p. 10) Confirm that testing of this product against ticks will occur in a lab setting and that only lab-raised (rather than wild) ticks will be used.
3. Clarify whether testing will last two or three days – the protocol discusses both. If testing with more than one species will occur on a single day, please clarify the process.
4. Clarify compensation for a subject who withdraws within the first eight hours of a test day. Are they compensated for the entire 8 hour period, or a pro-rated rate based on the length of their participation prior to withdrawing?
5. The protocol notes that female subjects' pregnancy status will be confirmed when they are exposed to the test substance or to wild mosquitoes in the field. Female subjects' pregnancy status should be confirmed anytime they are exposed to any mosquitoes, whether in the field or in the lab, or the test substance.
6. Confirm how and at what point during the consent process subjects' ages will be verified.
7. Describe how the researchers conducting the consent meetings will confirm that subjects have comprehended the study's purpose and conduct prior to being invited to complete the consent form. For example, subjects could be asked a standard set of questions about the study's conduct.
8. Upon arrival and before the test substance is applied, the study's medical monitor should assess each subject's skin to ensure they do not have any conditions that would render them ineligible to participate. Additionally, either by phone or on the day of testing, researchers should confirm that subjects remain eligible to participate and have complied with all pre-testing conditions.
9. Move the discussion of remuneration from the "risks and benefits" section of the protocol to a section on compensation.
10. Provide the rationale for collecting subjects' social security numbers.

11. Include in the protocol information about how payment will be made to subjects (cash, check, pre-paid card; mail or in person).
12. Revise the protocol to include information about how adverse events will be evaluated and reported, if necessary to the IRB. Who on the staff will determine whether an adverse event is serious, and whether it is study-related? What criteria will be used to make this determination? What are the qualifications of the person making the determination?
13. Revise the protocol and consent to acknowledge risks associated with COVID-19 that are not directly related to the activities monitored during the study, to describe the precautions that will be followed, and to indicate that the study's conduct will comply with all federal, state, and local requirements and guidance related to this virus outbreak in effect at the time of the study. Examples of precautions include: conducting consent virtually by videoconference, having all staff and subjects wear a mask/face covering, social distancing to the maximum extent possible, contacting subjects prior to the test day to assess their health and potential exposures to COVID, excluding subjects and staff who do not meet the CDC's screening criteria, and having a process in place to notify study staff and/or subjects if anyone they had contact with during the study becomes ill.
14. Revise the consent form to align with the revised requirements at 40 CFR 26.1116. The consent must begin with a concise and focused presentation of key information. The consent form should include more information about how the subject can withdraw from participation, such as how they will get back from the test site and whether their data will be used.
15. Revise the consent form section on "Pregnancy Risks". Delete the first sentence, and replace it with "Federal regulations prohibit females who are pregnant, nursing or lactating

Attachments:

1. Protocol Review
2. Completeness checklists
3. EPA's Power vs. Sample Size Calculation for Tick Repellency Studies
4. EPA Memorandum: Review of Response to 75-Day Letter Deficiencies in Support of an Efficacy Protocol with HSRB Review for 93616PA6 with 11% Oil of Lemon Eucalyptus (OLE) and 7.75% Methyl Nonyl Ketone as its Active Ingredients

Attachment 1 - EPA Protocol Review

Title: Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions

Date: February 17, 2020, amended version December 23, 2020.

Principal Investigator and any sub-investigators: Dr. Scott P. Carroll

Participating Laboratory: Carroll-Loye Biological Research; 711 Oak Avenue, Davis, CA 95616

Sponsor: Mimikai; 1564 Green Valley Road, Danville, CA 94526

Trial Monitoring Center: Carroll-Loye Biological Research; 711 Oak Avenue, Davis, CA 95616

IRB: Advarra IRB (Institutional Review Board)

6940 Columbia Gateway Drive, Suite 110

Columbia, MD 21046

1. Societal Value of Proposed Research

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

This study is designed to determine the CPT of an insect repellent, Mimikai Lilly Pilly, containing 11.0% OLE and 7.75% MNK as its active ingredients, against three tick species, *Ixodes scapularis*, *Dermacentor variabilis,* and *Amblyomma americanum.* The product will be tested in the laboratory on 25 subjects at the standard dose of 0.5g/600cm^2 for up to 14 hours. EPA requires efficacy testing of products claiming efficacy against disease vectors to support efficacy claims on product labels.

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 median CPT of a personal, skin-applied tick repellent product, containing the active ingredients OLE and MNK. This information does not currently exist. The proposed product has not been evaluated for its performance against ticks.

c) How would the study be used by EPA?

EPA requires product-specific efficacy data for registration of products claiming efficacy against pests of public health importance. Data generated by the proposed research will be used to characterize the duration of repellency of the proposed product in support of registration. EPA will review the proposed study to verify that it satisfies product-specific efficacy data requirements and it is acceptable for supporting efficacy claims on the product label.

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

EPA requires product-specific efficacy data to support product registration. No previous testing of 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 11.0% OLE and 7.75% MNK against three tick species - *Ixodes scapularis, Dermacentor variabilis,* and *Amblyomma americanum* - at the EPA standard application rate of 0.5g/600cm^2.

"*The hypothesis that the Test Material will significantly reduce the number of ticks Crossing treated versus untreated skin is not the objective of this study. The objective is to compute a reasonable estimate of median and mean with 95% confidence intervals for the duration between application and sufficient repellency breakdown such that for each tick species there are two ticks crossings on a subject within a half hour period. That pattern is here assessed for each tick species at a resolution of 15 minutes*" (§4.9).

(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 the 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 sample size is 25 subjects according to the EPA's recommended sample size of 25 test subjects for testing repellency against ticks (Protocol Appendix 8) The rationale is 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. "*Twenty-five subjects is the current EPA-recommended number for tick repellency studies involving novel repellent formulations applied to human skin. The Agency's recommendation is based on its in-house power analysis (see* Protocol Appendix 8*). EPA applied this power analysis to scenarios very similar to this protocol*." (§4.1).

(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 for testing repellency. The other arm will be treated with the test product and serve as the treatment group (§4.2). A positive control will not be used.

(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?

Each subject will be his/her own control. Treatment application to right or left arm will not be randomized. Treatment will be applied to non-dominant arm of subject. Participants (male and females) will be randomly assigned as either test or alternate subjects, maintaining an approximate 50:50 male to female ratio.

(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 for all test subjects for each tick species will be calculated using the Kaplan-Meier survival analysis.

(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 of 25 subjects per tick species is according to EPA recommendation. This recommendation is based on the results from EPA simulations where the desired K=0.7, the median CPT is 4 or 6 hours, the expected P5MR is 0.4. Under these conditions, 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 product 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 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 than indicated on the product label. For detailed information on the statistical simulation see Protocol Appendix 8.

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to repellent product in a spray formulation, containing 11.0%

OLE and 7.75% MNK and. The active ingredient in OLE is 65 % p-Methane-3,8-diol (PMD). The product will be applied at a rate of 0.5g/600cm^2 surface skin area. Exposure time to the test substance will last maximum of 14 hours for each day of testing per subject. Exposure time to ticks will last up to 13 hours. The product has been tested to satisfy Tier I Human Health Assessment data required for registration (MRIDs 510641-01 thru 510641-08) and is classified as toxicity category IV for all routes of exposure. 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 laboratory colonies. Ticks will only be used once with a single subject and used ticks will be destroyed. EPA recommends amending the protocol to include identity of tick supplier and procedure for destroying used ticks. EPA also recommends sourcing all three tick species from same supplier, if possible.

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

Efficacy data to satisfy product performance requirements and support label claims of repellency against ticks for this product are required by EPA for registration. EPA requires submission of product performance data for registration of 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 dose use for testing repellency (0.5g/600cm^2) approximates EPA standard dose (0.48 g/600 cm^2) used for testing repellency of pump sprays.

(c) What duration of exposure is proposed?

A day of testing will take a minimum of 4 hours and a maximum of 14 hours from time of product application. Repellency evaluation will take up to three days of testing per subject to complete testing on all three tick species. Proposed exposure periods consist of exposing ticks to untreated human skin for screening actively questing ticks and to treated forearms (three minutes per tick), at exposure intervals of 15 minutes for a maximum of 13 hours of repellency testing per tick species, or until the time point when repellent breakdown or CPT is reached by subject, whatever occurs first.

2.3 Endpoints and Measures

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

The efficacy endpoint is the First Conformed Crossing, defined as *'First Confirmed Crossing' (FCC) is one which is followed by another within 30 minutes*." (§1.1). Time to product failure is measured by the time of the FCC for each tick species for each subject (§1.1). Complete Protection Time or CPT is the measurement for residual repellency or time to product failure, measured by the FCC. CPT is defined as in §1.1 as "*the time between application of Test Material and the First Confirmed Crossing of an actively foraging tick from the untreated skin surface of a subject's hand 3 cm or more into the treated forearm skin area."* CPT is measured as a single time value for each subject for each tick species. The endpoints are appropriate to the questions being asked. Using the Kaplan-Meier estimator, the Median CPT (mCPT) will be calculated across all test

subjects exposed to each tick species.

(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.
- Forearm will be prepared for dose application. Skin surface area will be measured in advance, during first visit.
- Pre-test training on how to handle ticks will be conducted on first visit to familiarize subjects with tick behavior and determine subject's attractiveness to ticks.
- Both forearms of same subject will be employed to assess tick behavior on untreated and treated forearms.
- Pathogen-free ticks from laboratory colonies will be used for testing efficacy.
- Efficacy will be conducted on three species of ticks using same 25 test subjects for testing all three tick species.
- Tick movement, time from product application to time of first exposure will be recorded, and the start and stop times for each exposure period will be recorded.
- Research staff and study director will monitor testing, and data recording.
- Alternate subjects (8 alternate subjects) will be enrolled to ensure adequate sample size.
- A Quality Assurance Unit will be in place to monitor all study activities and data collection.
- There will be three test days for subjects testing all three tick species.
- Stopping rules and criteria for subject withdrawal are established.

(c) What QA methods are proposed?

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.

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

Sources of variation include tick species, tick activity, and attractiveness of subjects. These uncertainties will be addressed by each subject serving as their own control for qualifying ticks; using same subject for testing all three tick species; qualifying ticks to ensure they are questing, and using the lowest mCPT for the three tick species for the duration of efficacy**.** Ticks will be sourced from a single supplier, if possible.

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. The protocol proposes to ensure balance in subjects' gender (50/50 female/male) and recruitment will be conducted broadly to draw a diverse, representative sample of subjects.

(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?

Recruitment will take place in the Davis and Sacramento area of California. Volunteers will be recruited from interested subjects who meet the eligibility criteria, including speaking English, being between 18 and 60 years old, and having spent time outdoors.

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

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

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

Yes, if EPA recommendations are adopted.

3.2 Equitable Selection of Subjects

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

Inclusion criteria

- Age: 18-60 years
- Sex: Male/female
- Race: Any race
- Completed Consent Process
- Speak and read English

Exclusion criteria

- Known to be hypersensitive to tick bites
- Phobic of ticks
- Known to be allergic to topical repellents, essential oils of plants, or common cosmetics
- Known to be sensitive to any of the test product ingredients
- Poor physical condition
- Unwilling to submit to brief query about personal condition
- Use of topical repellent within 48 hours preceding the efficacy test
- Unwilling to refrain from use of perfumed products, alcoholic beverages or smoking after 9 PM the evening preceding the efficacy test and throughout that test
- Known to be pregnant or lactating
- Unable to deliver ticks to own left and right arms
- Unable to see ticks on skin or otherwise effectively monitor them on skin

- Spouse, immediate family member, student or employee of the Study Director, or Sponsor, or dependent of the Study Director, of a Study Director employee, of the Sponsor, or of a Sponsor employee
- Does not regularly spend time in outdoor settings
- Proves unattractive to ticks during tick handling training (study visit 1)
- Has participated in an interventional study (other than a biting arthropod repellency efficacy study) in the previous three months
- Prone to or suffering from rashes or other skin conditions including eczema, psoriasis, and sunburn

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

None. The protocol specifies that employees, managers, and spouses of employees of the researchers and of the Sponsor (MMIKAI), as well as students of the Study Director are not eligible to participate.

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

Recruitment does not target specifically any vulnerable populations.

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

Volunteers will be recruited in the areas where testing will be conducted. Advertisements will be posted in print and digitally. Either the research team conducting the study will recruit subjects, or an independent recruitment firm will used. The recruitment materials are included with the submission. Recruitment will be conducted until at least 44 individuals have agreed to attend a consent meeting.

Interested candidates can call the number on the advertisements and will be contacted by a member of the research team. The researcher will provide more information about the study and go over some of the eligibility criteria using an IRB-approved script. Candidates will self-report eligibility, and those who qualify will be invited to attend a one-on-one consent meeting.

Consent meetings will be held one-on-one with a member of the study team. 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. T

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

Subjects will be recruited through print and digital advertisements. There will be no connection or communication between the researchers and the potential subjects' employers, which minimizes the potential for coercion or undue influence. In addition,

students or employees of the study director are excluded from participation. Finally, any employees, managers, and spouses of employees of the researchers and the study sponsor are excluded from participation.

3.3 Remuneration of Subjects

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

The protocol notes that subjects will be compensated for their time spent participating in the study as follows: $25 per hour for participation in consenting, screening, and pre-test training. Subjects will receive $200 for the first 8 hours of participation in a field testing day and $25 per hour for participation beyond 8 hours. Alternates will receive $75 if they are not chosen to replace a subject and enroll in the study. Breaks for subjects between exposures and provision of snacks and drinks have been incorporated into the study design.

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

No.

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

No.

(d) How and when would subjects be paid?

The method of payment is unclear. The protocol notes that subjects will be paid at the end of each encounter.

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, containing the active ingredients OLE and MNK. The product formulation is classified as toxicity category IV for all routes of exposure (MRIDs 510641-03 thru 510641-08 in Protocol Appendix 6).

OLE is classified as toxicity category II for Eye irritation (MRID 446242-05); toxicity category III for acute oral (LD_{50} > 2,408 mg/kg (MRID 446242-03)); acute dermal (LD_{50} > 2,000 mg/kg (MRID 446242-04)), dermal irritation (MRID 446242-06), and toxicity category IV for Acute inhalation (LC_{50} > 2.06 mg/L (446241-04)). It is not a dermal sensitizer (MRID 446242-07) and no mutagenic ((MRID 446242-08).

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. OLE contains 65% PMD according

to EPA's risk assessment (EPA Memorandum Feb. 4, 1999; Biopesticide Registration of Citriodiol (100% pure, containing 65 % PMD)). A 90-Day dermal study in rats (MRID 444387-10) tested PMD (98.3 % pure) at increasing doses, 0, 1,000 and 3,000 mg/kg/day. The NOAEL = 1,000 mg/kg/day, and the LOAEL = 3,000 mg/kg/day. The endpoints for NOAEL and LOAEL are based on treated skin observations, erythema, edema, eschar, and histological observations in treated skin, increased acanthosis, and inflammation at the highest dose of 3,000 mg/kg/day. 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. 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.

The active ingredient MNK is classified as toxicity category III for acute dermal (LD_{50} > 2,000 mg/kg (MRIDs 419041-02 and 431638-01); eye irritation (MRIDs 419041-04), and dermal irritation (MRID 419041-05), and as toxicity category IV for acute oral (LD_{50} >5,000 mg/kg (MRID 419041-01)) and acute inhalation (LC_{50} > 5.43 mg/L (MRID 419041-03). MNK is a weak sensitizer (MRID 419041-06). The reported Lowest Adverse Effect Level (LOAEL) and No Adverse Effect Level (NOAEL) is >300 mg/kg/day in New Zealand white rabbits, based on 21-day sub-chronic dermal exposure (MRID 461103-01). The study limited testing to 300 mg/kg/day. The NOAEL for sub-chronic dermal irritation is 1,000 mg/kg/day (MRID 461103-01). A sub-chronic A 90-day inhalation study was not conducted because chronic inhalation effects are not expected based on low vapor pressure (4.49×10^{-2} Torr). For maternal and developmental toxicity, the reported NOAEL > 100 mg/kg/day at the highest dose tested. MNK is not mutagenic (U.S. EPA Reregistration Eligibility Decision (RED) for Methyl Nonyl Ketone. July 1995. 738-R-95-038 and EPA Preliminary Work Plan and Summary Document. MNK. Registration Review. March 28, 2012 in Protocol Appendix 6).

(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 end-use product to be used as skin applied repellent and it will be used consistent with the Directions for Use on the product label. The dose of application (0.5g/600 cm^2/ day) is lower than any NOAEL or LOAEL for OLE (*see* 4.1 Risk Characterization (a) above). For MOEs based on MNK, EPA recommends revising MOE calculations in §4.6 for single and double applications, and re-calculating MOEs for single and double applications to adults and children using LOC = for dermal irritation (*see* Attachment 4). For the purpose of repellency testing, the Agency considers the exposure of the subjects to the levels proposed for testing the test substance not to pose an unreasonable risk of adverse effects to subjects.

(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. To mitigate risks from exposure to ticks, ticks will be placed one at a time on subjects' arms and their behavior will be monitored closely. Ticks will be removed if they begin to bite and attach to the subject. To eliminate the risk of transmission of tick-borne disease, ticks will be sourced from pathogen-free colonies. Ticks will only be used once with a single subject. Used or discarded ticks will be destroyed.

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

Individuals who may be at an increased risk for adverse effects are not eligible to become subjects in this study, including individuals known to be allergic or sensitive to skin-applied insect repellents, and those with known skin conditions that could be exacerbated by study participation or with cuts/abrasions on areas that will be exposed during testing.

4.2 Risk minimization

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

The protocol discusses five risks to subjects as a result of study participation: exposure to the test material, exposure to ticks and tick-borne illness, physical stress of test conditions, and psychological risks associated with disclosure of pregnancy testing results. The protocol notes that risks will be minimized as follows.

Both active ingredients in the test product are registered with the EPA for use in skin-applied repellents at or above the concentrations used in this product. The EPA's science review concludes that the risks associated with exposure to the test substance during the study are low. To further minimize the risks, subjects will be enrolled only if they do not have a known sensitivity or allergy to insect repellents or common cosmetics. 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. Subjects will be trained to remove ticks before they begin to bite and attach to the subject. To eliminate the risk of transmission of tick-borne disease, ticks will be sourced from pathogen-free colonies. Ticks will only be used once with a single subject, and ticks will be destroyed after they are placed on a subject's arm.

Pregnancy testing will be conducted in private and only a single female member of the research team will discuss the results with the subject.

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

1. Consented duration is reached.
2. Test site becomes unsafe (in the Study Director's judgment)
3. Subject shows signs of tick hypersensitivity during test
4. Subject shows signs of hypersensitivity to test product during test
5. Subject asks to withdraw
6. Subject's treated limb receives Confirming Crossings for all target species that subject is testing on that day
7. Medical management is invoked for the subject
8. Subject becomes unattractive to ticks

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

"Medical management refers to research staff procedures for responding to observation of an adverse health condition in a subject, whether that observation is initially made by the affected subject, by another subject, or by a researcher. If the adverse health condition is judged by the Study Director as an emergency, a researcher will contact 9-1-1 by cellular or ground line telephone and cooperate with instructions from emergency personnel. If the Study Director judges the adverse health condition to not be an emergency, the Study Director will contact the physician on call for the study and comply with any instructions given. On the day of testing, a physician who has read the protocol and discussed the research with the Study Director will be on-call. Contact information for the nearest medical facilities and maps from the test site to the facilities will be prepared and on file before the day of testing." (p. 9)

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

"*All subjects are asked to contact the Study Director and a physician of their own choice at any time should they develop a skin rash (a delayed hypersensitivity reaction) within 7 days of the conclusion of the test day*." (p. 8)

(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?

See (c) above.

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

Expenses will be covered by Carroll-Loye Biological Research According to the

protocol, Carroll-Loye will cover the costs of medical treatment for study-related injuries that are not covered by the subject's insurance.

5. Benefits

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

There are no benefits to the subjects participating in this research study.

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

7. Independent Ethics Review

(a) What IRB reviewed the proposed research?

Advarra IRB.

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

Yes.

(c) Is this IRB registered with OHRP?

Yes.

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

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

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

Yes.

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

Yes.

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

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

8. Informed Consent

(a) Will informed consent be obtained from each prospective subject?

Yes.

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

Yes.

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

With the EPA's comments addressed, the consent materials will meet the requirements of 40 CFR 26.1116.

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

Recruitment is limited to subjects who can speak and understand English. No information on the literacy rate will be collected during this study.

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

All subjects and research staff will speak English, so there will not be any language barriers.

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

The protocol does not provide information about how subjects' comprehension of the materials will be assessed. EPA recommends that the protocol be revised to include this information prior to initiating the research.

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

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

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

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

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

9. Respect for Subjects

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

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

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

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

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

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

EPA has recommended that the protocol be revised to include more specific information on how withdrawn subjects will be transported back to the area where they parked, and how withdrawn subjects' data will be used.

Attachment 2 - Completeness Checklists

Checklist Associated with 40 CFR 26.1125
Submission of proposed human research for EPA review

Requirement	Y/N	Comments/Page Refs
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, and progress reports submitted by investigators, and reports of injuries to subjects.	Y	MIM-007 Feb 17, Appendix 9 MIM-007, Dec 23, Appendices 8 and 10
(2) Minutes of IRB meetings . . . in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; and a written summary of the discussion of controverted issues and their resolution.		Minutes not generated for review of MIM-007, Dec 23 version because it was reviewed under expedited review
(3) Records of continuing review activities, including the rationale for conducting continuing review of research that otherwise would not require continuing review as described in §26.1109(f)(1).	Y	
(4) Copies of all correspondence between the IRB and the investigators.	Y	MIM-007 Feb 17, Appendix 9 MIM-007, Dec 23, Appendices 8 and 10
(5) A list of IRB members in the same detail as described in §26.1108(a)(2).	Y	MIM-007 Feb 17, Appendix 9
(6) Written procedures for the IRB in the same detail as described in §26.1108(a)(3) and (4).	Y	On file with EPA
(7) Statements of significant new findings provided to subjects, as required by §26.1116(c)(5).	N/A	
The following additional information, to the extent not already included. A discussion of:		
(a)(1) The potential risks to human subjects	Y	MIM-007, Dec 23
(a)(2) The measures proposed to minimize risks to the human subjects	Y	MIM-007, Dec 23
(a)(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	MIM-007, Dec 23
(a)(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	MIM-007, Dec 23
(a)(5) The balance of risks and benefits of the proposed research.	Y	MIM-007, Dec 23
(b) All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.	Y	MIM-007, Dec 23
(c) Information about how subjects will be recruited, including any advertisements proposed to be used.	Y	MIM-007, Dec 23
(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	MIM-007, Dec 23
(e) All correspondence between the IRB and the investigators or sponsors.	Y	MIM-007, Dec 23 MIM-007 Feb 17, Appendix 8
(f) Official notification to the sponsor or investigator, in accordance with the requirements of this subpart, that research involving human subjects has been reviewed and approved by an IRB.	Y	MIM-007, Dec 23

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

Criterion	Y/N	Comment/Page Reference
Consent Process – 40 CFR 26.1116(a)		
(1) Before involving a human subject in research covered by this subpart, an investigator shall obtain the legally effective informed consent of the subject.	Y	
(2) An investigator shall seek informed consent only under circumstances that provide the prospective subject sufficient opportunity to discuss and consider whether or not to participate and that minimize the possibility of coercion or undue influence.	Y	
(3) The information that is given to the subject shall be in language understandable to the subject.	Y	
(4) The prospective subject must be provided with the information that a reasonable person would want to have in order to make an informed decision about whether to participate, and an opportunity to discuss that information.	Y	
(5) (i) Informed consent must begin with a concise and focused presentation of the key information that is most likely to assist a prospective subject in understanding the reasons why one might or might not want to participate in the research. This part of the informed consent must be organized and presented in a way that facilitates comprehension. (ii) Informed consent as a whole must present information in sufficient detail relating to the research and must be organized and presented in a way that does not merely provide lists of isolated facts, but rather facilitates the prospective subject's understanding of the reasons why one might or might not want to participate.	N	EPA provided comments and suggested revisions.
(6) No informed consent may include any exculpatory language through which the subject 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	
Basic Elements of Informed Consent – 40 CFR 26.1116(b) 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 that 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 that 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	
(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; and	Y	
(9) One of the following statements about any research that involves the collection of identifiable private information or identifiable biospecimens: (i) A statement that identifiers might be removed from the identifiable private information or identifiable biospecimens and that, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from the subject, if this might be a possibility; **or** (ii) A statement that the subject's information or biospecimens collected as part of the research, even if identifiers are removed, will not be used or distributed for future research studies.	Y	
Additional elements of informed consent – 40 CFR 26.1116(c) One or more of the following elements of information, when appropriate, 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) that 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	
(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 provided comments and suggested revisions.
(5) A statement that significant new findings developed during the course of the research that may relate to the subject's willingness to continue participation will be provided to the subject;	N/A	
(6) The approximate number of subjects involved in the study;	Y	
(7) A statement that the subject's biospecimens (even if identifiers are removed) may be used for commercial profit and whether the subject will or will not share in this commercial profit;	N/A	
(8) A statement regarding whether clinically relevant research results, including individual research results, will be disclosed to subjects, and if so, under what conditions; and	N/A	
(9) For research involving biospecimens, whether the research will (if known) or might include whole genome sequencing (*i.e.,* sequencing of a human germline or somatic specimen with the intent to generate the genome or exome sequence of that specimen).	N/A	
(h) 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	

Checklist associated with 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 (including in an electronic format) by the subject. A written copy shall be given to the subject.	Y	
(b) The informed consent form may be either of the following:		
(1) A written informed consent form that meets the requirements of §26.1116. The investigator shall give the subject adequate opportunity to read the informed consent form before it is signed; alternatively, this form may be read to the subject.	Y	
(2) A short form written informed consent form stating that the elements of informed consent required by §26.1116 have been presented orally to the subject, and that the key information required by §26.1116(a)(5)(i) was presented first to the subject, before other information, if any, was provided. The IRB shall approve a written summary of what is to be said to the subject. When this method is used, there shall be a witness to the oral presentation. Only the short form itself is to be signed by the subject. 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 must be given to the subject, in addition to a copy of the short form.	N/A	

EPA's Power vs. Sample Size Calculation for Tick Repellency Studies
Date: 3/30/2018

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

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[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.

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:

$K = 95\%\ LCL_{mCPT}/\text{estimated mCPT}$

where:

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

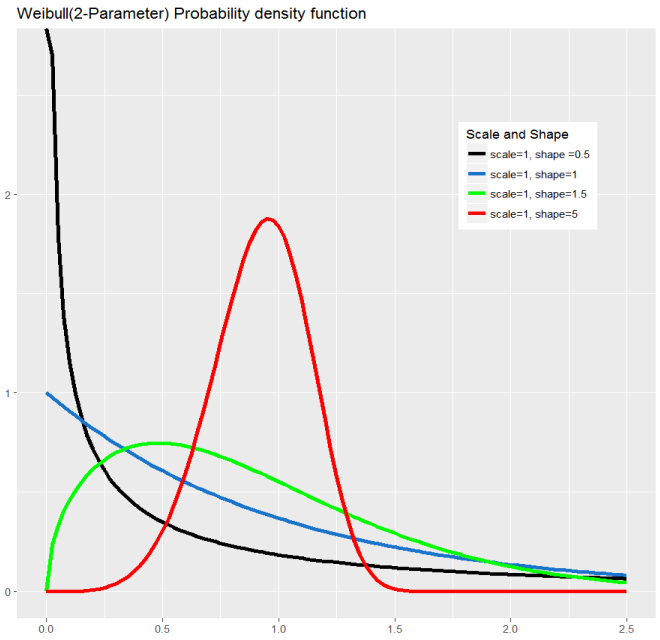


Figure A2. Cumulative Distribution Function (CDF) for Above Weibull PDF

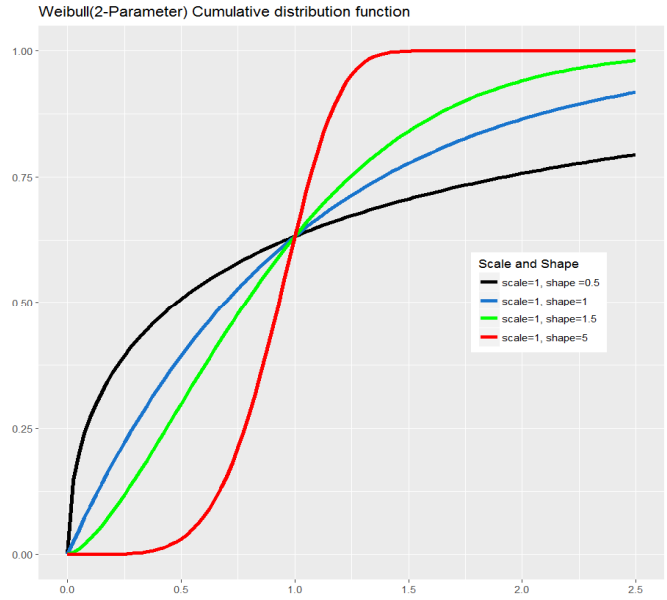
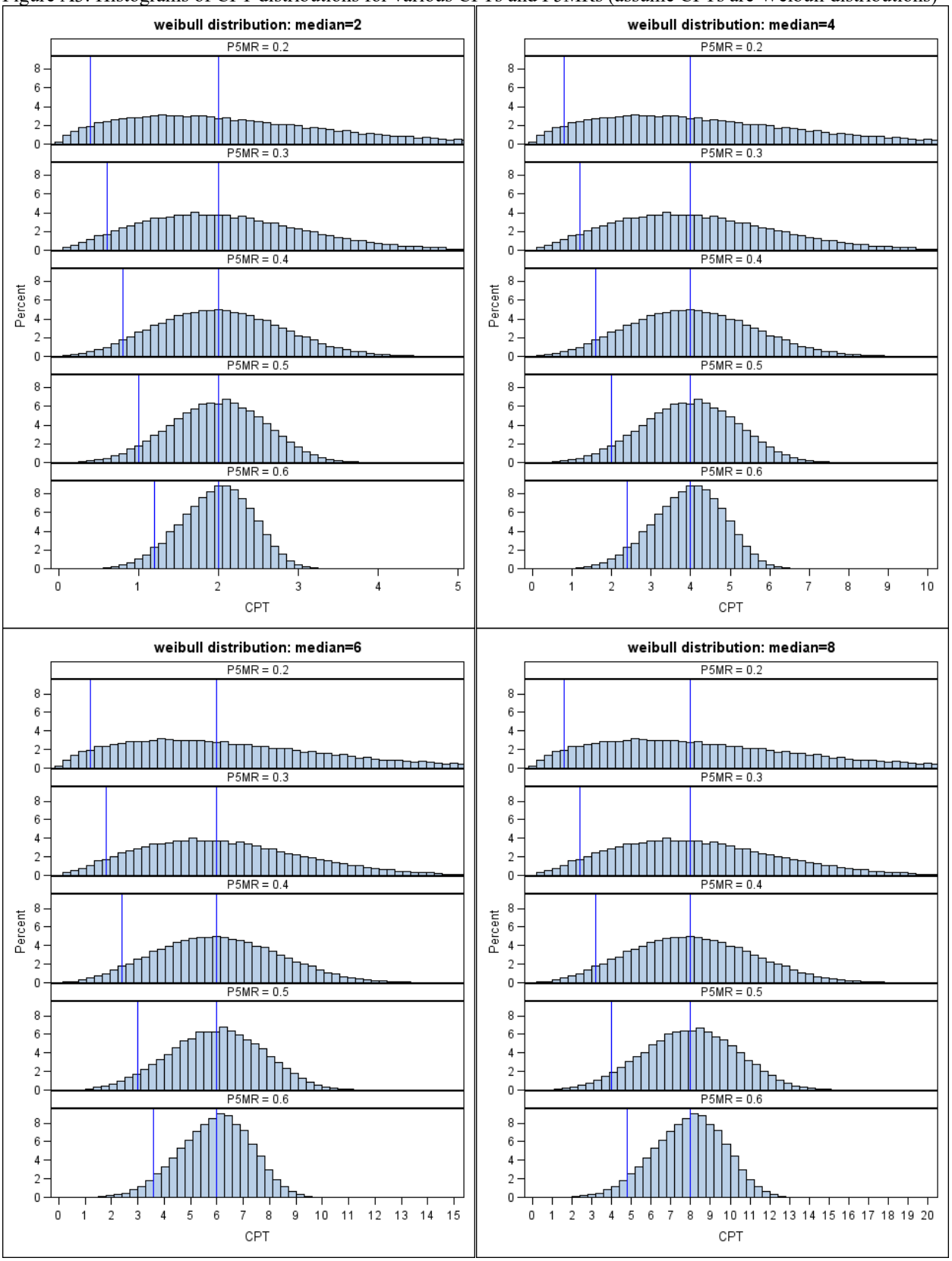


Figure A3: Histograms of CPT distributions for various CPTs and P5MRs (assume CPTs are Weibull distributions)



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 5th 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);
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dm log 'clear';%PowerCPT(med=2,  P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=21, nmax=30,
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dm log 'clear';%PowerCPT(med=2,  P5MRS=0.2 0.3 0.4 0.5 0.6 0.7 0.8, nmin=21, nmax=30,
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*=====> Create Figures and Print Results;


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                        Ticks.&dist._T15_M4_K%sysevalf(10*&K)_N&nmin._&nmax._D&maxt
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ods rtf close;
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Review of Response to 75-Day Letter Deficiencies in Support of an Efficacy Protocol with HSRB Review for 93616PA6 with 11% Oil of Lemon Eucalyptus (OLE) and 7.75% Methyl Nonyl Ketone as its Active Ingredients

Type of Data Review:	Human Health
Decision Number:	561586
Case Number:	00148508
EPA File Symbol Number:	93616PA6
Chemical Class:	Biochemical
PC Code:	040522, 044102
Tolerance Exemption Petition:	N/A
MRID Nos.:	N/A
PRIA Code:	M001

FROM: Sadaf Shaukat, Biologist
Risk Assessment Branch
Biopesticides & Pollution Prevention Division (7511P)

THRU: Angela Gonzales, Biologist
&
Shannon Borges, Branch Chief
Risk Assessment Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: Menyon Adams, Risk Manager
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

ACTION REQUESTED

Mimikai Inc. has submitted an application for the submission of an efficacy protocol using mosquitoes and ticks for Human Studies Review Board (HSRB) review. In response to an Agency 75-day deficiency letter dated October 28, 2020, they have submitted scientific rationale in order to request the Agency to lower the uncertainty factors and thus, the level of concern (LOC) for calculating margins of exposure (MOEs) for their proposed end-use product (EP) Mimikai Lilly Pilly Repellent (EPA File Symbol No. 93616PA6) with 11.0% Oil of Lemon Eucalyptus (OLE) and 7.75% Methyl Nonyl Ketone. Mimikai Lilly Pilly Repellent is a mosquito and tick repellent for skin and clothing. This memorandum contains the human health MOE discussion for the proposed EP, Mimikai Lilly Pilly Repellent.

EXECUTIVE SUMMARY

Methyl nonyl ketone (MNK; also known as 2-undecanone) is an organic compound that can be produced synthetically or extracted from various plant sources. Due to its strong odor, it is used primarily as an insect and animal repellent. The subject of this memo will be the potential for risk relative to the dermal irritation observed in a 21-day dermal toxicity (rabbit) study performed with methyl nonyl ketone as a TGAI when considering Mimikai's mosquito and tick protocols to be submitted to the HSRB.

In the 21-day dermal toxicity study of MNK in New Zealand white rabbits, the test doses were 1, 30, 100, or 300 mg/kg/day. The no adverse effect level (NOAEL) for dermal irritation is 100 mg/kg/day based on moderate to severe dermal irritation observed at 300 mg/kg/day with no systemic effects observed up to the highest dose tested. It is important to note that the application site was semi-occluded. Skin occlusion can enhance the hydration of the stratum corneum and thus exacerbate any irritant effects of the applied chemical. Therefore, the point of departure (i.e. the NOAEL) selected for risk assessment may be conservative in that the manner in which the EP will be applied to humans will not be occluded or semi-occluded. In addition, it is noteworthy that the rabbits were exposed 21 days whereas the human subjects will only be exposed for one day. Since the primary adverse effect is demonstrated to be localized dermal irritation and a lack of systemic toxicity has been demonstrated in this study and has been confirmed in the overall available toxicity database for MNK, the reduction of the standard 10x interspecies and 10x intraspecies uncertainty factors would be appropriate.[1]

In addition, because the dermal point of departure (POD) was based on irritation effects that can be localized to the area of contact, risk can be estimated based on a comparison of the dermal loading rate used in the 21-day dermal toxicity study to the application rate of the proposed product.

DERMAL RISK ASSESSMENT OVERVIEW

Reducing the Uncertainty Factors

The standard uncertainty factors used in the Agency's risk assessments are typically 10x to account for inter-species differences and 10x to account for intraspecies differences. A total uncertainty factor of 100 is the standard level of concern (LOC). However, based on toxicokinetic and toxicodynamic considerations, these 10x factors may be refined to 3x. Relevant information on considering Data-Derived Extrapolation Factors (DDEFs) for direct acting irritants and corrosive chemicals can be found in Section 2.5 of the 2001 report from the National Resource Council (NRC) *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals.* Specifically, Section 2.5.3.2.3 entitled "Mechanism or Mode of Action Is Unlikely to Differ Among Species" states the following:

"If evidence is available indicating that the mechanism or mode of action, such as direct-acting irritation or alkylation, is not expected to differ significantly among species, **an interspecies UF (uncertainty factor) of 3 is generally used.** The rationale for the selection of a UF should include the following:

1. A description of the mechanism of action.
2. A discussion of why the mechanism of action is unlikely or likely to differ.
3. Is bioavailability, metabolism, detoxification, elimination likely to be an issue?" (pp. 72-73)

Similarly, with respect to the intra-species UF, as noted in the NRC report in Section 2.5.3.3.4," In those cases in which the mode or mechanism of action is such that the response elicited by exposure to the chemical by different subpopulations is unlikely to differ, an intraspecies UF of 3-fold is generally used. Typically, this response involves a direct-acting mechanism of toxicity in which metabolic or physiologic differences are unlikely to play a major role."

[1] https://www.nap.edu/catalog/10122/standing-operating-procedures-for-developing-acute-exposure-guideline-levels-for-hazardous-chemicals

Compared to systemic effects, irritation responses are not expected to show as large a variation in severity and duration of response between or among mammalian species. In addition, although it is known that there are differences between animal species and among humans in the way a chemical may be absorbed, metabolized, and excreted, the lack of influence of these processes on an irritant response removes some of the characterization of uncertainty that is usually performed for systemic toxicants. The uncertainty factors for both the interspecies and intraspecies differences can be reduced to 3x each, making the LOC 10 for this specific case.

Lack of Systemic Toxicity

No known systemic toxicity has been associated with MNK exposure. MNK is part of a large class of molecules called ketones. Ketones are water-soluble molecules that are produced by the liver. They are absorbed through the gastrointestinal tract and rapidly eliminated from the blood. They are endogenous in humans as components of fatty acid and carbohydrate metabolism and have been detected in the blood. Generally, ketones are metabolized into innocuous substances, more specifically, they are reduced to secondary alcohols and excreted after their conjugation with glucuronic acid in the urine or bile.[2]

Although some liver and kidney effects were observed at 1000 mg/kg/day (limit dose) in a 90-day (gavage) rat toxicity study, no other systemic effects have been identified at more relevant doses.[3] In addition, no systemic or developmental toxicity was observed in a developmental toxicity study with range-finding data even at the limit dose of 1000 mg/kg/day.[4] Furthermore, the repeat dose toxicity NOAEL is 1087 mg/kg/day for an analog of methyl nonyl ketone, 2-heptanone, which contributes to the weight of evidence that dermal irritation is the primary mechanism of action, not systemic toxicity.[5]

Dermal Loading Calculations

For Mimikai's proposed end-use product, risk was estimated based on the dermal loading rate instead of body burden because the endpoint selected for dermal exposure is based on skin irritation, which is a superficial effect in a localized area rather than a systemic effect that occurs after absorption. Therefore, this method of risk estimation is more biologically relevant. Risk was estimated using the dermal loading rate in the 21-day dermal toxicity study (3.3 mg ai/cm^2) divided by the loading rate of the active ingredient on the skin provided by the applicant (0.064 mg ai/cm^2). The resulting risk estimate, or MOE is 52. Since 52 exceeds the LOC of 10, there is no risk of concern to the participants in the proposed mosquito and tick protocols.

Details of Calculations

In order to calculate the dermal loading rate in the 21-day dermal toxicity study, the dose of 100 mg/kg/day is multiplied by the average weight of the rabbit in the study, which was 3.3 kg. The resulting dose to the rabbit is 330 mg MNK/rabbit. This is then divided by the surface area of the exposed patch of skin of the rabbit which was 100 cm^2. This results in a dermal loading rate of 3.3 mg MNK/cm^2. This rate is then compared to the loading rate in the protocol which was 0.833 mg product/cm2. Since the protocol is using the actual product, the active ingredient percentage (7.75 % MNK) needs to be taken into consideration, so 0.833 is multiplied by 0.0775, resulting in a loading of 0.064 mg MNK/cm2 on the human subject. The loading rates are then compared, 3.3/0.064 to result in a MOE of 52.

[2] http://www.inchem.org/documents/jecfa/jecmono/v042je15.htm

[3] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2495

[4] U.S. EPA, 1992. DER: MRID 42225901 & 42225902

[5] https://www.sciencedirect.com/science/article/pii/S0278691519304235#bib26

CONCLUSION

In accordance with the NAS recommendations, the LOC for MNK may be refined to 10 as the primary toxic effect is irritation and there is a lack of systemic toxicity on MNK. The MOE of 52 exceeds the LOC, therefore there is no unacceptable risk to the human subjects in the proposed mosquito and tick protocols.

cc: Sadaf Shaukat, A. Gonzales, M. Adams, BPPD Science Review File, IHAD/ARS: