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 Field Evaluation of Skin-Applied Mosquito Repellent Product Containing Oil of Lemon Eucalyptus and Methyl Nonyl Ketone

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REF: Dr. Scott P. Carroll, Study Director. (2020) Protocol for "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes." Protocol No. MIM-006. Unpublished document sponsored by Mimikai, 1564 Green Valley Road, Danville, CA 94526. Dec. 23, 2020. 113p.

We have reviewed the referenced protocol for field testing of a skin-applied repellent product containing 11% Oil of Lemon Eucalyptus (OLE, also known as Citriodiol) and 7.75% 2-undecanone (methyl nonyl ketone or MNK) against wild populations of mosquito species within three genera, *Aedes, Culex* and *Anopheles,* from both scientific and ethical perspectives. 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

the EPA guideline OPPTS 810.3700: *Insect Repellents to be Applied to Human Skin*,[1] as well as the recommendations from the U.S. Environmental Protection Agency (EPA) and the Human Studies Review Board (HSRB). 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-004, 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the protocol application (93616PA1 Mosquito and Tick HSRB Efficacy Study Protocols; Decision Number: 543565) was withdrawn on March 22, 2019.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-006, 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 to determine the duration of efficacy of the test material in repelling mosquitoes relevant to US consumers, such as those listed on Table 1 "*Prominent target mosquito species in probable field study areas*" in §1.1 (p. 5), when applied at a typical consumer dose of 0.5g/600 cm^2.

1 EPA. Product Performance Test Guidelines; OPPTS 810.3700: Insect Repellents Applied to Human Skin. EPA 712-C-10-001. July 7, 2010. p. 25.

Efficacy Endpoints and Definitions

The efficacy endpoint is First Confirmed Landing (FCL) signaling time point to product failure. Complete Protection Time (CPT) measures duration of repellent efficacy from time of product application to time of FCL. FCL is defined as a landing that is followed by a second landing occurring within 30 minutes of the first. The confirmatory landing may occur during the same exposure period as the first landing or on the exposure period immediately following the exposure period when the first landing occurs. Landings are the action of a mosquito alighting on a subject's skin before they probe or bite (§1.1; p. 6).

If a first landing is followed by a period of low landings or a skipped period due to bad weather, the first landing will be counted as FCL (§4.8.4, p. 32).

Study Design

This is a proposed field study using human subjects for testing lasting efficacy of a repellent product, MIMKAI Lilly Pilly, at preventing mosquito landings on human hosts. The proposed test is designed to determine the complete protection time (CPT) of the product at a standard application rate of 0.5g/600cm^2, using a sample of 13 treated subjects and 2 untreated controls. Test subjects will be selected from a pool of informed and consenting volunteers that will be trained in the laboratory on the use of aspirators for catching landing mosquitoes before they bite. Subjects' attractiveness to mosquitoes will also be assessed in the lab prior to the field testing (§1.3.2, p. 8).

The product will be tested against wild mosquito populations at two ecologically distinct sites. Site selection is based on the following criteria: 1) diversity and abundance of targeted mosquito species within three genera, *Anopheles, Aedes,* and *Culex*; 2) absence of mosquito-borne diseases at least one month prior to test initiation, and 3) absence of reported mosquito-borne disease cases in humans within 25 miles of planned test site. Potential test sites may be located in northern and southern California, Minnesota, Louisiana, Arizona, and Florida, where different mosquito species within the genera *Culex, Anopheles* and *Aedes* are present (Table 1, *Prominent target mosquito species in probable field study areas*) (§1.1, p. 5). Site selection is also restricted to locations where West Nile Virus (WNV) or any other mosquito-borne pathogens have not been detected for at least one month prior to testing, and where screen shelters may be effectively erected and maintained for subject comfort and safety between exposures (§4.7.2; p. 27). The proposed plan for site monitoring consists of trapping approximately 1,000 mosquitoes of all species combined weekly for one month prior to test initiation. §1.3.3; p. 10). "*Local public health services will be contacted during the week before a field test. If there are Zika, dengue, or chikungunya disease cases reported in humans within 25 miles of the planned* [test] *site within the preceding month, the test will be moved, rescheduled, or both*" (§1.3.3; p. 9).

The study plan includes one visit to the laboratory prior to field testing and one day visit per field site. The visit to the laboratory will last from 1 to 1.5 hours and it will take place within 30 days of the repellency test day. The laboratory visit includes orientation, obtaining participants' consent, and taking measurements of the length and circumferences of subjects' limbs for calculation of their skin surface area. Subjects' attractiveness to mosquitoes will be confirmed during this visit (§1.3.2, p. 8). If less than two mosquitoes land within two

minutes, the subject will be considered unattractive to mosquitoes and disqualified for participating in testing (§4.7.7; pg 8). Participants will also spend approximately 15 to 30 minutes practicing the use of aspirators for catching landing mosquitoes and familiarizing themselves with mosquito landing behavior (Protocol Appendix 3). Subjects will learn how to use aspirators for immediate removal of mosquitoes before they bite (Protocol Appendix 3). Pre-test practice will take place in the laboratory using certified pathogen-free, unfed mosquitoes from pathogen-free, laboratory colonies (Protocol Appendix 10).

At each field site, repellency testing will be conducted for a period of up to 12 hours (16 hours, including travel time) (Informed Consent; p. 5 of 15). Testing may begin several hours post-application of the repellent product due to travel time (§4.8.4; p. 31). Prior to test initiation and throughout the test, two untreated control subjects will monitor ambient landing pressure for up to five minutes prior to each exposure period to ensure adequate mosquito activity for each exposure period to start. Adequate landing pressure is five landings within five minutes or less on each control subject (§4.7.4; p. 28). Immediately following the assessment of adequate landing pressure on controls, each treated subject will undergo five-minute exposures at 30 minute intervals until CPT occurs for that subject, signaling repellent failure, or the end of study period is reached, whatever occurs first (§4.8.4; p. 33).

To measure CPT, subjects will work in pairs. Twelve treated subjects will be paired with each other. The 13th subject will be paired with a member of the staff. If any subject withdraws, the remaining subject from the pair will be paired with the 13th subject. A member of the staff will pair with any unpaired test subject. Each control subject will be paired with a technician. Testing pairs will be separated from other pairs by at least 3m (10 ft.) apart.

Sample Size and Number of Subjects

The study protocol proposes to test the product on two ecologically distinct field sites using a sample size of 13 treated subjects, two untreated control subjects to monitor mosquito landing pressure during testing, and five alternate subjects for a total of 20 individual subjects per field site. This sample size is based on a power analysis by the EPA (*see* Protocol Appendix 8). Forty participants will be required to complete the testing protocol over two days at two sites (one day per site). Recruitment will be conducted until 50 people are qualified to ensure an adequate sample size if subjects become unavailable to participate between the time of consenting and the time of the field test.

Randomization

Randomization procedure is described in §3.2 (pp. 16-17) and §4.7 (p. 26). Enrollment will proceed until 25 male and female candidates are identified. Candidates will be assigned a unique sequential number by order of contact. The list of candidate numbers will be randomized (§3.2). There will be a total of 40 subjects. Two groups of 20 subjects (10 males and 10 females) will be randomly assigned to either one of the two field trials. Thirteen subjects (7 of one gender and 6 of another) will be randomly assigned as test subjects, and an additional two subjects will be assigned as control (one male and one female). The remaining five subjects will be used as alternates (§4.7). Whether forearms or lower legs will be used in testing depends on species of mosquitoes present at the site and their behavior (§4.7, p. 27).

Treatment will be applied to non-dominant arm and no randomization procedure is described for treatment application to lower legs (§4.7, p. 27).

Study Plan

The study plan includes one visit to the laboratory prior to field testing and one day visit per field site. The visit to the laboratory will last from 1 to 1.5 hours and it will take place within 30 days of the repellency test day.

Repellency testing at field site will take place during a second visit at two ecologically distinct field sites. One day test day at the field site is expected to last up to a maximum of 16 hours, including travel time to the site (Protocol Appendix 1) Limb preparation, obtaining additional protective clothing, and repellent application will take place prior to field exposures and field testing (§4.8.4). Field exposures to mosquitoes will be conducted in an area where target mosquito species are present and Zika, dengue, or chikungunya viruses have not been detected. "*The study will be conducted in areas where such viruses have not been detected by PCR-based screening of trapped mosquitoes from the test sites for at least a month"* (§1.3.3; p. 10). The proposed plan for site monitoring consists of trapping approximately 1,000 mosquitoes of all species combined weekly for one month prior to test initiation (§1.3.3; p. 10). "*Local public health services will be contacted during the week before a field test. If there are Zika, dengue, or chikungunya disease cases reported in humans within 25 miles of the planned* [test] *site within the preceding month, the test will be moved, rescheduled, or both*" (§1.3.3; p. 9).

Test Substance Application

The product, Mimikai Lilly Pilly Repellent, will be applied at the EPA standard dose of 0.5g/600 cm^2 for testing repellency. Prior to application, either forearms or legs will be cleansed using unscented soap, rinsed with 70% alcohol solution and towel dried. Individual doses will be calculated based on each individual subject's skin surface area and converted to volume using test substance specific gravity. The test substance will be dispensed from tuberculin (1 ml) syringes and evenly applied on treated skin in a light rubbing motion by researchers wearing surgical gloves. Multiple research personnel will make the applications to maintain consistency concerning time of application among treated subjects and each application will take approximately two minutes. Time of application will be recorded for each subject in data collection sheets (Protocol Appendix 4).

Estimation of Skin Surface Area

Whether arms or legs will be used for repellency testing will depend on identified species of mosquitoes (and their behavior) sampled at the test site. Limb surface area will be calculated from length of forearm or lower leg by its average circumference. Average circumference will be measured at four points: upper forearm or upper leg, lower forearm or lower leg, and two equally spaced points in between (§4.5; pg. 22). Data sheet in Protocol Appendix 4.

Margin of Exposure

The product, Mimikai Lilly Pilly Repellent, will be tested against mosquito species within three genera, *Anopheles, Aedes* and *Culex,* at the EPA standard dose of 0.5g/600cm^2.

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 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). Methyl nonyl ketone 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 Rules

The protocol includes the following circumstances or conditions for stopping:

- Bad weather, i.e., wind speed > 10 mph and/or rain
- Landing pressure is above or below acceptable levels, i.e., no more than 15% of all exposure periods are permitted to have less than five landings within five minutes per control subject, and no more than 15% of all exposure may be skipped or missed due to bad weather
- End of field test period or consented duration reached
- Safety reasons
- CPT is reached by subject, i.e., when FCL occurs. The landing confirming the first landing may occur within same exposure period or in the exposure period following period in which the first landing occurred (§4.9; p. 33).

Withdrawal Criteria

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 is not used. Participants may also be removed from the study without their consent for any reason, including, but not limited to the discretion of the Chief Investigator, where continued participation may jeopardize the safety of the participant or the integrity of the study, or the study is stopped by the sponsor and/or Study Director prior to completion. Subjects are asked to withdraw from the study if they prove to be unattractive to mosquitoes.

Criteria for Using Data from Withdrawn Subjects and Replacing Subjects During Field Testing

"*If subject withdraws during the field test and before the withdrawing subject has received a confirming landing, the subject's data will be retained if his or her total exposure duration is > 90% of the mean of subjects who did not withdraw, provided that not more than 3 of 13 subjects have so withdrawn*" (§4.7.7; p. 29). "*If more than 3 of 13 subjects withdraw prematurely, those with the briefest participation will be replaced first.*" (4.7.7; p. 30)

Data Collection

Data collected from each treated subject include the following measurements: 1) exposure delay between time of application and first exposure in minutes; 2) time period from first field exposure to FCL, or end of test, and 3) time period between time of application and time to FCL (§4.9; p. 33). For control subjects, number of landings occurring in five minutes or less, and time in seconds when each landing occurs from the beginning of the beginning of the exposure period. Mosquitoes landing on exposed skin as well as on the

protective clothing of both control and treated subjects will be collected during testing (§4.7.3; p. 28). Environmental conditions such as temperature, relative humidity, wind speed, light intensity, general cloudiness, and precipitation will be monitored hourly throughout the field trial (§4.7.6; p. 30). Data collection sheet is provided in Protocol Appendix 4.

Statistical Analysis

CPT is measured as one single value for each treated subject. Repellency data from each subject's CPTs will be analyzed using Kaplan-Meier survival analysis to calculate a mean and median CPT (mCPT) for the product within 95% CI. Data on landings on control subjects will be tabulated by individual and exposure period. Mean landing pressure will be calculated per control subject per exposure period. Data on landing pressure will not be analyzed statistically for estimation of CPT.

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

Subjects will be exposed to the test substance, lab-raised mosquitoes, and wild mosquitoes.

The test 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. Subjects will be exposed to the test product, Mimikai Lilly Pilly, for a maximum of 16 hours from time of product application if product is applied before traveling to test site. Repellency evaluation will take one day at each test site. No subject will test at more than one test site.

Mosquitoes employed for pre-test practice on handling aspirators will be pathogen-free sourced from laboratory-reared colonies.

Proposed exposure periods for repellency testing in the field with wild mosquitoes consist of exposing treated human skin to field mosquitoes for five minutes at 30 minute intervals until subject experiences CPT, signaling repellency failure, or testing is ended, whatever happens sooner. Proposed exposure periods for monitoring ambient landing pressure consist of exposing untreated human skin to field mosquitoes for up to five minutes at 30 minute intervals throughout the test. Testing sites will be selected based on diversity and abundance of targeted mosquito species and absence of mosquito-borne diseases for at least one month prior to test initiation, and absence of reported mosquito-borne disease cases in humans within 25 miles of planned test site.

Good Laboratory Practice (GLP) Compliance and Quality Assurance

This study will be independently audited by a QAU for compliance with Good Laboratory Practice Regulations (40 CFR 160). *"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" (§5; p. 35).

Compliance with FIFRA and EPA regulations

Data resulting from execution of this protocol as well as study conduct will be reviewed by US EPA and the 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 site locations: Repellency phase of the study may be conducted at field sites located in northern and southern California, Minnesota, Louisiana, Arizona, and Florida, where different mosquito species within the genera *Culex, Anopheles* and *Aedes* are present and active and vector-borne pathogens are not detected.
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 Informed Consent Form (ICF) concerning covering treated skin between exposure periods. Subjects should not be instructed to cover treated skin between exposure periods since this practice is likely to disturb the applied repellent.
2. Revise item #13 in §3.3.2, Exclusion Criteria (p. 18) that reads, "Has participated in another field repellency test day of this study in the previous 72 hours." The limit of 72 hours between testing days should not apply to the proposed study since each subject will not participate in more than one field test. This sentence should be removed from the protocol.
3. Assigning treatments to a treated subject's non-dominant arm is acceptable. However, non-dominant limb is not applicable to lower legs. Therefore, applications to lower legs should be randomly applied, and randomization process should be described.
4. The following paragraph, extracted from §4.8.4 on p. 31, needs revision: "Treatments may be applied at the field site or shortly before travel from the lab to the field site. Because field testing conditions are likely to be more fatiguing to subjects than conditions in the laboratory prior to departure to the field or conditions during transport to the field, if the Test Material is anticipated to remain effective for many hours, applications may be made several hours in advance of the first exposure period to reduce the probability of subjects needing to withdraw due to exhaustion before

receiving a confirmed landing. Exposures of the second treated limb will begin at the next exposure period, in order to produce a replacement estimate of Complete Protection Time." Since product applications will be conducted either at the laboratory prior to travel to the field site, or at the field site, it is possible to approximate the period between product application and first exposure. Furthermore, delaying field exposure to coincide with mosquito activity in the field and reduce subjects' exposures in the field may take several hours between application and first field exposure. During this time no CPT data will be collected, and potential CPT time points will be unknown. For this reason, it is not recommended to prolong delaying first field exposure unless the registrant establishes criterion for ensuring that CPT recorded after prolonged first exposure delay is accurate and unlikely to have occurred at an earlier time point. In addition, exposures of "the second treated limb" does not apply to this experimental design therefore, the statement should be deleted.

5. The study protocol should indicate that the following CPT data from treated subjects will be reported in tabulated form to include: 1) time (hours:minutes) when product was applied per subject; 2) start time of each field exposure per subject; 3) length of time (hours:minutes) between product application and first exposure per subject; 4) time when first landing occurred per subject; 5) time when second (confirmatory) landing occurred per subject; and 6) total number of hours:minutes from time of product application to time of first confirmed landing or CPT time-point per subject.
6. The study protocol should indicate that data from control subjects will be reported in tabulated form to include: 1) start time (hours:minutes) of each field exposure; 2) time and number of mosquitoes landing on each control; 3) total number of mosquitoes landing in five minutes per control; 4) total time (minutes) for five landings to occur (when five landings happen in less than five minutes).
7. Replace "LIBes" with "Landings" on the heading of raw data collection sheets for Controls. Furthermore, the purpose of the metric "repulsion" = 0 at the bottom of the data collection sheets for control is unclear and should be removed.
8. Add to exclusion criteria in §3.3.2 that those subjects that are proved to be unattractive to mosquitoes will be excluded from further participation in repellency testing.
9. Amend §4.7.2 on p. 29 to propose that only mosquitoes that land on exposed skin of both control and treated subjects will be collected and that those landing on protective clothing of subjects will not be collected. It is recommended that the same standard for collecting mosquitoes that land only on exposed skin of treated and control subjects be maintained. Adding a new metric to measure background foraging activity is beyond the objective of monitoring landing pressure by control subjects. Furthermore, trying to collect all landings might potentially distract subjects and staff members from paying closer attention to mosquitoes landing on exposed skin, which could potentially result in inaccurate measures of CPT and landing pressure.
10. Amend the rationale/justification for sample size and remove last paragraph on pp. 20 -21. Justification for sample size is based on EPA simulation for determination of sample size, provided in Protocol Appendix 8. A sample size of 13 would be adequate to ensure that the study includes enough subjects to return reliable results without including more subjects than necessary.
11. Specify that data from withdrawn subjects who are not replaced should be counted as censored data for statistical analysis.

12. Remove the statement “attempted to bite” on line 1584, §4.8.4 on p. 32, and replace it with “landing”.
13. Multiple technicians will conduct product applications to minimize time of application and make time of application more consistent among subjects, however variability in application can be attributed to multiple technicians applying the required volume per subject. It is recommended to ensure consistency in the application method.
14. There is a plan for skipping exposures due to bad weather. In addition, it is recommended to check weather conditions in advance to test day to avoid scheduling testing under bad weather.
15. Trap type should be added to description of methods for site monitoring.
16. In addition to specifying how the Study Director will coordinate with local agencies, please consider adding (as appendices) the SOPs for the mosquito control districts of the testing locations/sites to further clarify the methods of site surveillance.

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 research. Attachment 1 provides supporting details and a point-by-point evaluation of this protocol.

1. **Societal Value of Proposed Research**: This study is designed to determine the efficacy of a topically-applied mosquito repellent, Mimikai Lilly-Pilly Repellent, which contains OLE and MNK. The aim of this research is to provide the data resulting from this proposed study will be used to support registration of the new skin-applied repellent by EPA. Efficacy at preventing mosquitoes from landing on each subject will be expressed as CPT, which is defined as the time between application of the repellent product and the occurrence of the first mosquito landing on the treated skin followed by a second landing within 30 minutes. The CPT data from each subject will be combined and analyzed to determine the mCPT, which will be used to develop product labeling. Research with human subjects is justified because sufficiently reliable non-human methods for testing the efficacy of topically-applied repellents have not been developed. The research has societal value because people are at risk of contracting mosquito-borne diseases, and such risks can be mitigated by the use of insect repellent products.

2. **Subject Selection**: The protocol calls for testing each product with 13 subjects, with an approximately equal number of males and females. An additional two individuals will participate in the testing as untreated controls, monitoring mosquito landing pressure immediately prior to each exposure period. In addition, five subjects will be enrolled as alternates, to take the place of any test subjects who withdraw before or on the day of testing (at least two subjects of each gender). A total of 20 individuals (13 test subjects, 2 untreated controls, and 5 alternates) will be selected to test each product. Therefore, a total of 40 subjects would be needed assuming each individual participates only in a single test day.

   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 50 individuals agree to attend a consent meeting, with a target of at least 25 males and 25 females.

The protocol includes a number of proposed testing locations. Table 1 provides a listing mosquito species and geographical areas, and the protocol notes that "the timing and exact location of mosquito populations desirable for repellent testing may vary from year to year." (p. 5) Regardless of the testing location or timing, similar recruitment procedures will be followed. Either the study staff or a recruitment service local to the study area will initiate the recruitment process. Recruitment will be conducted via electronic and posted advertisements in the area surrounding a study site. 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.

Prior to field testing, subjects will participate in a mosquito attractiveness test and training on how to use an aspirator. To verify subjects' attractiveness to mosquitoes, they will place an arm into a 12 in x 12 in x 18 in cage with 10 female Aedes aegypti mosquitoes. Subjects will wear a nitrile glove to protect their hand during the testing period. The forearm will be untreated and exposed to the mosquitoes. The protocol notes that two mosquitoes must land on a subject's arm within two minutes for the subject to be deemed sufficiently attractive to mosquitoes to continue in the testing. Subjects who are not deemed attractive to mosquitoes based on this assessment will be withdrawn from further study participation. Following the attractiveness assessment, subjects will be trained to aspirate mosquitoes in 24 in x 24 in x 24 in screened cage. During this training, subjects will expose a small area on the forearm to the mosquitoes in the cage. Research staff will train subjects on how to watch mosquitoes approach and land, and how to quickly remove landing mosquitoes. The training will be repeated at least three times, or until the subject demonstrates that they can use the aspirator competently. Subjects who cannot show competence in aspiration will be withdrawn from the study.

3. **Informed Consent**: During the recruitment period, interested candidates will contact study staff via phone or email to learn more about the study and to self-evaluate whether they meet the eligibility criteria. Those who are interested in continuing with enrollment will be invited to meet 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.

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

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

   To mitigate risks from exposure to the test material, the test product uses materials that have been evaluated by EPA for toxicity and found to lave low acute risk profiles. Individuals have had allergic reactions to insect repellents and cosmetics in the past are excluded from participation. Subjects with a history of or who are suffering from rashes or other skin conditions that could be exacerbated by exposure to the test substance are also excluded from participation. Any subject who shows signs of adverse reactions to the test substance during the course of the study will be removed from participation. To remove the test substance at the end of the test day (or upon withdrawal), treated skin will be washed with soap and water and rinsed with ethanol prior to the subject's departure from the test site.

   To mitigate risks from exposure to mosquitoes and disease vectors, subjects will be trained to aspirate landing mosquitoes before they probe or bite. For control subjects, aspiration during field testing will be done by a member of the research team. The protocol identifies the potential vector-borne diseases to which subjects could be exposed. The field testing sites will be monitored weekly for a month prior to the testing for relevant vector-borne viruses, and approximately 1,000 mosquitoes captured during each week of the monitoring phase will be pooled and tested for pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified.

   Risks of exposure to mosquitoes will also be mitigated by having all subjects wear clothing, gloves, and head nets to protect untreated areas during the test periods. The protocol recommends that subjects wear Tyvek suits for protection; however, wearing this protective gear exposes the subjects to higher risk of heat-related illness. To minimize the risk of contracting any mosquito-borne diseases during the lab-based mosquito attractiveness test, the cages will be populated with mosquitoes from a colony reared in the laboratory and certified to be disease free.

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

To protect subjects against the physical risks associated with the test environment, subjects will be protected from biting insects while not actively participating in a test period. Each field site will have a large, screened shelter, along with fans and evaporative coolers if needed. The protocol identifies the risks associated with wearing a Tyvek suit and proposes to mitigate the risks by having study staff monitor subjects for signs of heat-related illness so they can provide assistance immediately. Subjects will have access to food and beverages, and restrooms. Individuals who are in poor physical health, which could make the challenges of participating in a test day in an outdoor environment will be excluded during the screening process. A certified first aider will be present during training and testing to provide emergency assistance if required.

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 following their participation. 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.

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

6. **Risk/Benefit Balance**: The protocol describes measures to minimize risk to subjects while maintaining the robustness of the scientific design. With to the risk mitigation measures put in place and the EPA's comments addressed, the residual risk to

subjects is low and reasonable in light of the potential benefits of the data to society.

7. **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.

8. **Independent Ethics Review**: On December 24, 2020, Advarra IRB approved the protocol dated December 23, 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.

G. Compliance with Applicable Ethical Standards

This is a protocol for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to 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.

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. During field testing, the protocol proposes that subjects wear Tyvek suits to protect from insect bites. EPA notes that wearing Tyvek suits can present an increased risk of heat-related illness to subjects. Both EPA and HSRB have recommended that subjects in field tests wear long-sleeved pants and shirts, head nets, and gloves during testing to prevent mosquito landing/biting on areas other than the test area. In January 2021, during review of a protocol for field testing a skin-applied repellent against mosquitoes, the HSRB recommended against subjects wearing Tyvek suits. EPA recommends revising the protocol to note that subjects will wear clothing to protect the untreated areas during the field tests, that hats and head nets will be provided, and that subjects will be instructed and reminded to wear light, loose-fitting clothing on the test day.
2. The protocol notes that subjects will be offered the option to participate as an untreated control, rather than being randomly assigned as a test or control subject. The consent process discussion notes that untreated control subjects will be consented before treated subjects so that those who do not want to participate as an untreated control may consent to participate as a treated subject. In another section, 4.7, the protocol notes that the control subjects will be randomly chosen. Revise the protocol to be consistent. EPA recommends randomizing subjects' assignments to minimize potential bias.
3. Separate consent forms are provided for subjects participating as an untreated research subject and as a treated research subject. As subjects will not be assigned until they are enrolled in the study and do not have a choice in which role they play in the study (treated subject, control, alternate), revise into a single consent form that covers both treated and untreated control subjects.
4. The consent process should be revised to include a step-by-step description and demonstration of the procedures involved with the attractiveness test, training on aspirating mosquitoes, and field testing. The subjects should be shown how the test substances will be applied to their leg for the future testing, be informed that they will wear gloves to protect their hands and head nets to protect the head, face and neck, and be shown how to aspirate mosquitoes.
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. Move the discussion of remuneration from the "risks and benefits" section of the protocol to a section on compensation. Remuneration is not a benefit associated with the study.
9. 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.
10. Provide information on how subjects will be transported to and from the test site. Will subjects travel in their own vehicles or be transported by the study staff members? If subjects do not use their own transportation, how will subjects who experience CPT before the test day end be handled?
11. The protocol notes that the testing may occur at two geographically distinct areas. Include a description of how recruitment will be handled if the two test sites are not located in close proximity such that a single pool of recruits could be used to populate both test days (p. 16-17).
12. Provide the rationale for collecting subjects' social security numbers.
13. 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?
14. Clarify expectations and compensation for alternates. How long are alternates expected to remain at the test site?
15. Include in the protocol information about how payment will be made to subjects (cash, check, pre-paid card; mail or in person).
16. The protocol notes that "[a]lternate subjects may return later to replace subjects that initiate testing but withdraw before useful data are generated" (p. 20). What are the specific conditions under which an alternate would be enrolled as a subject after a test day begins? What are the implications for the difference in time of application for the original subjects and alternate subjects? What are the criteria for "useful data"?
17. 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?
18. 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.
19. 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.

20. The consent form notes that "you may take a ten-minute break from exposure every hour" (p. 45). Please explain how this break works with the monitoring periods of five minutes every 30 minutes. Additionally, please explain how a 10 minute break every hour will impact the stopping rules of the study, specifically the number of periods that can be missed.
21. 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
22. Remove any references to "bites" (e.g., p. 30) "landing with intent to bite" or "LIBes" (*see*, e.g., p. 77).

Attachments:

1. EPA Protocol Review (Protocol MIM-006, dated December 23, 2020)
2. Completeness checklists
3. EPA's Power Analysis/Sample Size of Field-Based Mosquito Repellency Studies (July 2017)
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: *Field Evaluation of Skin-Applied Mosquito Repellent Product Containing OLE and Methyl Nonyl Ketone*

Protocol Date: December 23, 2020

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

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1. Societal Value of Proposed Research

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

The purpose of the proposed study is to assess the duration of a skin-applied insect repellent product for preventing mosquito landings on human hosts. The objective of the study is to determine the duration of efficacy of the test material for repelling mosquitoes relevant to US consumers, such as those listed on Table 1, "Prominent target mosquito species in probable field study areas" (§1.1; p. 5), when applied at a typical consumer dose of 0.5g / 600cm^2.

EPA requires testing of products claiming efficacy against public health pests, especially those that may vector 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 mCPT of a personal, skin-applied repellent product containing the active ingredients OLE and MNK against mosquitoes. This information does not currently exist.

The rationale for testing is to collect data to establish a mCPT, based on the most conservative mCPT of species tested at two field sites. Data generated by the proposed research will be used to characterize the lasting repellency of the product proposed for registration as measured by the most conservative mCPT time value. A standardized protocol will enable the EPA to receive consistent and scientifically reliable data about the complete protection time for the product. Field testing data will provide information about the length of repellency time after treatment before the first confirmed landing by a mosquito occurs.

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

EPA requires product-specific efficacy data for registration of skin applied insect repellent products according to recommendations from the EPA OPPTS 810.3700: Insect Repellents to be Applied to Human Skin guidelines. The proposed product has not been evaluated for its performance against mosquitoes. 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 mosquitoes under the proposed use pattern has been conducted.

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

Human subjects are required because they represent the target system for the test material, and sufficiently reliable non-human models for repellency testing have not been developed.

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 a novel insect repellent, containing 11.0% OLE and 7.75% MNK against species of mosquitoes within the genera *Anopheles*, *Aedes* and *Culex* at the EPA standard application rate of 0.5g/600 cm^2.

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

The objective cited may be achieved by the study if the protocol is revised and amended in accordance with 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 13 separate treated subjects per test site. A sample size of 13 subjects for calculating the mCPT is based on EPA's recommended sample size of 13 test subjects for testing repellency against mosquitoes in the field. The EPA simulation for determination of sample size is described in Protocol Appendix 8. The rationale is that a sample size of 13 would be adequate to ensure that the study includes enough subjects to return reliable results without including more subjects than necessary.

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

Two negative control subjects will be employed to monitor adequate landing pressure at the site for testing repellency. Control subjects for the purpose of monitoring mosquito activity will not be factored in the statistical analysis of the data.

(c) How is the study blinded?

The study is not blinded. Each product will be tested separately. The investigator and subjects will be aware of the identity of the test substance on each day of testing. Observations are based on timing of mosquito landings.

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

Randomization procedure is described in §3.2 (pp. 16-17) and §4.7 (p. 26). Enrollment will proceed until 25 male and female candidates are identified. Candidates will be assigned a unique sequential number by order of contact. The list of candidate numbers will be randomized (§3.2). There will be a total of 40 subjects. Two groups of 20 subjects (10 males and 10 females) will be randomly assigned to either one of the two field trials. Thirteen subjects (7 of one gender and 6 of another) will be randomly assigned as test subjects, and two as control (one male and one female). The remaining five subjects will be used as alternates (§4.7). Whether forearms or lower legs will be used in testing depends on species of mosquitoes present at the site and their behavior (§4.7, p. 27). Treatment will be applied to non-dominant arm and no randomization procedure is described for treatment application to lower legs (§4.7, p. 27). EPA recommends describing randomization process for randomly assigning treatment to left or right lower legs of subjects.

(e) Can the data be statistically analyzed?

Yes. See (f) below.

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

The mCPT of all test subjects at two test sites will be calculated using the Kaplan-Meier survival analysis. EPA recommends the duration of protection for the repellent product to be the lowest mCPT of test conducted at 2 sites. EPA recommends rounding down mCPT value to the nearest whole number. For example, three hours and 45 minutes would be listed on the label as three hours.

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

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

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

The sample size of 13 subjects is acceptable based on the results from EPA's simulations. For detailed information on the statistical simulation see Attachment 3.

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to repellent product in a spray formulation, containing 7.75% MNK and 11.0% OLE. 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 will last maximum of 16 hours for 1 day of testing per subject. The product has been tested to satisfy Tier I Human Health Assessment data required for registration (MRIDs 510641-01 through 510641-08) and is classified as toxicity category IV for all routes of exposure. Subjects will be exposed to natural populations of field mosquitoes where relevant vector-borne pathogens have not been detected. During the laboratory pre-test practice training, participants will be exposed to pathogen-free mosquitoes from pathogen-free laboratory colonies.

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

Efficacy data are required to characterize product performance and support efficacy claims against mosquitoes on product label. 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?

EPA's accepted standard dose (0.5g/600cm^2) will be used for testing repellency. This dose is based on an analysis of the dosimetry results from repellent studies reviewed by EPA and HSRB since 2006.2 EPA considers the dose of 0.5g product/600cm^2 of skin to be an appropriate product dose for testing bag-on-valve spray canister type of product under this protocol.

(c) What duration of exposure is proposed?

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.

A day of testing will take up to 12 hours in the field and a maximum of 16 hours, including travel time to the field site. EPA recommends specifying time from product application to time of first exposure period. Repellency evaluation will take one day per site of testing per subject. Proposed exposure periods consist of exposing treated skin to field mosquitoes for five minutes at 30 minute intervals until the time point to repellent breakdown or CPT is reached by treated subject, or end of test, whatever happens sooner. Control subjects will monitor landing pressure by exposing untreated skin to field mosquitoes for five minutes or less if five landings per control subject is achieved in less than five minutes. Exposure periods for monitoring landing pressure will precede exposure periods for efficacy determination.

2.3 Endpoints and Measures

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

The efficacy endpoint is defined as the time of product failure measured by the time of the First Confirmed Landing (FCL). FCL is defined as a landing followed by a second landing within 30 minutes of the first. CPT is the measurement for residual repellency or time to product failure from time of product application. CPT is measured as a single value for each subject. CPT is measured by the FCL. The endpoints are appropriate to the questions being asked. Using the Kaplan-Meier estimator, the mCPT will be calculated for all test subjects at each site.

(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 the study.
- Limbs will be prepared for dose application. Skin surface area will be measured in advance, during first visit.
- Pre-test training on how to capture landing mosquitoes using aspirators will be conducted on first visit. The pre-test training will be used to determine subject's attractiveness to mosquitoes. Subjects' attractiveness to mosquitoes will be assessed prior to conducting pre-test training. EPA recommends expanding inclusion/exclusion criteria for adding exclusion from test participation subjects that are proved to be unattractive to mosquitoes.
- Two control subjects will be employed to monitor adequate landing pressure throughout the repellency test.
- Pathogen-free and unfed mosquitoes from pathogen-free laboratory colonies will be used for assessment of subjects' attractiveness to mosquitoes and pre-test practice on how to use aspirators in the laboratory.
- Efficacy will be conducted at two ecologically distinct sites, using 13 treated, two untreated and five alternate subjects per site.
- Number of mosquito landings, timing when landings occur, time from product application to time of first exposure, and time of each exposure thereafter will be recorded.
- Mosquitoes landing of subjects during field testing will be collected and saved for taxonomical identification and pathogen screening.
- Treated and control subjects will work in pairs. Research staff will work with test and control subjects during field testing, assisting them with data recording.

- Alternate subjects (five alternate subjects) will be enrolled to ensure adequate sample size.
- A Quality Assurance Unit will be in place to monitor all study activities and data collection.
- There will be two test days of testing, one day at each field site.
- Stopping rules, criteria on missing periods and CPT determination, and criteria for subject withdrawal, use of withdrawn subjects' data, and replacement of withdrawn subjects and are proposed.

(c) What QA methods are proposed?

This study will be independently audited by a QAU for compliance with Good Laboratory Practice Regulations (40 CFR 160). *"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"* (§5; p. 33).

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

Sources of variation include mosquito species and activity at two ecologically distinct sites, and attractiveness of subjects to mosquitoes. These uncertainties will be addressed by control subjects monitoring landing pressure throughout the test, by determination of subject's attractiveness to mosquitoes prior to efficacy testing, and by using the lowest (most conservative) mCPT per field site for the duration of efficacy.

3. Subject Selection

3.1 Representativeness of Sample

The population of repellent users is presumed to be diverse in age, gender, physical size, general health, attractiveness to biting insects, and other characteristics. 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?

Volunteers will be recruited in the areas local to the test site locations, including options listed in Table 1 of the protocol. 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. The study will be replicated at two sites, representatives of different mosquito habitats. Power analysis is employed for estimation of adequate sample size to calculate a reliable estimate of mCPT. Randomization plan to allocate subjects to treatments, maintaining a 50:50 distribution of sex ratio is described in §3.2 (pp. 16-17) and §4.7 (p. 26).

3.2 Equitable Selection of Subjects

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

The inclusion/exclusion criteria are complete and appropriate assuming the EPA's comments, identified in red below, are incorporated (Protocol, pp. 17-18)

Inclusion criteria

- *18-60 years old*
- *Male or female*
- *Any race*
- *Completed consent process, including provide written consent*
- *Speak and read English*

Exclusion criteria

- *Known to be hypersensitive or allergic to mosquito bites or arthropod bites or stings.*
- *Phobic of biting insects or insect bites.*
- *Known to be allergic to insect repellents, essential oils of plants, or common cosmetics.*
- *Known to be sensitive to any of the Test Material ingredients.*
- *Poor physical condition.*
- *Unwilling to submit to brief query about personal condition.*
- *Use of insect 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 see biting insects on skin or otherwise effectively monitor and remove biting insects that contact skin.*

- *Is the spouse, an immediate family member, 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, or a student of the Study Director*
- *Does not regularly spend time in outdoor settings*
- ~~*Has participated in another field repellency test day of this study in the previous 72 hours.*~~
- *Has participated in an interventional study (other than a biting arthropod repellency efficacy study) in the previous 3 months.*
- *Prone to or suffering from rashes or other skin conditions including eczema, psoriasis, and sunburn.*
- *Not attractive to mosquitoes during the mosquito attractiveness test*

(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; the protocol includes a list of potential test sites in Table 1. Advertisements will be posted on Craigslist and community bulletin boards. Either the research team conducting the study will recruit subjects, or an independent recruitment firm will used depending on the location of the study. The recruitment materials are included with the submission. Recruitment will be conducted until at least 50 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.

(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 mosquito 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 through 510641-08 in Protocol Appendix 6) and therefore is determined to be of low acute toxicity.

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 446242-06), and toxicity

category IV for Acute Inhalation (LC50 > 2.06 mg/L (446241-04)). It is not a dermal sensitizer (MRID 446242-07) and not 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 No Adverse Effect Level (NOAEL) = 1,000 mg/kg/day, and the Lowest Adverse Effect 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. 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 risk assessment for sub-chronic dermal exposure reported the LOAEL and the NOAEL > 300 mg/kg/day 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. For sub-chronic dermal irritation in the same study, the NOAEL > 100 mg/kg/day and < 300 mg/kg/day (MRID 431103-01). 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. Methyl nonyl ketone 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).

For Mimikai's proposed end-use product, the EPA estimated the risk 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, 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.

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 needs to be taken into consideration, so 0.833 is multiplied by 0.0775, resulting in 0.064 mg/MNK/human subject. The loading rates are then compared, 3.3/0.064 to result in an MOE of 52.

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

Risks to subjects include the risk of exposure to field mosquitoes, 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 applied for testing repellency (0.5g/600cm^2/day) is greater than any LOC for OLE and MNK. EPA considers the exposure of the subjects to the levels proposed for testing the repellent product not to pose an unreasonable risk of adverse effects to subjects.

(d) Does the research proposal adequately identify anticipated risks to human subjects and their likelihood of occurrence? How was this likelihood estimated?

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

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

Individuals who may be at an increased risk for adverse effects are not eligible to become subjects in this study, including individuals known to be allergic or sensitive to skin-applied insect repellents, and those with known skin conditions 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 specified in the protocol to minimize risks to subjects?

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

To mitigate risks from exposure to the test material, the test product uses materials that have been evaluated by EPA for toxicity and found to lave low acute risk profiles. Individuals have had allergic reactions to insect repellents and cosmetics in the past are excluded from participation. Subjects with a history of or who are suffering from rashes or other skin conditions that could be exacerbated by exposure to the test substance are also excluded from participation. Any subject who shows signs of adverse reactions to the test substance during the course of the study will be removed from participation. To remove the test substance at the end of the test day (or upon withdrawal), treated skin will be washed with soap and water and rinsed with ethanol prior to the subject's departure from the test site.

To mitigate risks from exposure to mosquitoes and disease vectors, subjects will be trained to aspirate landing mosquitoes before they probe or bite. For control subjects, aspiration during field testing will be done by a member of the research team. The protocol identifies the potential vector-borne diseases to which subjects could be exposed. The field testing sites will be monitored weekly for a month prior to the testing for relevant vector-borne viruses, and approximately 1,000 mosquitoes captured during each week of the monitoring phase will be pooled and tested for pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified.

All subjects will wear clothing, gloves, and head net to protect untreated areas during the test periods. The protocol recommends that subjects wear Tyvek suits for protection; however, wearing this protective gear exposes the subjects to higher risk of heat-related illness. To minimize the risk of contracting any mosquito-borne diseases during the lab-based mosquito attractiveness test, the cages will be populated with mosquitoes from a colony reared in the laboratory and certified to be disease free.

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

To protect subjects against the physical risks associated with the test environment, subjects will be protected from biting insects while not actively participating in a test period. Each field site will have a large, screened shelter, along with fans and evaporative coolers if needed. The protocol identifies the risks associated with wearing a Tyvek suit and proposes to mitigate the risks by having study staff monitor subjects for signs of heat-related illness so they can provide assistance immediately. Subjects will have access to food and beverages, and restrooms. Individuals who are in poor physical health, which could make the challenges of participating in a test day in an outdoor environment will be excluded during the screening process. A certified first aider will be present during training and testing to provide emergency assistance if required.

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 following their participation. 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.

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

Testing will be stopped for all subjects when the maximum testing duration is reached, the Study Director determines that the test site is unsafe, landing pressure falls below the minimum level needed or rises to a level that is uncomfortable or unsafe for subjects, certain weather conditions occur (wind, rain), or three consecutive periods have been skipped for any reason.

For individuals, stopping rules are invoked when the subject asks to withdraw, subjects are unattractive to mosquitoes, subject experienced an FLC, the subject exhibits sensitivity to mosquito bites or the test substance during testing, or medical management is invoked for the subject (p. 30).

(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"* (pp. 11-12).

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

See the responses to 4.2(b) and 4.2(c).

(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?

"*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. In addition, subjects are instructed to contact a medical practitioner of their own choice and inform the Study Director should they experience symptoms known to be associated with mosquito-borne viral diseases within a month after the test as described in the Informed Consent Document*." (§1.3.6).

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

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?

As a result of the data from this study, society will benefit from the availability of insect repellent products.

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

Society, the EPA, and registrants would benefit from this research. Society will benefit from repellent products that protect against bites from insects that carry vector-borne illnesses. The EPA will benefit from the submission of data that provides information about the product's efficacy in the field, which can be used to inform labeling and provide accurate information to users. The study sponsor will benefit by generating data that could result in product registration.

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

With the proposed changes, as well as the comments from EPA and HSRB addressed, the research is likely to generate scientifically valid results which would lead to the realization of the societal benefits as the product can be registered and labeling for the public can be updated and accurate.

6. Risk/Benefit Balance: 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 in section 3.5. 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-006 Feb 17, Appendix 8 MIM-006, 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-006, 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-006 Feb 17, Appendix 8 MIM-006, 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-006 Feb 17, Appendix 8
(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-006, Dec 23
(a)(2) The measures proposed to minimize risks to the human subjects	Y	MIM-006, Dec 23
(a)(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	MIM-006, Dec 23
(a)(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	MIM-006, Dec 23
(a)(5) The balance of risks and benefits of the proposed research.	Y	MIM-006, 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-006, Dec 23
(c) Information about how subjects will be recruited, including any advertisements proposed to be used.	Y	MIM-006, 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-006, Dec 23
(e) All correspondence between the IRB and the investigators or sponsors.	Y	MIM-006, Dec 23 MIM-006 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-006, 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	

Power Analysis/Sample Size of Field-Based Mosquito Repellency Studies
EPA Office of Pesticide Programs
July 2017

Objective

To determine the sample size N such that mosquito 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; such true **variation** of the Complete Protection Time (CPT) distribution will be expressed by the Weibull distribution family and a parameter, P5MR, defined as the 5^{th} percentile/mCPT.

The model simulation was prepared in anticipation of receipt of a proposed mosquito repellency protocol concerning IR3535 to be reviewed by the HSRB at a future date. EPA anticipates that most, if not all, future mosquito repellency studies with similar study designs can likely use this approach – and the simulation results described here - to determine the required sample size to achieve the stated objectives.

In order to develop estimates of a required sample size for a mosquito repellency study to achieve certain stated efficacy criteria and estimate a complete protection time (CPT)[1], it is necessary to determine the distribution of mosquito repellent failure times (generally considered to be time to first landing with intent to bite). However, the underlying distribution of the CPT of a product being tested in a mosquito 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 mosquito repellency studies. From past submitted studies that have been examined, the EPA has found CPTs display a left skewed distribution in some of the datasets.

On this basis, EPA assumed for this sample size determination exercise that a distribution of mosquito repellent failure times follows a Weibull distribution. A Weibull distribution is commonly used in reliability engineering and failure 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: **κ** (the "shape" parameter and sometimes referred to in some parameterizations as "a") and λ (the scale parameter and sometimes referred to as "b").[2] The PDF (probability density function) and CDF (cumulative distribution

[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 landing with intent to bite (FCLIB). The FCLIB is considered to be when one landing is followed by another landing within 30 minutes. The first landing is confirmed by the second landing.

[2] 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.

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 1 and 2 for some illustrative **κ** and λ values.

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

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

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

where: mCPT= 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

<u>Re-parameterization of Standard Weibull Equation</u>

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 ($\boldsymbol{\kappa}$ (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 2 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 1 below compares these two parameterizations for the example PDF and CDF distributions shown in Figures 1 and 2, respectively, for the **κ** and λ parameterizations shown there, illustrating the conversion to this new parameterization:

Table 1. Re-parameterization of Weibull Distribution Parameters from Traditional (**κ**, λ) to Revised (P5MR, mCPT) for Example Weibull Distributions Appearing in Figures 1 and 2.

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 failure 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 failure rate over time possibly suggesting failure 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 failure 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 2, 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 approaches 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.

An example of the (varied) kinds of distributional "shapes" associated with various parameterizations is shown in Figure 2 as histograms of the CPT. More specifically, Figure 2 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, and 0.6) for the (assumed) Weibull Distribution[3] . As seen in Figure 2, 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

[3] Other simulations were performed for the lognormal, normal, and uniform distributions, with the latter one (particularly) done as a form of sensitivity analysis but these are not discussed in this report; the simulation outputs, however, are provided in Appendix 4. Note that the power estimates for a given sample size from the Weibull and Lognormal distributions are similar.

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 for the field exercise to achieve a desired set of aims regarding precision around the estimate of the mCPT. These judgments are based in part on available data and past experiences[4] and in part on general thoughts regarding consumer 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 greater than a given acceptable K (a scalar which measures the precision of the estimates in estimating the mCPT). Such mosquito 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 > K (and the trial is considered to be a "success" in the power calculation)

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

Ideally, a mosquito 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 and spread of the CPT

[4] See Appendix 2 for Weibull parameters fit to previous mosquito efficacy field data that the EPA has evaluated for a similar design and experimental set-up. In general, the values found in these (prior) studies support the values selected here to be used for the simulation

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 Field Mosquitoes Repellent Study

In mosquito field repellency studies, test subjects are exposed in the field for 5-minute intervals immediately following product application and then for 5 minutes every 30 minutes until a "first confirmed landing" occurs. For subjects who receive confirmed landings, the CPTs are set as 0 if the first confirmed landing occurs during the first 5 minutes after application of the repellent; otherwise, the CPTs are rounded down to the nearest half hour (i.e., the starting time of the exposure period in which the first confirmed landing occurs). For those subjects for which there are no confirmed landings 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 simulate the field 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 ≤ 5, 6-35, 36-65, 66-95, … 576-605 minutes, the CPTs are set to be 0-, 0.5-, 1-, 1.5- hours…10 hours, respectively, to simulate the study design in which each study participant is exposed for 5 of every 30 minutes until the first confirmed mosquito landing. If the randomly generated CPTs are greater than 10 hours (or 605 minutes), they are considered in the calculation to be (right) censored at 10 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, and 8 hours; P5MR = 0.2, 0.4, 0.5, 0.6, 0.7, and 0.8; sample size per dataset = 10, 11, 12 … 20; and the lowest acceptable K = 0.6, 0.7, and 0.8; all assuming that CPT follows a Weibull distribution[5].

[5] Such calculations were similarly done for the lognormal distribution, normal distribution, and uniform distribution, but are not discussed further in this report. The SAS output from these calculations and various associated tables and graphs, however, is shown in Appendix 4 for completeness.

Results of Simulation

Tables 1, 2, and 3 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, respectively. These are shown for various values of mCPT (ranging from 2 to 8 hours), P5MR (ranging from 0.2 to 0.8), and Sample Size (ranging from 10 to 20). 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 a 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.

Figures 4, 5, and 6 present visually the same results in Tables 1, 2, and 3 (as power curves rather than tables).

As can be seen within each Table or Figure, the power of a study to achieve a given acceptable ratio K value (e.g., 0.6, 0.7, or 0.8 representing 95% LCL_{mCPT}/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). 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 Figures or 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% LCL_{mCPT}/mCPT > K" decreases since stricter requirements for a "success" are being levied.

The SAS Code used to generate the simulated data and the associated tables and graphs are presented in Appendix 3. Note - as described earlier - that simulations were also performed for the lognormal, normal, and uniform distributions, in part to serve as a sensitivity analysis and these are presented in the Appendix 4 for completeness, but are not discussed further here.

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

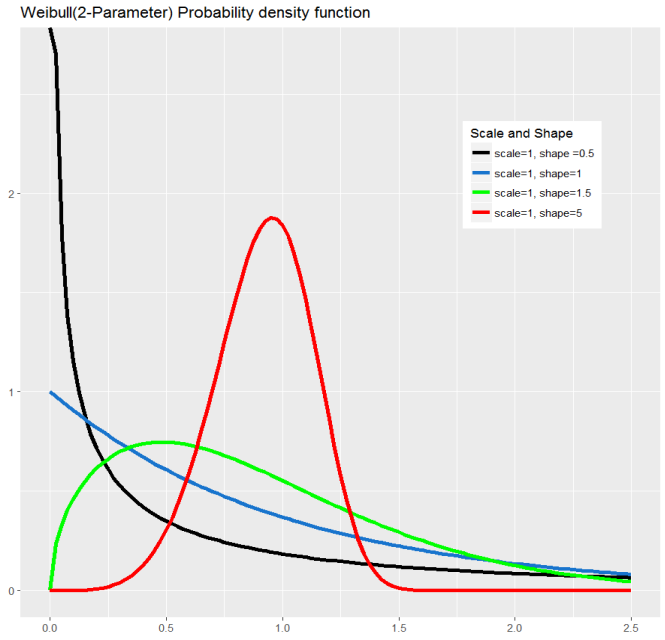


Figure 2. Cumulative Distribution Function (CDF) for Above Weibull PDF

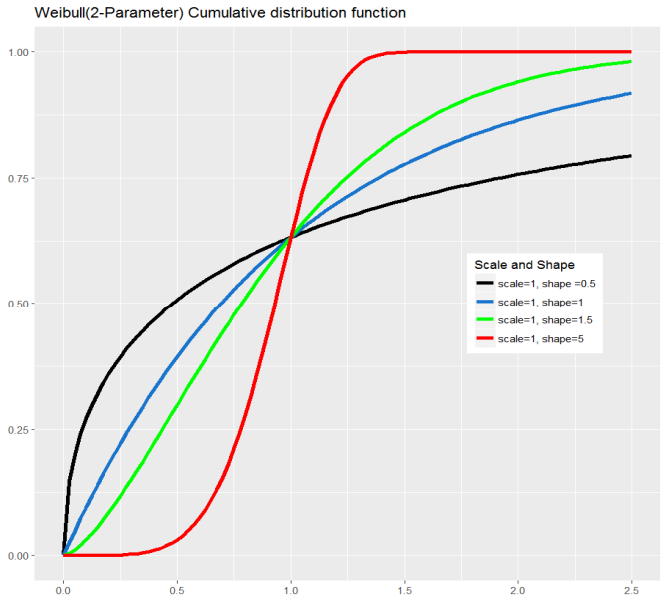


Figure 3: Histograms of CPT distributions for various CPTs and P5MRs (assume CPTs are Weibull distributions)

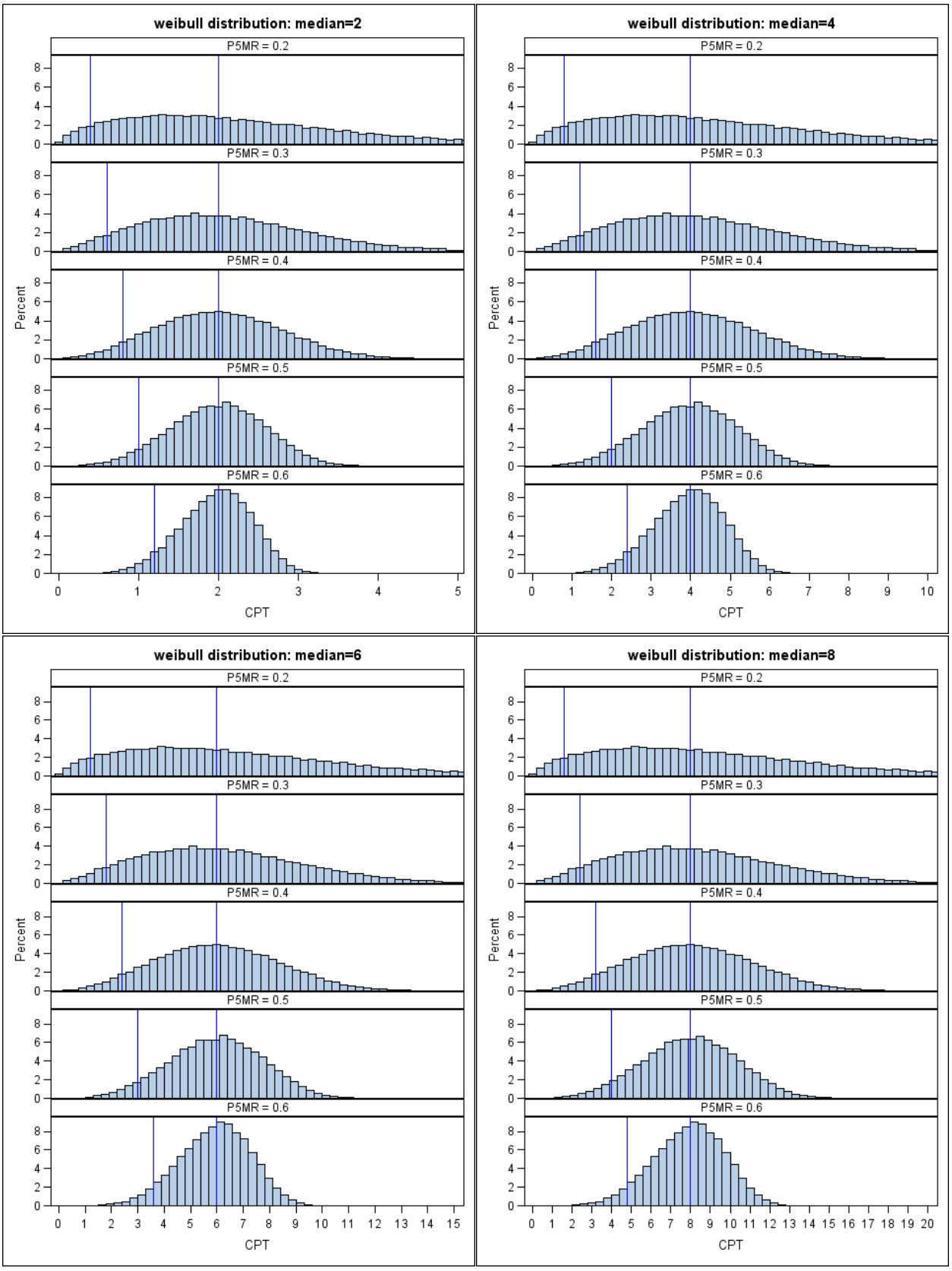


Table 1: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		_10	_11	_12	_13	_14	_15	_16	_17	_18	_19	_20
2	0.2	0.071	0.291	0.207	0.473	0.362	0.369	0.502	0.494	0.637	0.626	0.521
	0.4	0.297	0.691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

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

Table 2: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Weibull distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.022	0.096	0.066	0.199	0.121	0.128	0.217	0.211	0.304	0.299	0.222
	0.4	0.132	0.415	0.299	0.607	0.484	0.522	0.634	0.677	0.751	0.767	0.668
	0.5	0.267	0.633	0.516	0.789	0.697	0.748	0.803	0.851	0.868	0.895	0.838
	0.6	0.476	0.813	0.732	0.881	0.845	0.864	0.885	0.919	0.893	0.926	0.918
	0.7	0.694	0.895	0.876	0.861	0.889	0.847	0.850	0.888	0.799	0.837	0.888
	0.8	0.768	0.780	0.821	0.673	0.750	0.591	0.652	0.699	0.566	0.622	0.694
4	0.2	0.016	0.075	0.053	0.166	0.109	0.088	0.190	0.171	0.276	0.245	0.177
	0.4	0.103	0.332	0.267	0.517	0.452	0.402	0.620	0.555	0.752	0.681	0.638
	0.5	0.210	0.525	0.468	0.715	0.685	0.624	0.830	0.776	0.914	0.866	0.848
	0.6	0.402	0.736	0.714	0.886	0.880	0.833	0.955	0.923	0.979	0.966	0.969
	0.7	0.673	0.914	0.917	0.971	0.975	0.962	0.987	0.986	0.982	0.988	0.995
	0.8	0.927	0.970	0.987	0.945	0.971	0.946	0.931	0.955	0.892	0.922	0.958
6	0.2	0.047	0.083	0.066	0.158	0.105	0.083	0.174	0.150	0.247	0.218	0.149
	0.4	0.079	0.294	0.225	0.473	0.387	0.356	0.556	0.507	0.690	0.636	0.566
	0.5	0.172	0.483	0.406	0.679	0.622	0.573	0.779	0.735	0.887	0.841	0.806
	0.6	0.335	0.697	0.649	0.861	0.851	0.804	0.938	0.909	0.977	0.963	0.956
	0.7	0.607	0.894	0.885	0.975	0.970	0.958	0.994	0.989	0.997	0.996	0.997
	0.8	0.897	0.987	0.992	0.988	0.995	0.993	0.989	0.993	0.978	0.987	0.995
8	0.2	0.309	0.234	0.297	0.289	0.320	0.210	0.347	0.251	0.392	0.306	0.306
	0.4	0.180	0.321	0.294	0.497	0.435	0.379	0.598	0.521	0.726	0.654	0.592
	0.5	0.206	0.499	0.439	0.692	0.645	0.603	0.804	0.762	0.904	0.867	0.830
	0.6	0.357	0.731	0.684	0.892	0.872	0.831	0.957	0.933	0.983	0.978	0.966
	0.7	0.634	0.922	0.907	0.985	0.981	0.976	0.998	0.994	0.998	0.998	0.999
	0.8	0.913	0.996	0.995	0.999	0.999	0.998	0.997	1.000	0.994	0.997	0.999

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

Table 3: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Weibull distribution)												
Median (hours)	P5MR	Sample size										
		_10	_11	_12	_13	_14	_15	_16	_17	_18	_19	_20
2	0.2	0.007	0.032	0.027	0.064	0.044	0.027	0.079	0.061	0.115	0.088	0.071
	0.4	0.035	0.119	0.103	0.219	0.188	0.140	0.275	0.227	0.355	0.287	0.258
	0.5	0.068	0.210	0.185	0.339	0.297	0.251	0.418	0.364	0.506	0.460	0.425
	0.6	0.133	0.341	0.306	0.496	0.473	0.407	0.594	0.560	0.678	0.670	0.641
	0.7	0.251	0.561	0.532	0.679	0.692	0.598	0.753	0.753	0.749	0.775	0.809
	0.8	0.455	0.696	0.728	0.654	0.728	0.562	0.648	0.694	0.565	0.620	0.692
4	0.2	0.004	0.026	0.012	0.053	0.027	0.022	0.054	0.045	0.085	0.065	0.042
	0.4	0.026	0.093	0.080	0.186	0.151	0.103	0.254	0.182	0.346	0.245	0.228
	0.5	0.060	0.170	0.165	0.315	0.297	0.202	0.436	0.315	0.546	0.413	0.414
	0.6	0.135	0.317	0.320	0.494	0.499	0.374	0.650	0.529	0.760	0.643	0.639
	0.7	0.295	0.548	0.565	0.726	0.754	0.651	0.863	0.784	0.914	0.854	0.873
	0.8	0.619	0.828	0.867	0.884	0.923	0.864	0.913	0.922	0.886	0.909	0.947
6	0.2	0.038	0.033	0.027	0.055	0.037	0.027	0.053	0.033	0.076	0.058	0.039
	0.4	0.022	0.098	0.072	0.206	0.135	0.115	0.234	0.196	0.341	0.289	0.214
	0.5	0.054	0.205	0.154	0.364	0.281	0.248	0.438	0.382	0.567	0.493	0.425
	0.6	0.133	0.383	0.335	0.572	0.525	0.473	0.694	0.626	0.812	0.748	0.716
	0.7	0.316	0.646	0.614	0.819	0.818	0.750	0.918	0.874	0.965	0.943	0.938
	0.8	0.670	0.916	0.917	0.967	0.974	0.962	0.986	0.984	0.977	0.985	0.993
8	0.2	0.301	0.193	0.270	0.198	0.264	0.155	0.250	0.157	0.244	0.171	0.206
	0.4	0.122	0.136	0.141	0.227	0.182	0.142	0.267	0.208	0.340	0.292	0.229
	0.5	0.082	0.209	0.165	0.368	0.282	0.256	0.434	0.392	0.561	0.505	0.424
	0.6	0.124	0.390	0.321	0.588	0.514	0.490	0.688	0.655	0.823	0.779	0.710
	0.7	0.299	0.683	0.610	0.857	0.808	0.794	0.915	0.909	0.966	0.963	0.939
	0.8	0.644	0.940	0.909	0.989	0.978	0.981	0.994	0.995	0.993	0.995	0.998

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

Figure 4: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Weibull distributions)

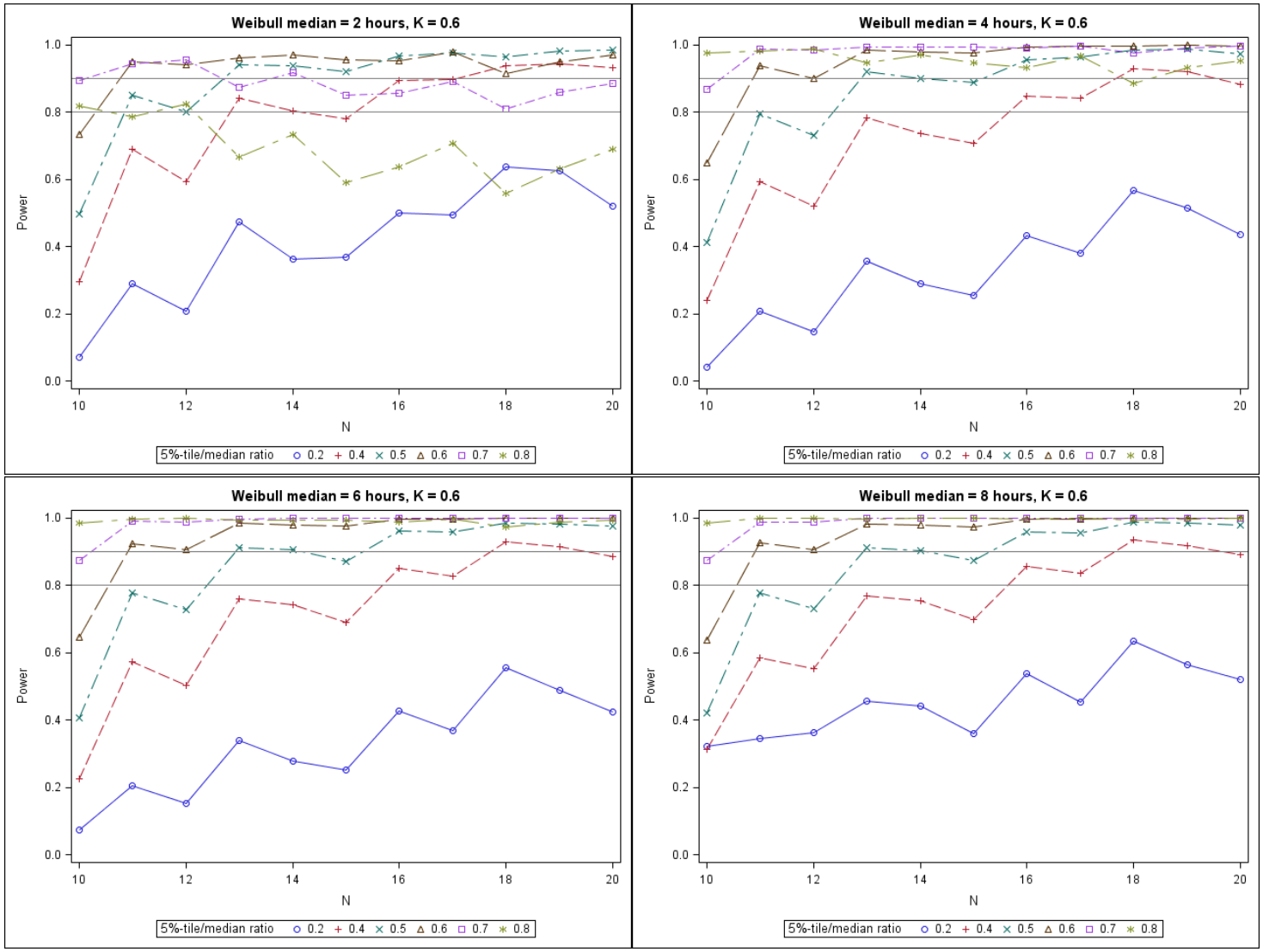


Figure 5: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Weibull distributions)

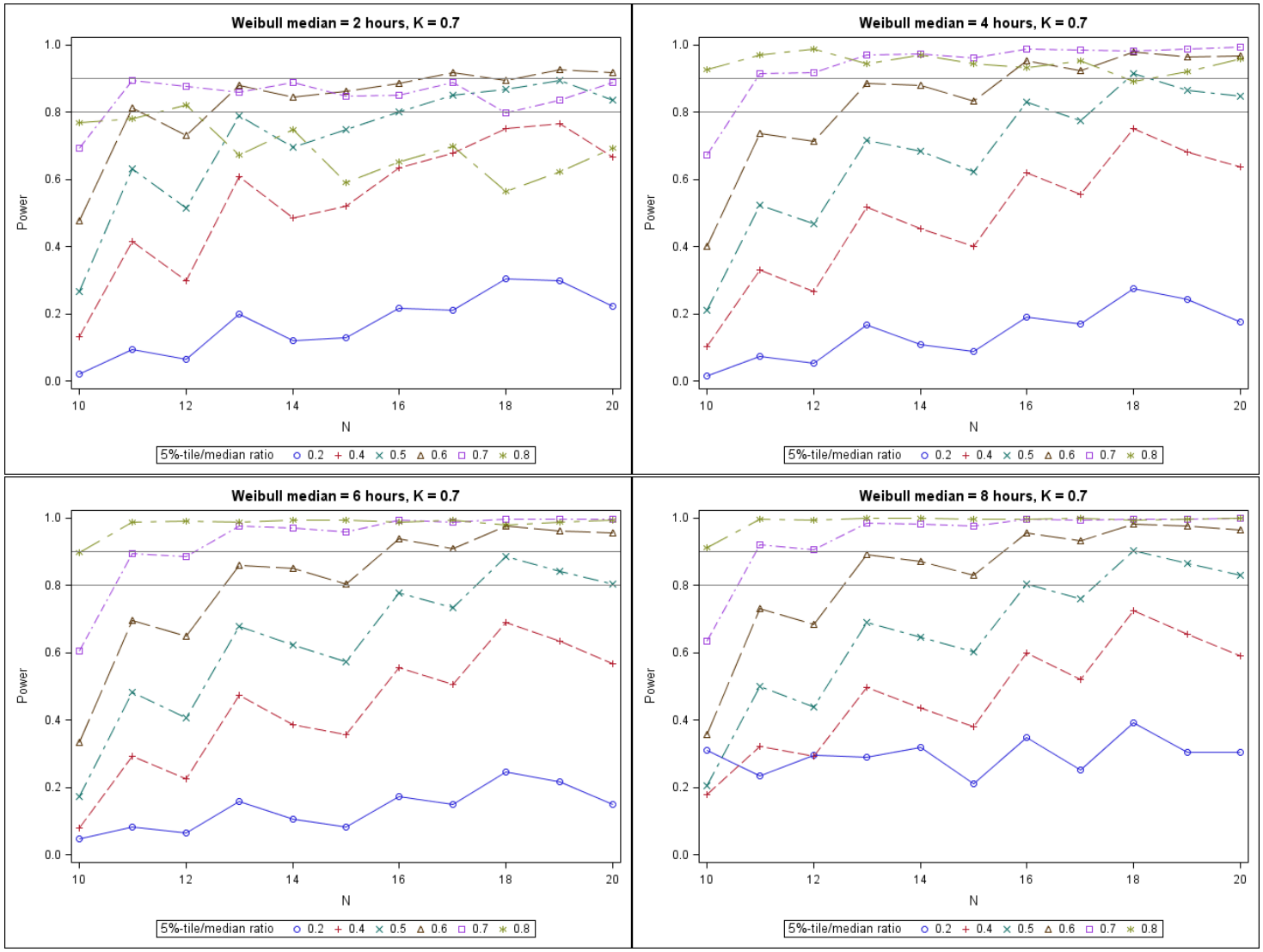
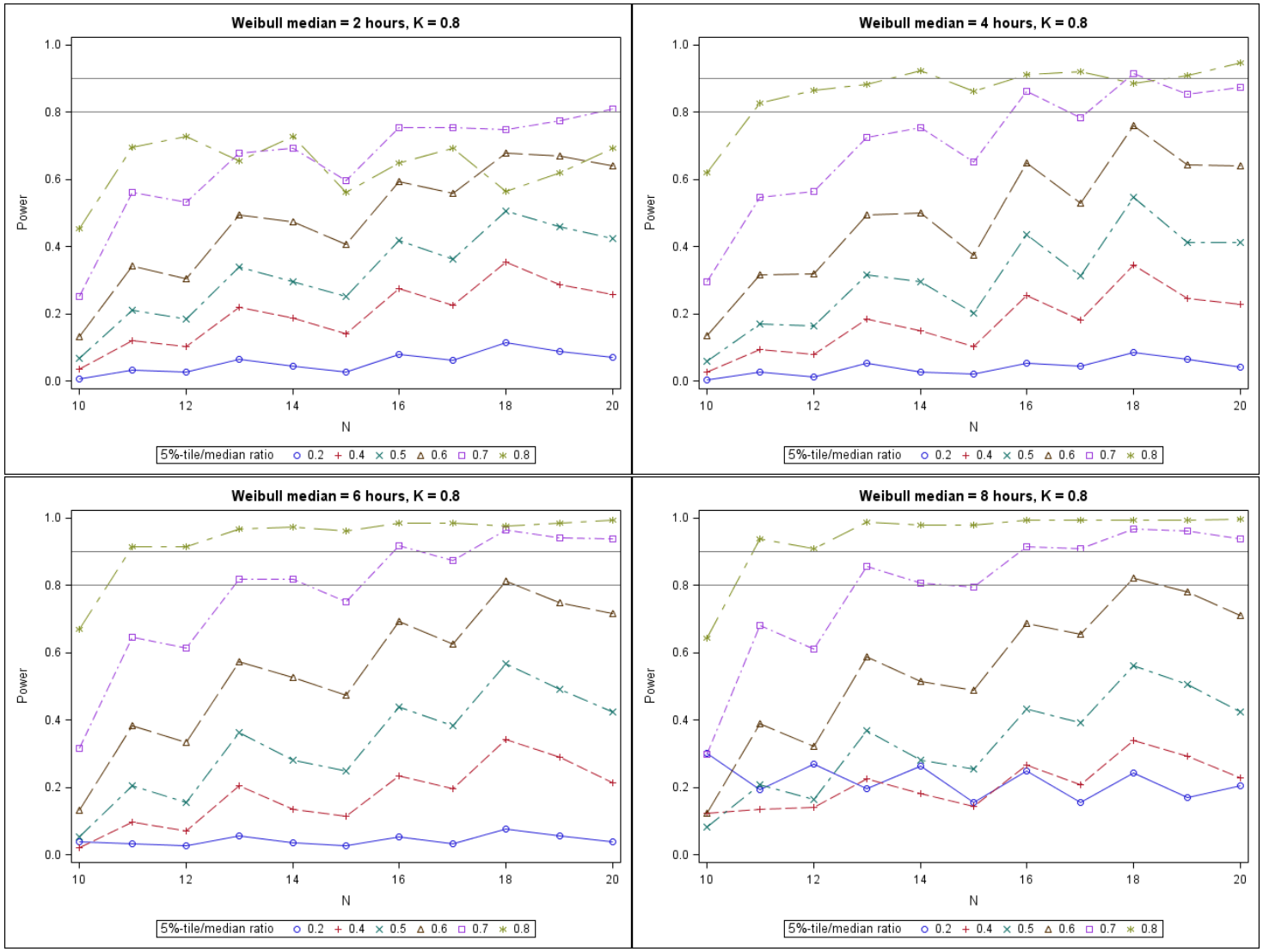


Figure 6: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Weibull distributions)



APPENDIX 1

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

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

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

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)

APPENDIX 2

Product	Location	Sample size	Est. mCPT (95% CI)	Ratio of 95% LCL/est. mCPT	Est. Weibull (shape κ; scale λ)	Est. P5MR
A	1	10	7.5 (4.0, 8.0)	0.53	6.602; 7.777	0.674
	2	10	8.5 (4.5, 10.0)	0.53	5.855; 8.624	0.641
B	1	10	12.0 (6.0, 12.0)	0.50	4.311; 10.669	0.547
	2	10	12.0 (8.5, 12.0)	0.71	10.424; 11.516	0.779
C	1	10	7.5 (4.0, 9.0)	0.53	4.430; 8.146	0.556
	2	8	5.0 (2.5, 5.5)	0.50	5.318; 4.915	0.613
D	1	10	2.0 (1.5, 2.0)	0.75	7.004; 2.135	0.690
	2	10	2.5 (1.0, 3.5)	0.40	3.557; 2.8970	0.481
E	1	10	8.25 (6.0, 10.0)	0.73	7.609; 8.733	0.710
	2	10	8.0 (3.5, 8.5)	0.44	4.009; 7.442	0.522

APPENDIX 3

SAS codes

```
*============================================================*
* Programmer: James Nguyen, USEPA                            *
*                                                            *
* Project: Mosquito Repellency Studies                       *
*                                                            *
* Purpose: Power Analysis/sample size calculation            *
*                                                            *
* Description:                                               *
*     - distributions: Weibull, Normal, Lognormal, Uniform   *
*     - create histograms of the distributions               *
*     - SAS Procedures: PROC LIFETEST and PROC ICLIFETEST    *
*                                                            *
* Review Date: 4/10/2017                                        *
*============================================================*;
options formdlim="=" ps=90 ls=90 nonumber nodate;

libname MOS "C:\Users\JNguyen\Desktop\MOS";

%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 Histogram(MED=, P5MRS=, dist=, 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);


        Data Parameters;
                MED = &MED;
                %do i = 1 %to &N;
                        P5MR = &&P5MR&i;
                        P5 = MED*P5MR;
                        output;
```

```
		%end;
		label MED = "P50";
	run;

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

	data simmer;
		call streaminit(&seed);
		set parameters;
		do i = 1 to 50000;
			%generate;
			output;
		end; *i;
		drop i a b;
	run;

	title "&dist distribution: median=&MED";
	Proc SGPANEL data = Simmer;
		panelby P5MR/rows=&N;
		Histogram CPT/binwidth=%sysevalf(2.5*&MED/50);
		refline P5 /axis=x lineattrs=(pattern=1 thickness=1 color=blue);
		refline MED/axis=x lineattrs=(pattern=1 thickness=1 color=blue);
		colaxis values = (0 to %sysevalf(2.5*&MED) by 1);
	run;
	Proc datasets nolist; save sasmacr; run;quit;
%Mend;title;

%Histogram(MED=2,  P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=4,  P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=6,  P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=8,  P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);
%Histogram(MED=10, P5MRS=0.2 0.3 0.4 0.5 0.6, dist=weibull, seed=279420);



%Macro Histogram1(MED=, P5MR=, seed=);

	Data Parameters;
		MED = &MED;
		P5MR = &P5MR;
		P5 = MED*P5MR;

		do i = 1 to 4;
			if i = 1 then Distribution = "Lognormal";
			if i = 2 then Distribution = "Normal";
			if i = 3 then Distribution = "Uniform";
			if i = 4 then Distribution = "Weibull";
			%distParam;
			output;
		end;
		label MED = "P50" P5MR="5%-tile/median"; drop i;
	run;

	data simmer;
		call streaminit(&seed);
		set parameters;
		do i = 1 to 50000;
			%generate;
			output;
		end; *i;
		drop i a b;
	run;

	title "median=&MED    P5MR=&P5MR" ;
	Proc SGPANEL data = Simmer;
		panelby Distribution/rows=4;
```

```
                Histogram CPT/binwidth=%sysevalf(2.5*&MED/50);
                refline P5 /axis=x lineattrs=(pattern=1 thickness=1 color=blue);
                refline MED/axis=x lineattrs=(pattern=1 thickness=1 color=blue);
                colaxis values = (0 to %sysevalf(2.5*&MED) by 1);
        run;
        Proc datasets nolist; save sasmacr; run;quit;
%Mend;title;

%Histogram1(MED=2,  P5MR=0.2, seed=279420);
%Histogram1(MED=2,  P5MR=0.4, seed=279420);
%Histogram1(MED=2,  P5MR=0.5, seed=279420);
%Histogram1(MED=2,  P5MR=0.6, seed=279420);
%Histogram1(MED=2,  P5MR=0.7, seed=279420);



%Macro CPT;
        CPT=CPT*60;
        if CPT <= 5 then do;
                LT = 0;                RT = 0;   CPT= 0;             censor = 0;
        end;
        else if CPT >= &maxT*60 then do;
                LT = &maxT*60; RT=.;   CPT=&maxT*60;  censor = 1;
        end;
        else do;
                LT = 30*floor((CPT-5)/30)+5; RT = 30*ceil((CPT-5)/30);      CPT = RT;
        censor = 0;
        end;

        CPT = CPT/60;
        LT = LT/60;
        RT = RT/60;
%Mend;title;


%Macro power;
        ods select none;
        %if &censor=right %then %do;
                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;
        %end;
        %if &censor=interval %then %do;
                ods output quartiles=MPT;
                Proc iclifetest data = simmer(keep=MED P5MR N Sim LT RT) method=turnbull
impute(seed=1234);
                        by MED P5MR N Sim;
                        time (LT, RT);
                run;
        %end;
        ods select default;

        Proc datasets nolist; delete simmer; run;quit;

        Data MPT;
                set MPT;
                if percent = 50;
                power = (LowerLimit >= &K*Estimate);
                %if &censor=right %then %do; Censor = "right";%end;
                %if &censor=interval %then %do; Censor = "interval"; %end;
        run;

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

```
%Mend;title;

%Macro Mosquito(med=, P5MRS=, nmin=,nmax=,maxT=,K=,dist=,censor=,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 simmer MPT &dist&MED; quit;

	%end;

	Data MOS.&dist._&censor._MED&MED._K%sysevalf(100*&K);
		set All_&dist&MED;
	run;

	Proc datasets nolist; save sasmacr; run;quit;

%Mend;


dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= weibull, censor=right, NSim=4000, seed=561);
```

```
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= weibull, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= weibull, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= weibull, censor=right, NSim=4000, seed=561);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= weibull, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= weibull, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= weibull, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= weibull, censor=right, NSim=4000, seed=352);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= weibull, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= weibull, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= weibull, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= weibull, censor=right, NSim=4000, seed=352);


dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Lognormal, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Lognormal, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Lognormal, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Lognormal, censor=right, NSim=4000, seed=561);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Lognormal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Lognormal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Lognormal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Lognormal, censor=right, NSim=4000, seed=352);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Lognormal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Lognormal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Lognormal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Lognormal, censor=right, NSim=4000, seed=352);


dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Normal, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Normal, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Normal, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Normal, censor=right, NSim=4000, seed=561);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Normal, censor=right, NSim=4000, seed=352);
```

```
dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Normal, censor=right, NSim=4000, seed=352);


dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.6, dist= Uniform, censor=right, NSim=4000, seed=561);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Uniform, censor=right, NSim=4000, seed=352);

dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.8, dist= Uniform, censor=right, NSim=4000, seed=352);


/*
dm log 'clear';%Mosquito(med=2, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=4, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=6, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
dm log 'clear';%Mosquito(med=8, P5MRS=0.2 0.4 0.5 0.6 0.7 0.8, nmin=10, nmax=20, maxT=10,
K = 0.7, dist= Weibull, censor=interval, NSim=4000, seed=352);
*/






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


libname MOS "C:\Users\JNguyen\Desktop\MOS";
%let folder=C:\Users\JNguyen\Desktop\MOS;

%Macro SGPLOT(distribution=, K=);
        title "&distribution median = 2 hours, K = 0.&K";
        Proc SGPLOT data = MOS.&distribution._right_med2_k&k.0;
                scatter x = N y = Power/group = P5MR;
                series x = N y = Power/group = P5MR;
                refline 0.8 0.9/axis=y;
                yaxis min=0 max=1;
        run;
```

```
        title "&distribution median = 4 hours, K = 0.&K";
        Proc SGPLOT data = MOS.&distribution._right_med4_k&k.0;
                scatter x = N y = Power/group = P5MR;
                series x = N y = Power/group = P5MR;
                refline 0.8 0.9/axis=y;
                yaxis min=0 max=1;
        run;
        title "&distribution median = 6 hours, K = 0.&K";
        Proc SGPLOT data = MOS.&distribution._right_med6_k&k.0;
                scatter x = N y = Power/group = P5MR;
                series x = N y = Power/group = P5MR;
                refline 0.8 0.9/axis=y;
                yaxis min=0 max=1;
        run;
        title "&distribution median = 8 hours, K = 0.&K";
        Proc SGPLOT data = MOS.&distribution._right_med8_k&k.0;
                scatter x = N y = Power/group = P5MR;
                series x = N y = Power/group = P5MR;
                refline 0.8 0.9/axis=y;
                yaxis min=0 max=1;
        run;
%Mend;

%Macro print(distribution=, K=);
        data &distribution._K&K;
                set MOS.&distribution._right_med2_k&k.0
                        MOS.&distribution._right_med4_k&k.0
                        MOS.&distribution._right_med6_k&k.0
                        MOS.&distribution._right_med8_k&k.0;
        run;
        Proc transpose data = &distribution._K&K out = &distribution._K&K(drop=_NAME_);
                by MED P5MR;
                ID N;
                var Power;
        run;
        title "&distribution K=0.&K.0";
        Proc print data = &distribution._K&K noobs label; format _: 6.3; run;
%mend;



%SGPLOT(distribution=Weibull, K=6);
%SGPLOT(distribution=Weibull, K=7);
%SGPLOT(distribution=Weibull, K=8);

%SGPLOT(distribution=Lognormal, K=6);
%SGPLOT(distribution=Lognormal, K=7);
%SGPLOT(distribution=Lognormal, K=8);

%SGPLOT(distribution=Normal, K=6);
%SGPLOT(distribution=Normal, K=7);
%SGPLOT(distribution=Normal, K=8);

%SGPLOT(distribution=Uniform, K=6);
%SGPLOT(distribution=Uniform, K=7);
%SGPLOT(distribution=Uniform, K=8);



ods rtf file = "&folder\&dist Median=&MED K=&K..rtf" bodytitle;
%print(distribution=Weibull, K=6);
%print(distribution=Weibull, K=7);
%print(distribution=Weibull, K=8);

%print(distribution=Lognormal, K=6);
%print(distribution=Lognormal, K=7);
%print(distribution=Lognormal, K=8);

%print(distribution=Normal, K=6);
%print(distribution=Normal, K=7);
%print(distribution=Normal, K=8);
```

```
%print(distribution=Uniform, K=6);
%print(distribution=Uniform, K=7);
%print(distribution=Uniform, K=8);
ods rtf close;
```

APPENDIX 4

Table 4-1. Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Lognormal distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.045	0.223	0.126	0.344	0.239	0.257	0.355	0.362	0.440	0.467	0.353
	0.4	0.236	0.580	0.476	0.776	0.666	0.678	0.815	0.812	0.900	0.903	0.869
	0.5	0.449	0.820	0.770	0.937	0.902	0.899	0.958	0.962	0.982	0.985	0.984
	0.6	0.768	0.969	0.955	0.979	0.988	0.980	0.980	0.991	0.963	0.981	0.988
	0.7	0.964	0.971	0.985	0.933	0.961	0.931	0.926	0.948	0.886	0.915	0.940
	0.8	0.894	0.867	0.914	0.801	0.851	0.761	0.797	0.839	0.743	0.792	0.827
4	0.2	0.048	0.169	0.103	0.259	0.188	0.162	0.295	0.254	0.381	0.343	0.279
	0.4	0.176	0.472	0.411	0.682	0.595	0.578	0.749	0.735	0.851	0.844	0.796
	0.5	0.367	0.729	0.662	0.895	0.834	0.828	0.924	0.927	0.973	0.972	0.960
	0.6	0.638	0.932	0.896	0.984	0.977	0.972	0.989	0.992	0.998	0.999	0.997
	0.7	0.919	0.996	0.991	0.999	0.999	0.998	0.997	0.999	0.995	0.998	0.999
	0.8	0.994	0.992	0.997	0.976	0.990	0.981	0.971	0.984	0.949	0.971	0.979
6	0.2	0.175	0.207	0.202	0.283	0.240	0.199	0.343	0.266	0.417	0.355	0.304
	0.4	0.180	0.474	0.400	0.677	0.600	0.561	0.751	0.706	0.845	0.827	0.794
	0.5	0.360	0.703	0.665	0.876	0.826	0.804	0.930	0.916	0.971	0.963	0.956
	0.6	0.635	0.917	0.900	0.982	0.976	0.964	0.992	0.993	0.999	0.998	0.999
	0.7	0.922	0.994	0.993	1.000	0.999	0.999	1.000	1.000	0.999	1.000	1.000
	0.8	0.999	0.999	1.000	0.998	0.999	0.998	0.996	0.999	0.993	0.998	0.999
8	0.2	0.408	0.389	0.438	0.449	0.470	0.371	0.535	0.418	0.594	0.501	0.487
	0.4	0.378	0.567	0.551	0.739	0.697	0.635	0.813	0.766	0.886	0.864	0.842
	0.5	0.469	0.742	0.731	0.898	0.868	0.831	0.942	0.924	0.979	0.967	0.963
	0.6	0.680	0.923	0.919	0.987	0.983	0.966	0.994	0.992	0.998	0.999	0.998
	0.7	0.929	0.994	0.994	1.000	1.000	0.999	1.000	1.000	1.000	1.000	1.000
	0.8	0.999	1.000	1.000	1.000	1.000	0.999	1.000	1.000	0.999	1.000	1.000

Table 4-2: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Lognormal distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.008	0.048	0.034	0.110	0.070	0.063	0.117	0.106	0.172	0.159	0.106
	0.4	0.072	0.285	0.197	0.465	0.355	0.390	0.489	0.530	0.608	0.619	0.519
	0.5	0.197	0.529	0.419	0.697	0.591	0.641	0.699	0.750	0.783	0.811	0.720
	0.6	0.440	0.749	0.648	0.856	0.775	0.816	0.844	0.893	0.877	0.907	0.874
	0.7	0.677	0.878	0.831	0.896	0.895	0.870	0.886	0.933	0.855	0.909	0.926
	0.8	0.800	0.866	0.905	0.796	0.849	0.744	0.795	0.843	0.737	0.786	0.837
4	0.2	0.024	0.056	0.037	0.110	0.064	0.058	0.107	0.093	0.160	0.139	0.090
	0.4	0.057	0.227	0.173	0.411	0.312	0.297	0.462	0.423	0.593	0.541	0.469
	0.5	0.157	0.430	0.368	0.627	0.561	0.521	0.714	0.669	0.819	0.765	0.745
	0.6	0.353	0.666	0.643	0.838	0.827	0.769	0.909	0.890	0.958	0.928	0.935
	0.7	0.692	0.894	0.906	0.966	0.970	0.945	0.988	0.983	0.992	0.993	0.996
	0.8	0.954	0.986	0.993	0.977	0.991	0.979	0.970	0.985	0.945	0.965	0.982
6	0.2	0.161	0.128	0.142	0.153	0.149	0.099	0.165	0.118	0.200	0.163	0.121
	0.4	0.068	0.210	0.157	0.368	0.279	0.258	0.402	0.361	0.542	0.487	0.403
	0.5	0.127	0.379	0.300	0.591	0.487	0.466	0.646	0.614	0.775	0.727	0.669
	0.6	0.284	0.623	0.559	0.809	0.773	0.735	0.880	0.867	0.941	0.912	0.914
	0.7	0.608	0.879	0.873	0.964	0.959	0.944	0.988	0.981	0.997	0.995	0.997
	0.8	0.941	0.993	0.996	0.996	0.999	0.998	0.998	0.999	0.995	0.997	0.999
8	0.2	0.394	0.311	0.381	0.331	0.382	0.274	0.390	0.296	0.407	0.320	0.333
	0.4	0.273	0.317	0.326	0.461	0.415	0.331	0.525	0.432	0.628	0.540	0.500
	0.5	0.260	0.439	0.406	0.621	0.577	0.504	0.712	0.650	0.824	0.771	0.731
	0.6	0.362	0.663	0.617	0.844	0.822	0.775	0.904	0.891	0.959	0.941	0.933
	0.7	0.653	0.905	0.898	0.979	0.975	0.972	0.993	0.992	0.999	0.999	0.998
	0.8	0.959	0.999	0.998	0.999	1.000	1.000	1.000	1.000	0.999	1.000	1.000

Table 4-3 Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Lognormal distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.005	0.022	0.020	0.043	0.031	0.019	0.050	0.032	0.074	0.044	0.033
	0.4	0.021	0.067	0.062	0.144	0.117	0.087	0.178	0.134	0.252	0.193	0.164
	0.5	0.043	0.142	0.125	0.253	0.212	0.157	0.288	0.246	0.378	0.334	0.283
	0.6	0.094	0.253	0.219	0.414	0.364	0.301	0.487	0.448	0.571	0.549	0.510
	0.7	0.210	0.491	0.465	0.661	0.649	0.571	0.734	0.750	0.771	0.797	0.809
	0.8	0.534	0.794	0.824	0.780	0.833	0.723	0.792	0.839	0.736	0.785	0.836
4	0.2	0.019	0.022	0.014	0.042	0.025	0.021	0.029	0.028	0.048	0.046	0.019
	0.4	0.014	0.073	0.044	0.146	0.101	0.075	0.155	0.119	0.228	0.173	0.140
	0.5	0.036	0.129	0.109	0.237	0.207	0.151	0.311	0.226	0.412	0.300	0.303
	0.6	0.095	0.253	0.256	0.399	0.404	0.297	0.540	0.409	0.654	0.513	0.532
	0.7	0.258	0.462	0.496	0.649	0.666	0.558	0.782	0.688	0.861	0.770	0.801
	0.8	0.623	0.779	0.834	0.880	0.914	0.845	0.926	0.914	0.923	0.916	0.951
6	0.2	0.157	0.098	0.119	0.088	0.108	0.061	0.095	0.054	0.096	0.066	0.061
	0.4	0.028	0.068	0.046	0.138	0.092	0.073	0.144	0.125	0.203	0.184	0.121
	0.5	0.033	0.141	0.094	0.266	0.187	0.176	0.292	0.257	0.408	0.355	0.285
	0.6	0.078	0.292	0.228	0.469	0.397	0.358	0.541	0.482	0.680	0.606	0.560
	0.7	0.253	0.552	0.513	0.737	0.721	0.655	0.839	0.799	0.917	0.862	0.877
	0.8	0.680	0.882	0.904	0.962	0.971	0.946	0.989	0.982	0.992	0.992	0.996
8	0.2	0.392	0.279	0.366	0.270	0.348	0.244	0.332	0.245	0.315	0.234	0.289
	0.4	0.240	0.194	0.230	0.235	0.245	0.169	0.267	0.193	0.305	0.237	0.214
	0.5	0.173	0.203	0.193	0.301	0.266	0.195	0.337	0.275	0.435	0.368	0.306
	0.6	0.145	0.301	0.251	0.495	0.414	0.369	0.538	0.516	0.680	0.639	0.558
	0.7	0.250	0.588	0.502	0.773	0.714	0.707	0.837	0.841	0.916	0.888	0.876
	0.8	0.660	0.910	0.901	0.978	0.973	0.971	0.993	0.990	0.998	0.998	0.997

Table 4-4: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Normal distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.139	0.454	0.358	0.655	0.556	0.540	0.722	0.701	0.824	0.820	0.770
	0.4	0.326	0.711	0.653	0.877	0.825	0.818	0.916	0.912	0.960	0.962	0.952
	0.5	0.506	0.858	0.833	0.946	0.934	0.922	0.966	0.974	0.967	0.981	0.985
	0.6	0.730	0.951	0.949	0.963	0.978	0.962	0.958	0.979	0.934	0.957	0.969
	0.7	0.925	0.957	0.976	0.908	0.940	0.901	0.900	0.923	0.842	0.882	0.915
	0.8	0.875	0.846	0.899	0.768	0.821	0.723	0.760	0.812	0.695	0.753	0.791
4	0.2	0.098	0.360	0.291	0.569	0.476	0.448	0.646	0.618	0.775	0.757	0.692
	0.4	0.253	0.635	0.562	0.838	0.764	0.749	0.880	0.874	0.950	0.949	0.926
	0.5	0.415	0.800	0.747	0.938	0.901	0.899	0.965	0.960	0.989	0.990	0.982
	0.6	0.638	0.936	0.913	0.991	0.983	0.975	0.992	0.994	0.996	0.998	0.999
	0.7	0.888	0.993	0.987	0.996	0.998	0.995	0.994	0.998	0.988	0.996	0.997
	0.8	0.991	0.988	0.995	0.967	0.983	0.973	0.959	0.980	0.934	0.958	0.970
6	0.2	0.088	0.344	0.272	0.552	0.461	0.426	0.648	0.594	0.777	0.743	0.693
	0.4	0.246	0.607	0.558	0.820	0.761	0.719	0.889	0.864	0.956	0.936	0.932
	0.5	0.408	0.780	0.745	0.930	0.905	0.876	0.966	0.955	0.990	0.986	0.984
	0.6	0.638	0.927	0.918	0.987	0.982	0.973	0.996	0.995	1.000	0.998	1.000
	0.7	0.893	0.993	0.992	1.000	0.999	0.998	1.000	1.000	0.999	1.000	1.000
	0.8	0.997	0.999	0.999	0.996	0.998	0.997	0.994	0.998	0.988	0.996	0.997
8	0.2	0.231	0.395	0.362	0.574	0.504	0.459	0.667	0.605	0.780	0.756	0.705
	0.4	0.303	0.618	0.578	0.821	0.765	0.723	0.891	0.860	0.959	0.941	0.934
	0.5	0.422	0.785	0.759	0.933	0.910	0.879	0.967	0.956	0.991	0.986	0.987
	0.6	0.646	0.927	0.920	0.989	0.984	0.972	0.997	0.994	1.000	0.999	0.999
	0.7	0.896	0.992	0.991	1.000	1.000	0.998	1.000	1.000	1.000	1.000	1.000
	0.8	0.997	1.000	1.000	1.000	1.000	0.999	0.999	1.000	0.998	0.999	1.000

Table 4-5: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Normal distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.044	0.216	0.143	0.390	0.287	0.293	0.429	0.441	0.566	0.559	0.450
	0.4	0.136	0.465	0.347	0.662	0.549	0.592	0.684	0.727	0.790	0.813	0.728
	0.5	0.262	0.627	0.521	0.803	0.710	0.744	0.801	0.857	0.857	0.892	0.840
	0.6	0.460	0.796	0.712	0.891	0.836	0.854	0.877	0.925	0.883	0.920	0.916
	0.7	0.690	0.885	0.860	0.882	0.895	0.855	0.867	0.915	0.822	0.879	0.908
	0.8	0.795	0.843	0.886	0.761	0.819	0.706	0.759	0.811	0.689	0.743	0.801
4	0.2	0.033	0.175	0.130	0.340	0.257	0.216	0.405	0.346	0.535	0.469	0.413
	0.4	0.111	0.369	0.317	0.581	0.516	0.466	0.683	0.622	0.811	0.746	0.709
	0.5	0.214	0.526	0.475	0.742	0.704	0.643	0.833	0.797	0.915	0.871	0.870
	0.6	0.391	0.718	0.697	0.882	0.881	0.821	0.944	0.925	0.976	0.957	0.964
	0.7	0.683	0.905	0.915	0.971	0.979	0.957	0.990	0.988	0.990	0.993	0.996
	0.8	0.940	0.983	0.993	0.966	0.984	0.968	0.961	0.978	0.926	0.957	0.973
6	0.2	0.028	0.153	0.105	0.300	0.215	0.191	0.345	0.301	0.483	0.426	0.347
	0.4	0.085	0.328	0.259	0.550	0.455	0.416	0.619	0.580	0.754	0.708	0.646
	0.5	0.174	0.486	0.416	0.710	0.648	0.600	0.794	0.764	0.886	0.851	0.827
	0.6	0.334	0.681	0.635	0.863	0.837	0.798	0.927	0.914	0.970	0.951	0.952
	0.7	0.607	0.892	0.885	0.971	0.969	0.958	0.991	0.989	0.997	0.996	0.998
	0.8	0.925	0.991	0.995	0.994	0.999	0.996	0.997	0.998	0.992	0.996	0.998
8	0.2	0.185	0.222	0.214	0.351	0.296	0.231	0.416	0.335	0.522	0.448	0.390
	0.4	0.162	0.350	0.308	0.560	0.492	0.428	0.653	0.603	0.784	0.730	0.680
	0.5	0.205	0.494	0.439	0.726	0.678	0.621	0.809	0.785	0.909	0.875	0.851
	0.6	0.358	0.713	0.662	0.890	0.870	0.835	0.945	0.932	0.981	0.971	0.970
	0.7	0.642	0.916	0.902	0.985	0.980	0.980	0.996	0.996	0.999	0.999	0.998
	0.8	0.943	0.998	0.998	0.999	1.000	0.999	1.000	1.000	0.998	0.999	1.000

Table 4-6: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Normal distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.013	0.069	0.049	0.142	0.109	0.081	0.176	0.136	0.250	0.193	0.154
	0.4	0.032	0.142	0.120	0.259	0.214	0.167	0.311	0.259	0.401	0.352	0.305
	0.5	0.067	0.210	0.183	0.355	0.314	0.245	0.426	0.382	0.504	0.469	0.433
	0.6	0.116	0.328	0.291	0.502	0.464	0.387	0.578	0.559	0.661	0.650	0.628
	0.7	0.239	0.537	0.508	0.683	0.685	0.601	0.741	0.776	0.760	0.799	0.824
	0.8	0.512	0.768	0.801	0.745	0.802	0.685	0.756	0.807	0.689	0.741	0.800
4	0.2	0.008	0.051	0.035	0.112	0.084	0.055	0.140	0.098	0.208	0.138	0.123
	0.4	0.029	0.114	0.093	0.224	0.192	0.133	0.304	0.214	0.420	0.293	0.295
	0.5	0.055	0.182	0.169	0.320	0.305	0.221	0.451	0.323	0.572	0.432	0.443
	0.6	0.118	0.289	0.310	0.479	0.484	0.363	0.633	0.503	0.750	0.620	0.632
	0.7	0.271	0.512	0.528	0.695	0.720	0.616	0.827	0.744	0.892	0.824	0.853
	0.8	0.618	0.800	0.844	0.892	0.923	0.854	0.927	0.925	0.911	0.920	0.952
6	0.2	0.005	0.051	0.031	0.115	0.070	0.054	0.129	0.104	0.196	0.155	0.105
	0.4	0.022	0.120	0.088	0.247	0.179	0.159	0.297	0.247	0.410	0.347	0.287
	0.5	0.044	0.206	0.163	0.377	0.297	0.265	0.453	0.389	0.584	0.509	0.457
	0.6	0.111	0.357	0.307	0.558	0.502	0.444	0.664	0.600	0.799	0.721	0.691
	0.7	0.285	0.605	0.573	0.796	0.786	0.716	0.885	0.861	0.949	0.907	0.921
	0.8	0.680	0.898	0.913	0.968	0.977	0.955	0.990	0.986	0.990	0.993	0.996
8	0.2	0.164	0.131	0.143	0.164	0.163	0.101	0.190	0.135	0.245	0.177	0.146
	0.4	0.097	0.152	0.128	0.260	0.204	0.158	0.298	0.248	0.411	0.353	0.283
	0.5	0.082	0.213	0.171	0.389	0.303	0.265	0.440	0.401	0.589	0.536	0.445
	0.6	0.119	0.362	0.290	0.591	0.496	0.465	0.660	0.635	0.789	0.759	0.695
	0.7	0.273	0.638	0.564	0.827	0.773	0.760	0.887	0.891	0.945	0.933	0.918
	0.8	0.658	0.923	0.909	0.983	0.976	0.978	0.995	0.995	0.998	0.998	0.998

Table 4-7: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Uniform distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.059	0.266	0.177	0.434	0.319	0.331	0.471	0.450	0.576	0.586	0.491
	0.4	0.242	0.561	0.468	0.727	0.657	0.664	0.792	0.772	0.876	0.870	0.824
	0.5	0.429	0.788	0.736	0.923	0.880	0.889	0.955	0.949	0.981	0.982	0.974
	0.6	0.987	0.995	0.994	0.995	0.995	0.993	0.990	0.998	0.981	0.990	0.996
	0.7	0.996	0.990	0.994	0.970	0.981	0.968	0.960	0.977	0.931	0.961	0.975
	0.8	0.900	0.904	0.934	0.851	0.886	0.793	0.849	0.875	0.813	0.851	0.883
4	0.2	0.038	0.183	0.126	0.336	0.253	0.226	0.385	0.340	0.499	0.467	0.397
	0.4	0.175	0.446	0.372	0.641	0.577	0.547	0.723	0.675	0.814	0.807	0.743
	0.5	0.357	0.681	0.627	0.851	0.796	0.802	0.906	0.883	0.947	0.948	0.917
	0.6	0.693	0.919	0.876	0.975	0.956	0.960	0.986	0.984	0.996	0.996	0.991
	0.7	1.000	1.000	1.000	1.000	1.000	0.999	0.999	1.000	0.999	0.999	1.000
	0.8	0.999	0.997	1.000	0.996	0.996	0.994	0.990	0.998	0.981	0.990	0.996
6	0.2	0.037	0.161	0.114	0.313	0.228	0.198	0.368	0.312	0.485	0.442	0.384
	0.4	0.159	0.437	0.359	0.626	0.559	0.541	0.722	0.660	0.819	0.784	0.740
	0.5	0.371	0.653	0.620	0.823	0.797	0.766	0.895	0.853	0.946	0.930	0.910
	0.6	0.674	0.899	0.879	0.971	0.960	0.951	0.987	0.985	0.996	0.996	0.992
	0.7	0.970	0.998	0.999	0.999	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	0.999	0.999	1.000	0.999	0.999	1.000
8	0.2	0.291	0.302	0.323	0.423	0.390	0.299	0.499	0.375	0.564	0.491	0.458
	0.4	0.314	0.493	0.463	0.665	0.625	0.561	0.747	0.668	0.834	0.796	0.751
	0.5	0.452	0.687	0.668	0.835	0.813	0.784	0.905	0.872	0.949	0.934	0.918
	0.6	0.744	0.903	0.902	0.968	0.965	0.952	0.991	0.983	0.995	0.995	0.992
	0.7	0.977	0.998	0.997	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	1.000

Table 4-8: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Uniform distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.016	0.079	0.052	0.168	0.117	0.098	0.184	0.186	0.277	0.267	0.197
	0.4	0.073	0.242	0.172	0.417	0.310	0.335	0.445	0.489	0.570	0.601	0.491
	0.5	0.180	0.470	0.371	0.645	0.540	0.584	0.662	0.711	0.725	0.780	0.683
	0.6	0.543	0.731	0.636	0.803	0.711	0.767	0.783	0.839	0.826	0.874	0.808
	0.7	0.624	0.847	0.757	0.890	0.853	0.855	0.885	0.914	0.896	0.931	0.918
	0.8	0.820	0.904	0.914	0.845	0.885	0.803	0.841	0.882	0.813	0.856	0.882
4	0.2	0.014	0.066	0.041	0.145	0.100	0.073	0.162	0.145	0.266	0.220	0.155
	0.4	0.055	0.203	0.148	0.351	0.285	0.251	0.425	0.379	0.557	0.494	0.432
	0.5	0.136	0.365	0.305	0.551	0.499	0.442	0.648	0.594	0.746	0.690	0.655
	0.6	0.354	0.600	0.572	0.750	0.761	0.670	0.854	0.807	0.908	0.872	0.874
	0.7	0.782	0.850	0.876	0.934	0.947	0.896	0.973	0.954	0.989	0.976	0.980
	0.8	1.000	0.999	1.000	0.994	0.995	0.995	0.992	0.995	0.984	0.991	0.996
6	0.2	0.016	0.055	0.035	0.129	0.079	0.062	0.141	0.120	0.224	0.190	0.125
	0.4	0.045	0.180	0.118	0.313	0.244	0.209	0.367	0.334	0.493	0.450	0.371
	0.5	0.116	0.330	0.252	0.496	0.421	0.384	0.572	0.525	0.672	0.638	0.581
	0.6	0.280	0.549	0.493	0.713	0.683	0.616	0.799	0.764	0.875	0.848	0.828
	0.7	0.665	0.839	0.846	0.931	0.933	0.896	0.970	0.956	0.992	0.979	0.982
	0.8	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	0.999	1.000	1.000
8	0.2	0.268	0.210	0.267	0.257	0.268	0.179	0.297	0.215	0.358	0.267	0.244
	0.4	0.203	0.248	0.240	0.367	0.331	0.245	0.434	0.367	0.542	0.467	0.400
	0.5	0.203	0.356	0.318	0.513	0.467	0.394	0.615	0.554	0.713	0.660	0.612
	0.6	0.338	0.576	0.550	0.741	0.722	0.661	0.838	0.806	0.902	0.877	0.854
	0.7	0.693	0.892	0.882	0.959	0.956	0.942	0.982	0.980	0.995	0.990	0.990
	0.8	0.996	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 4-9: Results of power analysis when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Uniform distribution)												
Median (hours)	**P5MR**	**Sample size**										
		_10	**_11**	**_12**	**_13**	**_14**	**_15**	**_16**	**_17**	**_18**	**_19**	**_20**
2	0.2	0.009	0.031	0.023	0.065	0.053	0.031	0.081	0.051	0.120	0.091	0.070
	0.4	0.027	0.070	0.057	0.144	0.113	0.084	0.178	0.128	0.253	0.190	0.162
	0.5	0.047	0.121	0.119	0.227	0.192	0.142	0.275	0.211	0.344	0.278	0.246
	0.6	0.131	0.205	0.200	0.313	0.283	0.221	0.375	0.322	0.476	0.409	0.373
	0.7	0.142	0.364	0.315	0.530	0.483	0.418	0.609	0.595	0.703	0.692	0.651
	0.8	0.641	0.864	0.871	0.837	0.879	0.795	0.841	0.880	0.813	0.856	0.882
4	0.2	0.003	0.017	0.009	0.048	0.024	0.019	0.050	0.038	0.081	0.063	0.033
	0.4	0.013	0.061	0.038	0.121	0.090	0.066	0.138	0.103	0.205	0.160	0.122
	0.5	0.031	0.112	0.080	0.195	0.159	0.114	0.249	0.185	0.342	0.251	0.231
	0.6	0.092	0.198	0.194	0.309	0.325	0.211	0.428	0.317	0.528	0.403	0.428
	0.7	0.303	0.374	0.415	0.523	0.564	0.428	0.673	0.562	0.755	0.654	0.675
	0.8	0.766	0.735	0.806	0.805	0.860	0.769	0.902	0.839	0.916	0.874	0.899
6	0.2	0.006	0.015	0.009	0.041	0.019	0.017	0.042	0.029	0.069	0.054	0.029
	0.4	0.010	0.049	0.028	0.110	0.069	0.050	0.120	0.101	0.189	0.160	0.106
	0.5	0.026	0.101	0.065	0.197	0.142	0.114	0.214	0.201	0.327	0.284	0.219
	0.6	0.074	0.238	0.164	0.368	0.298	0.271	0.421	0.382	0.541	0.480	0.426
	0.7	0.246	0.461	0.422	0.608	0.611	0.521	0.732	0.656	0.808	0.751	0.748
	0.8	0.773	0.824	0.860	0.913	0.939	0.880	0.966	0.940	0.986	0.970	0.978
8	0.2	0.261	0.178	0.247	0.181	0.220	0.141	0.215	0.139	0.214	0.146	0.159
	0.4	0.175	0.129	0.162	0.175	0.176	0.100	0.203	0.141	0.245	0.184	0.143
	0.5	0.134	0.144	0.135	0.214	0.185	0.128	0.253	0.206	0.338	0.282	0.223
	0.6	0.119	0.224	0.182	0.354	0.289	0.254	0.418	0.378	0.529	0.492	0.407
	0.7	0.223	0.485	0.419	0.652	0.594	0.564	0.730	0.701	0.803	0.794	0.748
	0.8	0.679	0.868	0.851	0.939	0.942	0.916	0.976	0.968	0.991	0.984	0.987

Figure 4-1: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Lognormal distributions)

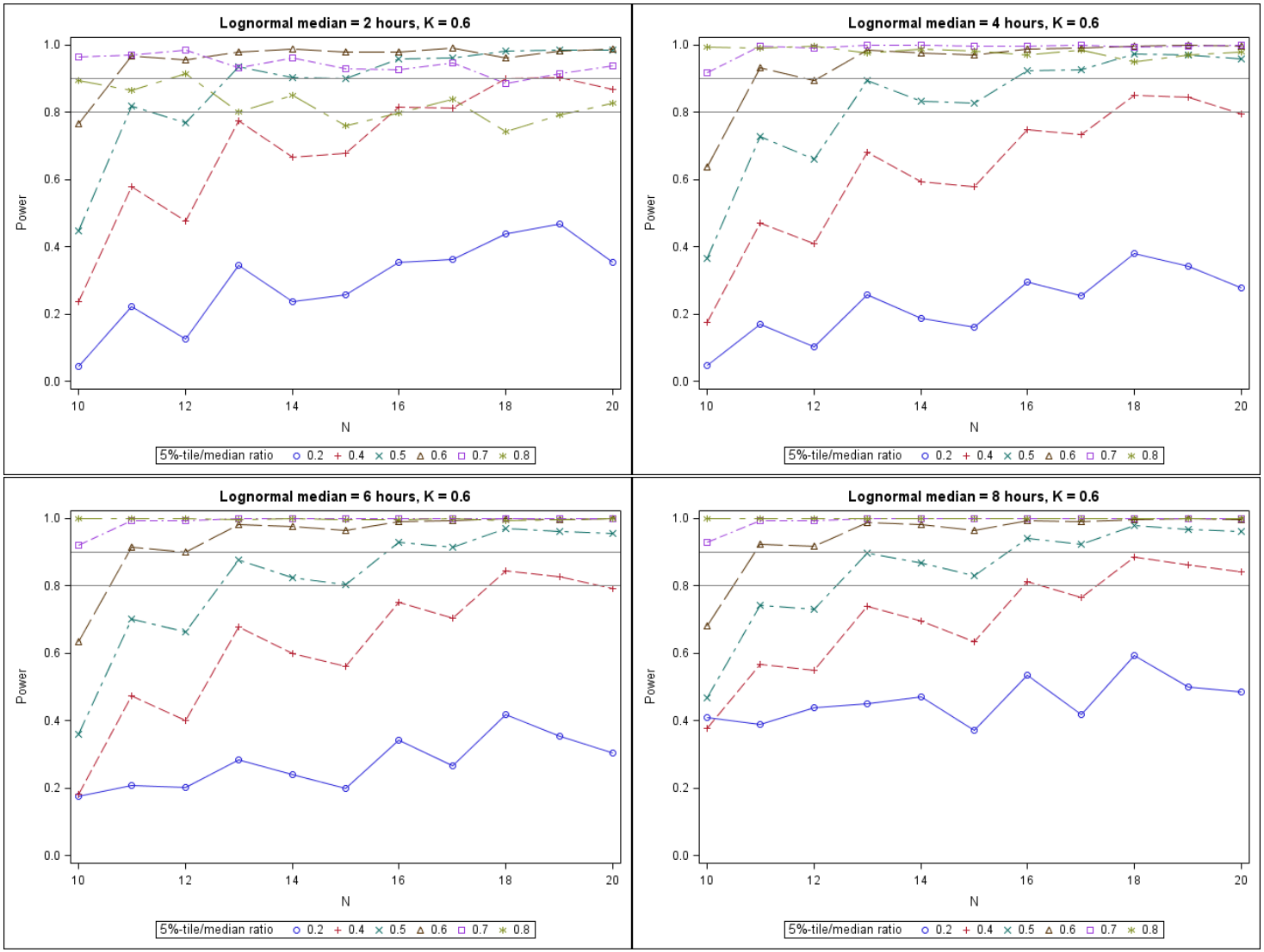


Figure 4-2: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Lognormal distributions)

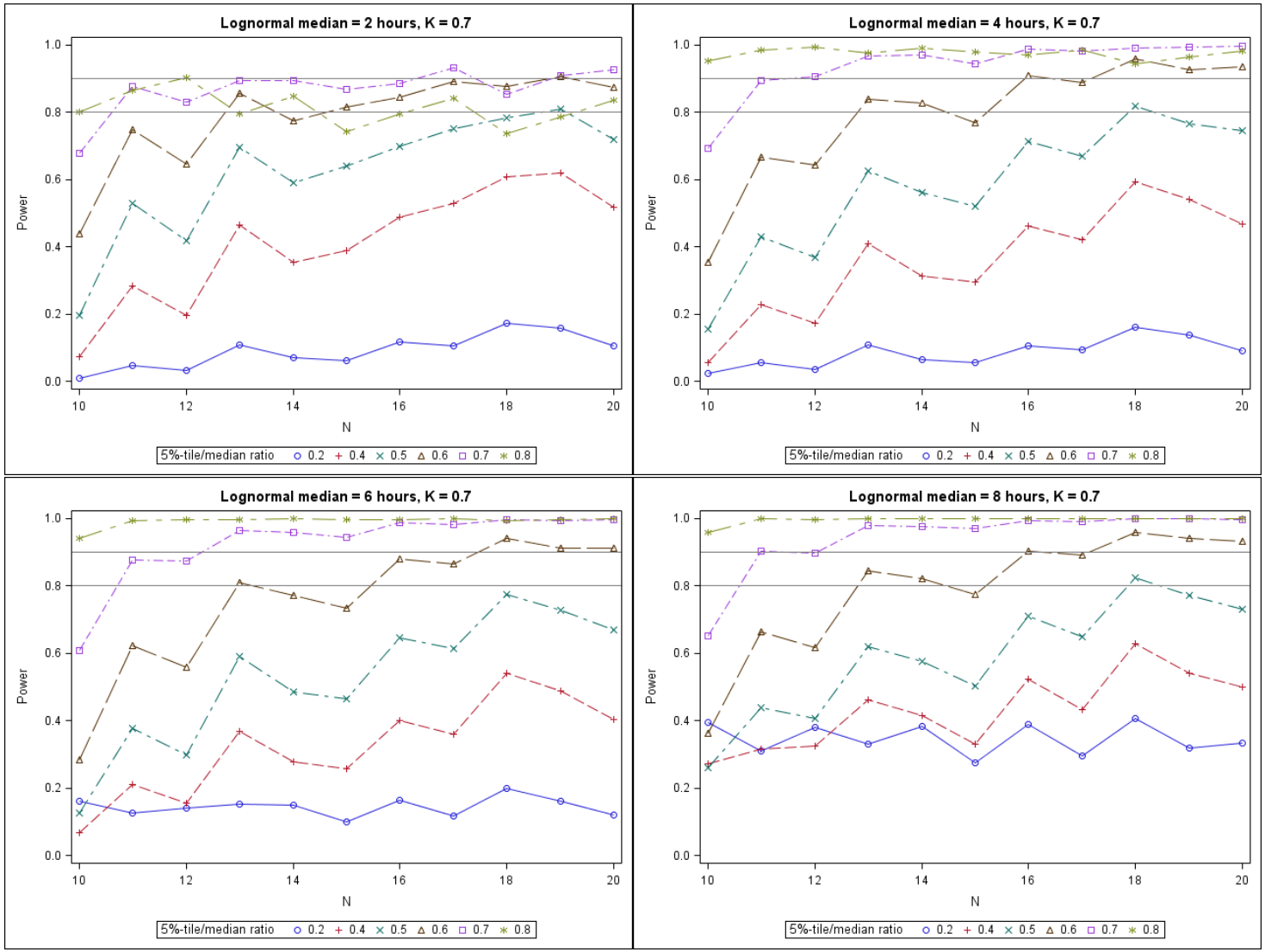


Figure 4-3: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Lognormal distributions)

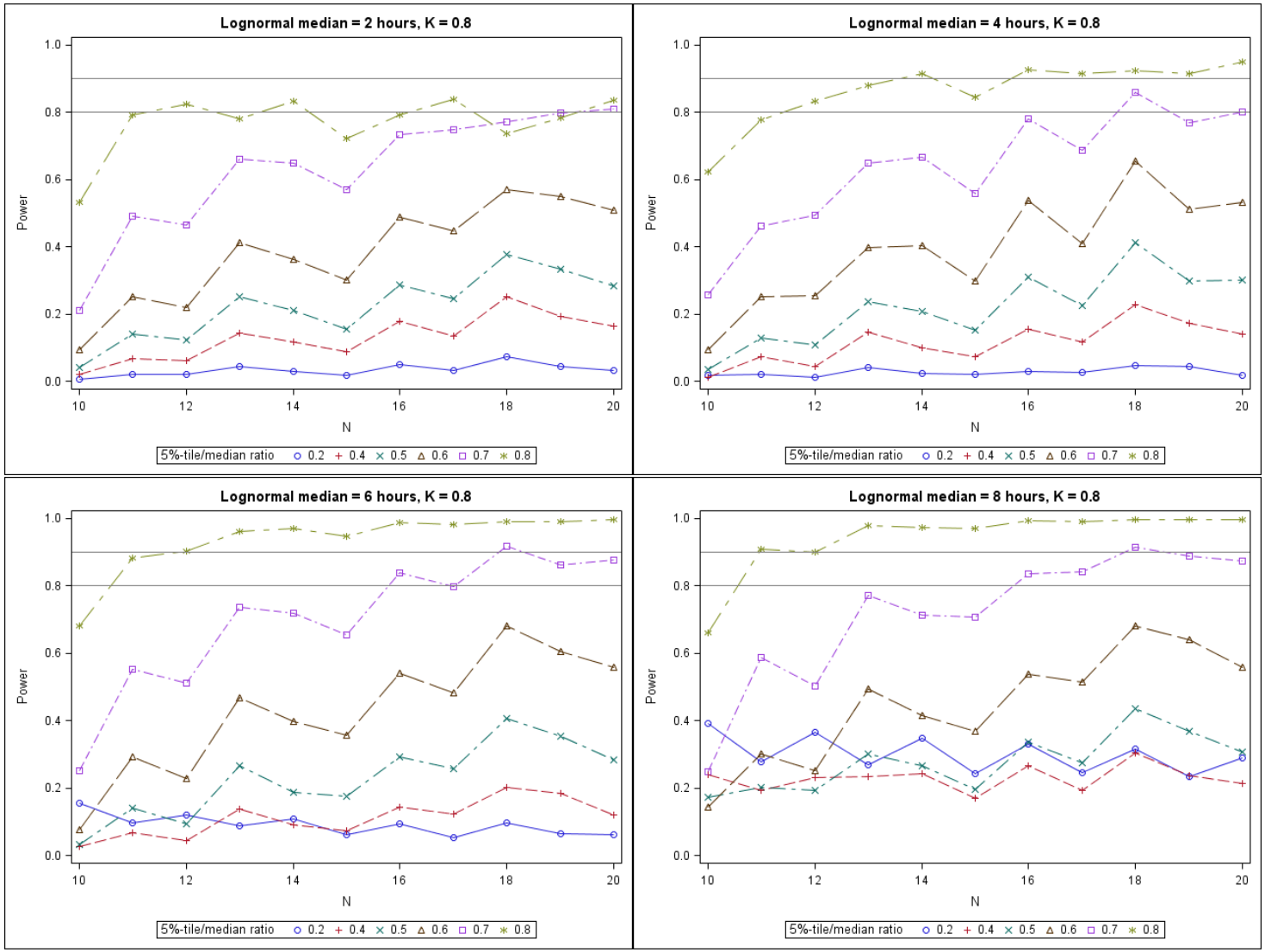


Figure 4-4: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Normal distributions)

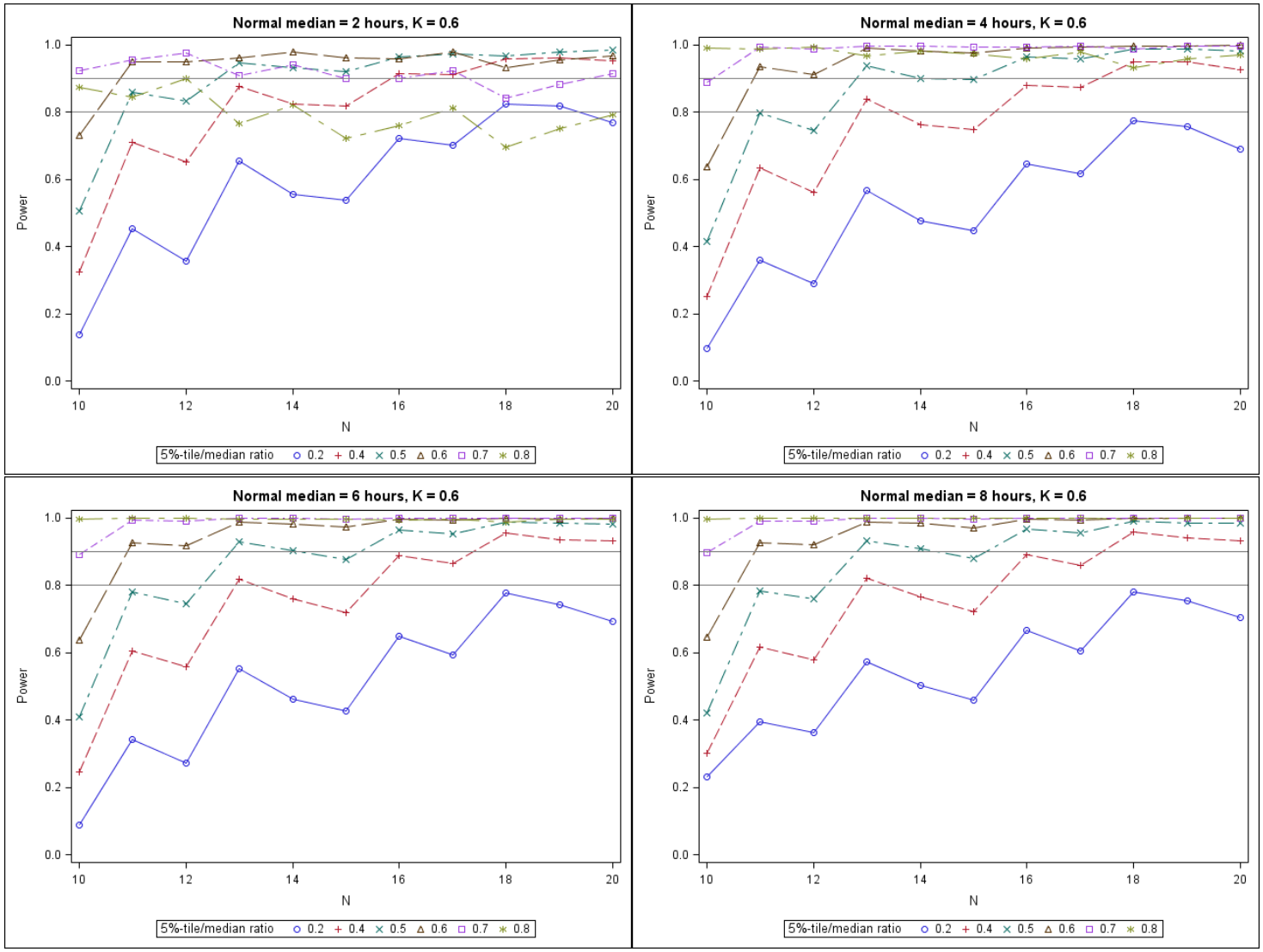


Figure 4-5: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Normal distributions)

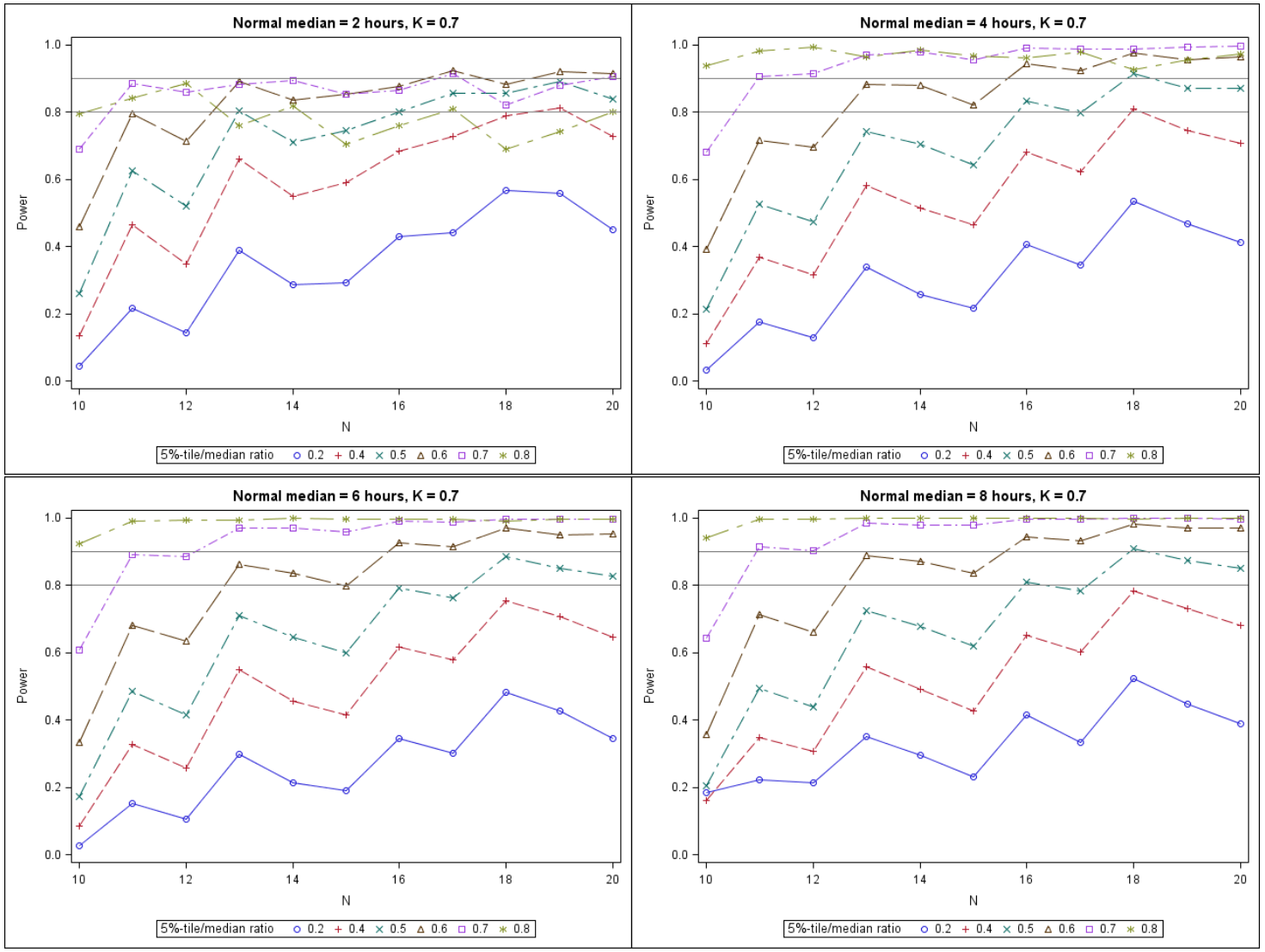


Figure 4-6: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Normal distributions)

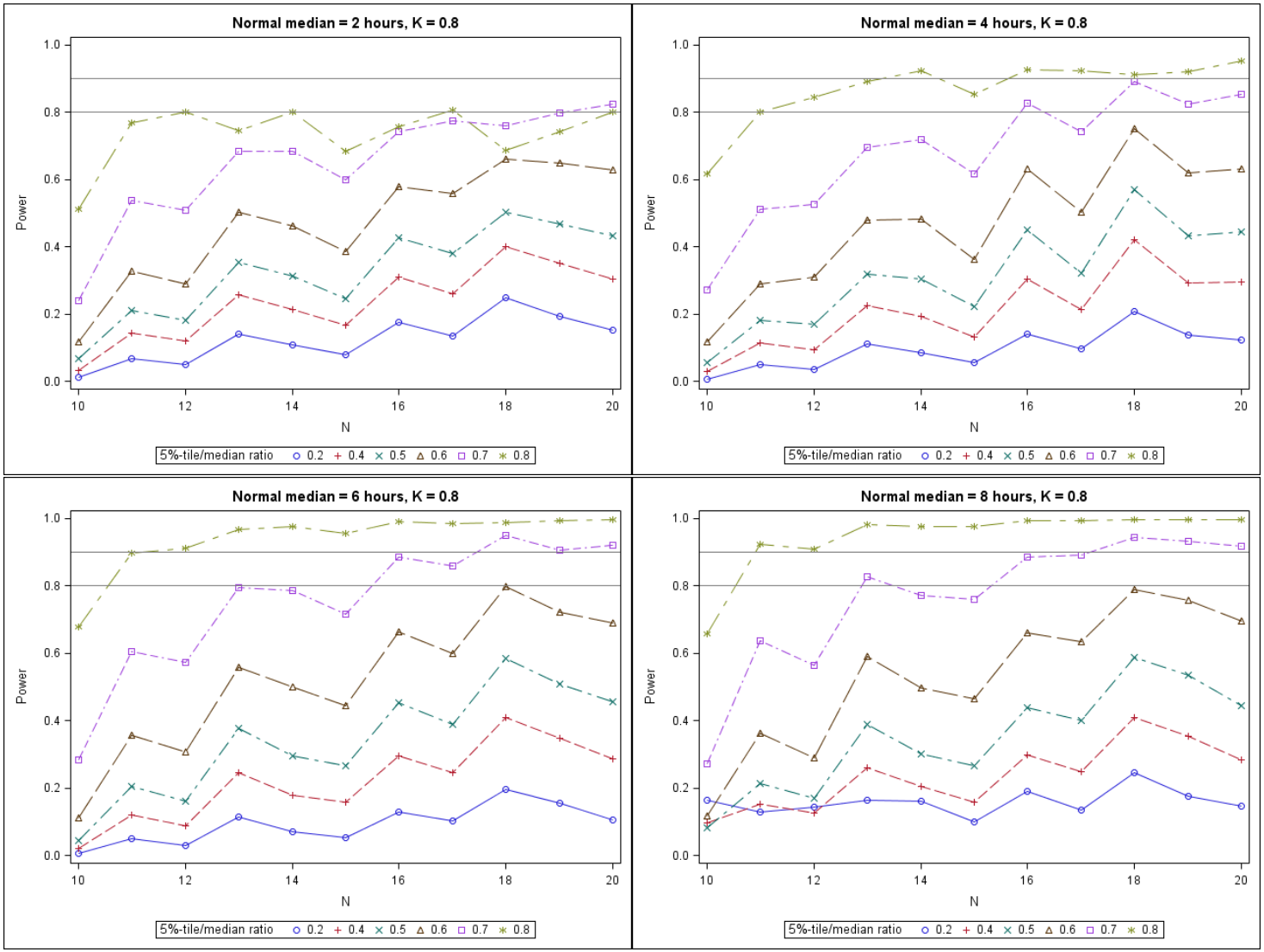
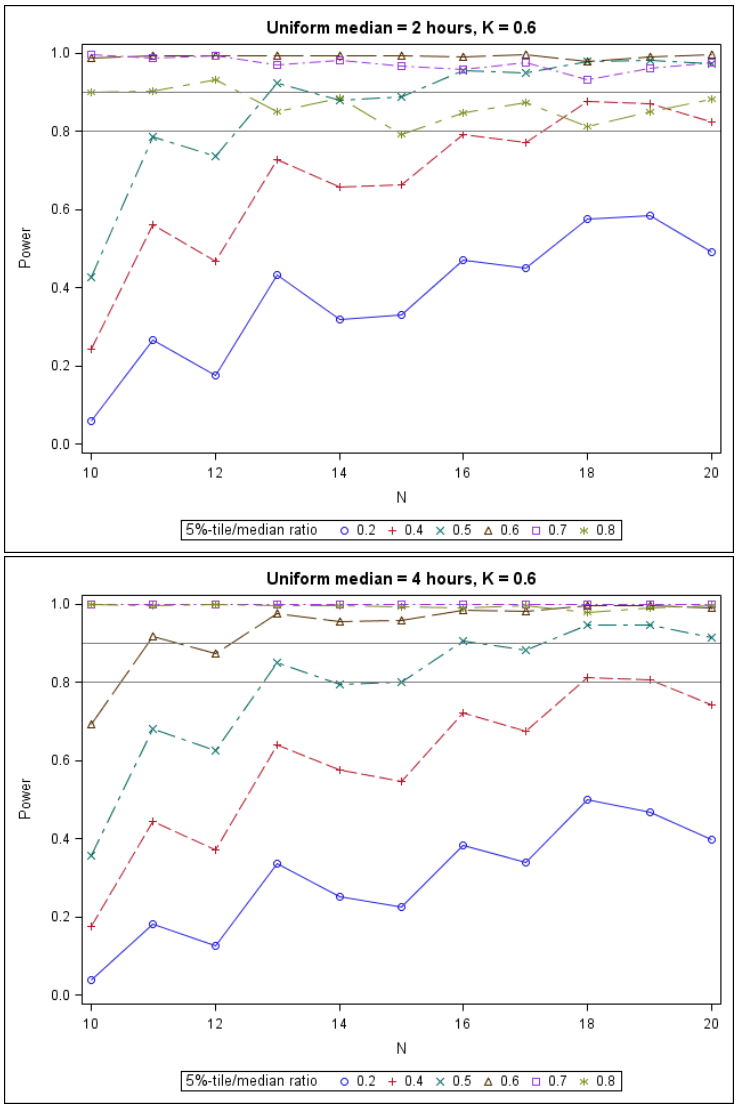


Figure 4-7: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.6 (Uniform distributions)



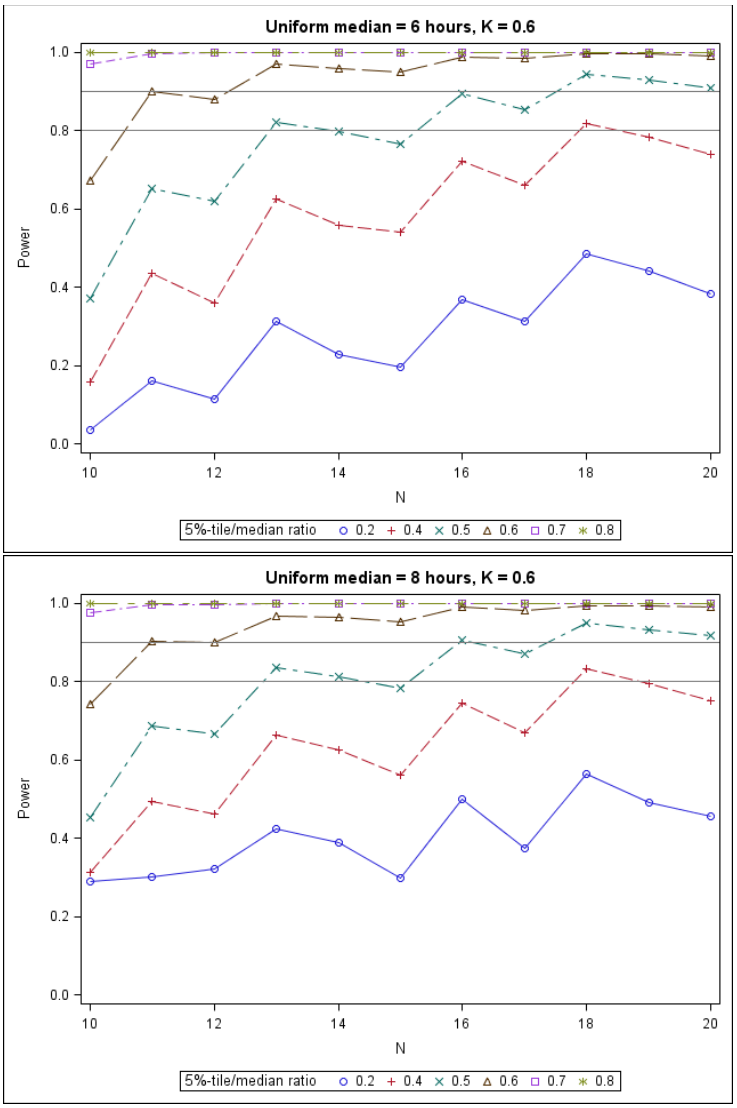
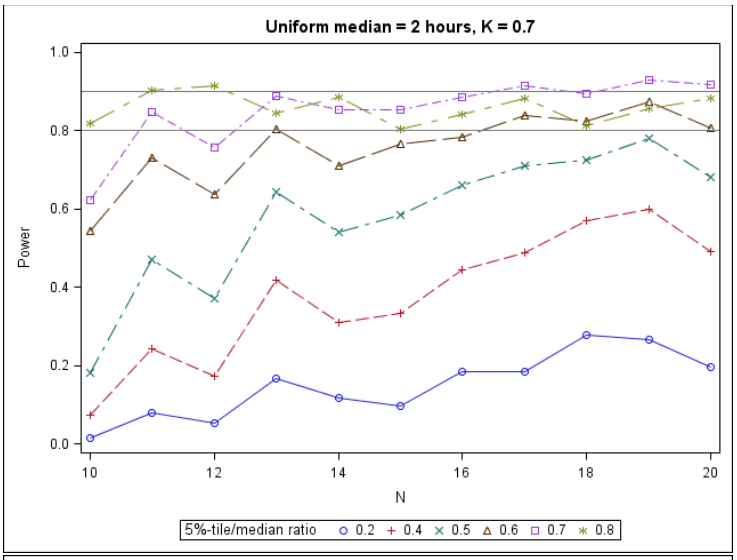
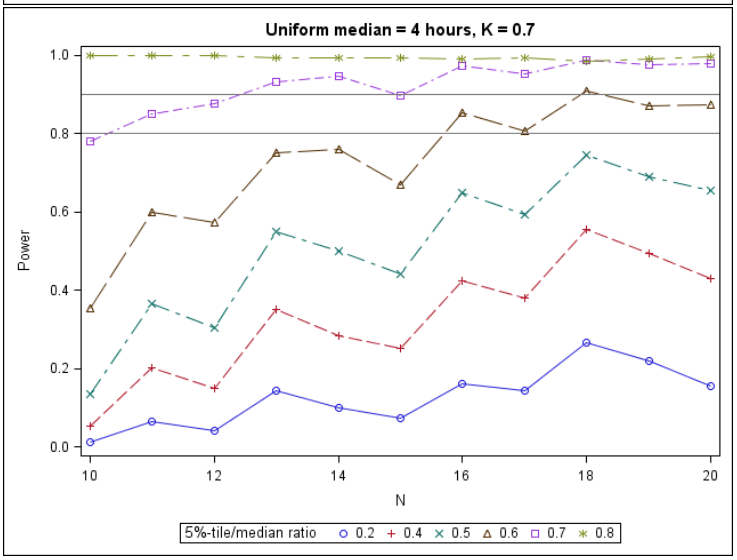


Figure 4-8: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.7 (Uniform distributions)


Uniform median = 2 hours, K = 0.7
Power
1.0
0.8
0.6
0.4
0.2
0.0
10
12
14
16
18
20
N
5%-tile/median ratio 0.2 0.4 0.5 0.6 0.7 0.8


Uniform median = 4 hours, K = 0.7
Power
1.0
0.8
0.6
0.4
0.2
0.0
10
12
14
16
18
20
N
5%-tile/median ratio 0.2 0.4 0.5 0.6 0.7 0.8

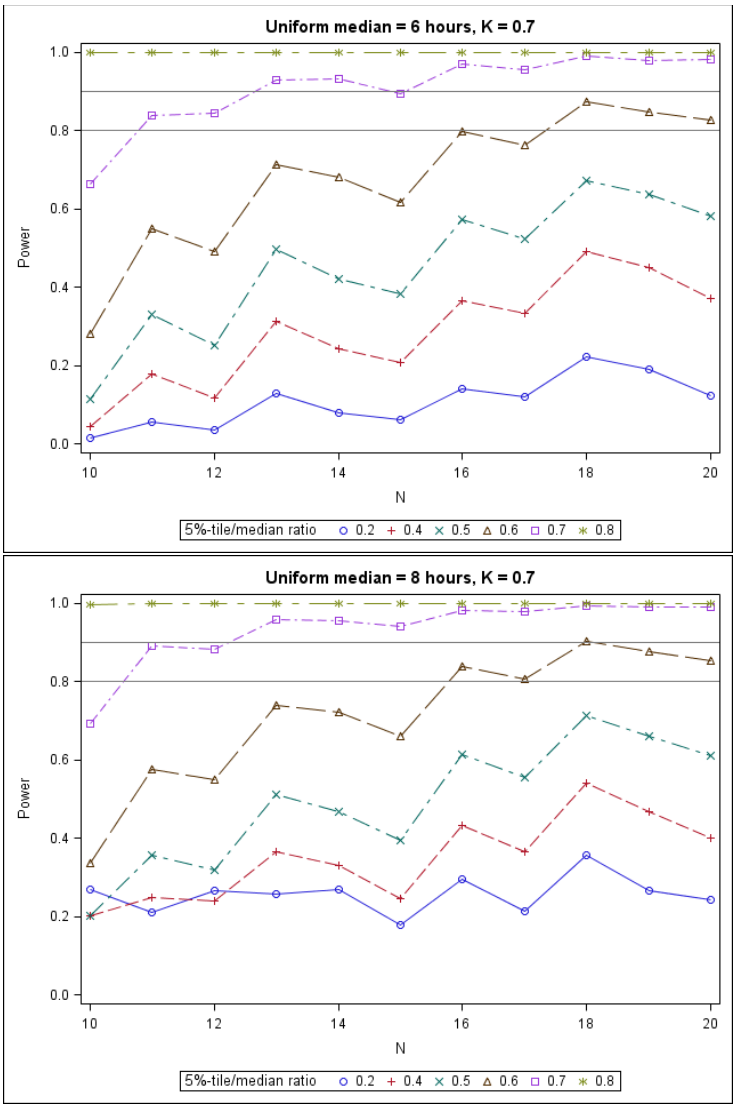
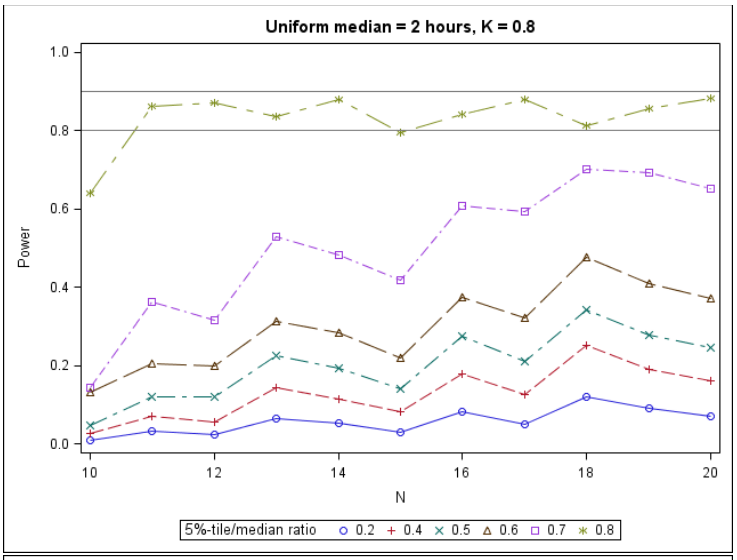
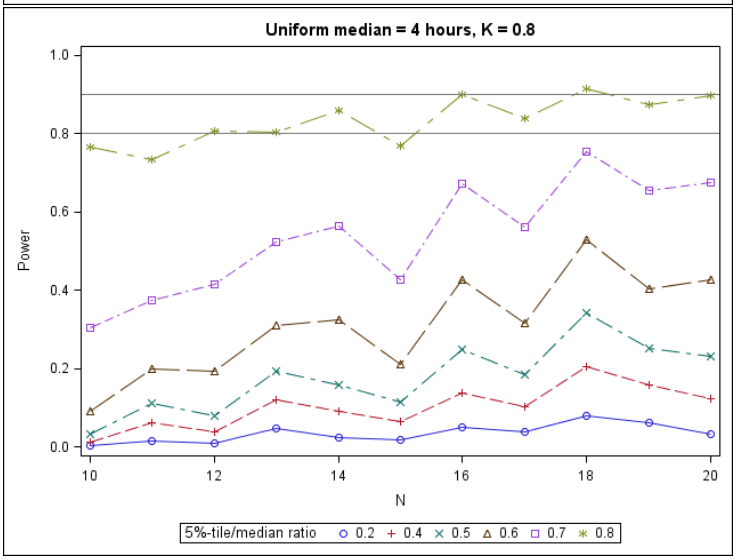
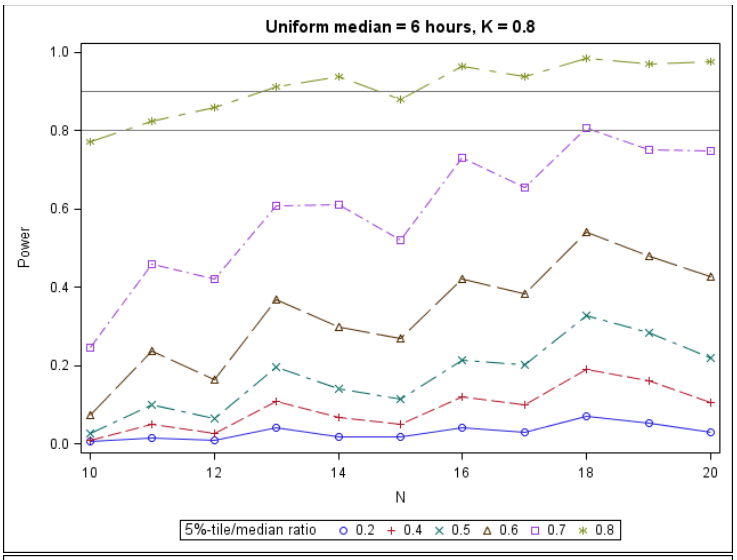
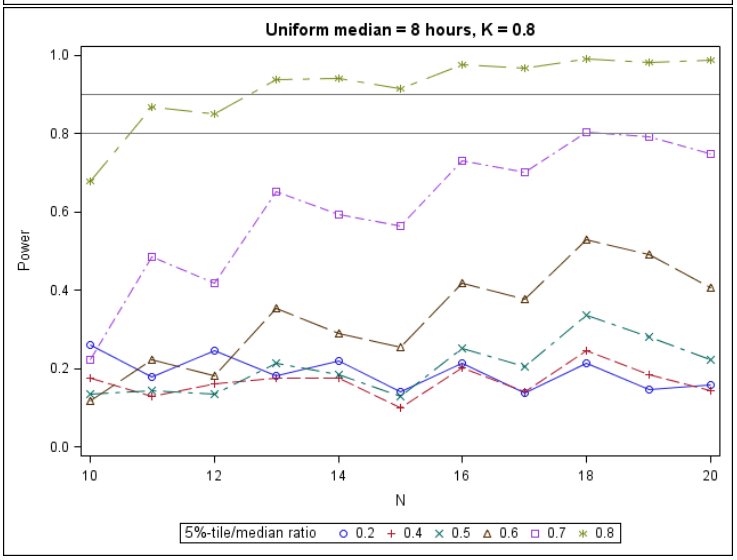


Figure 4-9: Power curves of study design when the lowest acceptable ratio 95% LCL_{mCPT}/mCPT = 0.8 (Uniform distributions)


Uniform median = 2 hours, K = 0.8
Power
1.0
0.8
0.6
0.4
0.2
0.0
10
12
14
16
18
20
N
5%-tile/median ratio 0.2 0.4 0.5 0.6 0.7 0.8


Uniform median = 4 hours, K = 0.8
Power
1.0
0.8
0.6
0.4
0.2
0.0
10
12
14
16
18
20
N
5%-tile/median ratio 0.2 0.4 0.5 0.6 0.7 0.8


Uniform median = 6 hours, K = 0.8
Power
1.0
0.8
0.6
0.4
0.2
0.0
10
12
14
16
18
20
N
5%-tile/median ratio 0.2 0.4 0.5 0.6 0.7 0.8


Uniform median = 8 hours, K = 0.8
Power
1.0
0.8
0.6
0.4
0.2
0.0
10
12
14
16
18
20
N
5%-tile/median ratio 0.2 0.4 0.5 0.6 0.7 0.8

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: