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

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

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MEMORANDUM

SUBJECT: Science and Ethics Review of a Protocol for Field Evaluation of Two Topically-Applied Insect Repellent Products Containing IR3535

FROM: Clara Fuentes, Ph.D., Entomologist
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs

Helen Hull-Sanders, Ph.D., Entomologist
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs

Michelle Arling, Human Research Ethics Review Officer
Office of the Director
Office of Pesticide Programs

TO: Linda Hollis, Chief, Biochemical Pesticides Branch
Biopesticide and Pollution Prevention Division
Office of Pesticide Programs

REF: Healy, Kristen. Protocol for "Field evaluation of two topically applied insect repellent products containing IR3535 against mosquitoes in Louisiana." Unpublished document. April 17, 2020. MRID 1052016.

The EPA has reviewed the referenced submission for field testing for two topically-applied repellent products (AKIVA 20 lotion and wipe) containing IR3535 against mosquitoes in Louisiana from both scientific and ethics perspectives. This study is sponsored by LivFul, Inc. and will be conducted by the London School of Hygiene and Tropical Medicine's ARCTEC and Louisiana State University (LSU). This review assesses the scientific aspects of the proposed research for a product performance study to evaluate the efficacy of skin-applied insect repellent products in terms of the recommendations of the EPA Guideline *Insect Repellents to be Applied to Human Skin*[1] and the EPA Human Studies Review Board (HSRB). Ethical aspects of the proposed

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

research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L and the EPA HSRB. A full point-by-point review of the protocol is included as

Field testing of these products has been conducted previously at two test sites.[2] For this study, the proposed site will be selected from locations in Louisiana to replace data from a previous study at one Florida site where data were insufficient to support protection time for more than three hours due to low landing pressure from predominantly *Culex* species. Product registration requires data from two distinct sites. Data resulting from this study will be combined with existing data that support a complete protection time (CPT) of 14 hours for the lotion and of 13 hours for the wipe.[3]

The submission included two versions of a protocol, one dated 17 April 2020 and one dated 6 May 2020. This review is of the 17 April 2020 version of the protocol.

A. Summary Assessment of Scientific Aspects of the Proposed Research

Objectives

The objective of the proposed study is to determine the efficacy and duration of protection of two skin-applied insect repellent products at preventing mosquito landings on human hosts. (Protocol, p. 5, §2)

Efficacy Endpoints and Definitions

The repellency endpoint is the first confirmed landing (FCL), used to measure residual repellency or CPT, which is the time period between application of the repellent product and the occurrence of the FCL signaling repellency failure. The FCL is defined as a single landing followed by a second landing within 30 minutes of the first recorded landing. Thus, an FCL can occur within the same exposure period as the first landing, or during the exposure period immediately following the exposure period in which the first landing occurred. Each exposure period lasts five minutes; exposure periods occur at 30 minute intervals. (Protocol, p. 12, §3.1; and p. 12, §7.2)

Study Design

This is a single-center, one-site field study. A subject may enroll to test the lotion, wipe, or both products. Under the proposed study design, testing each product requires a subject to participate in a single test day. A subject who enrolls to test both products may be involved for up to two days. The proposed field test is designed to characterize the residual efficacy of each product, AKIVA 20 Lotion and AKIVA 20 Wipes, applied at a standard rate of 1 g/600 cm^2 on a sample of 13 treated subjects. Residual efficacy will be measured for each subject from time of product

[2] EPA. Fuentes and Bohnenblust. Science Review of Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR335 Against Mosquitoes in Florida. October 3, 2019. Presented to the HSRB on October 23, 2019. https://www.epa.gov/sites/production/files/2019-10/documents/1a._science_review_of_ir3535_for_hsrb_10-3-19.pdf

[3] *Id.*

application to the time point at which FCL occurs, signaling repellency failure. Two untreated controls will monitor mosquito landing activity throughout the test. Site selection is based on the presence of mosquito species within the genera *Aedes, Anopheles* and *Culex*, and absence of mosquito-borne diseases. (Protocol, pp. 22-23, §6.6) Test subjects will be selected from a pool of 40 informed and consenting volunteers who qualify for repellency testing based on their attractiveness to mosquitoes and their ability to use aspirators (Protocol, pp. 21-22) and other eligibility criteria. (Protocol, p. 16-17)

The study plan includes pre-test activities to be conducted in the laboratory, and repellency testing to be conducted in the field. Pre-test activities will take two days to complete; the first day visit will consist of obtaining participants' consent. (Protocol, p. 15-16, §4.3) Following consent, subjects will have a second visit with the study staff to confirm attractiveness to mosquitoes, to learn how to use an aspirator, and to have the surface area of their lower legs measured. (Protocol, p. 21-22, §6.2-§6.4) Subjects will be screened in the laboratory for their attractiveness to mosquitoes, using 78 pathogen-free *Aedes aegypti* mosquitoes from pathogen-free laboratory colonies. The pathogen-free status of the mosquitoes will be confirmed by removing a subset from the colony to test for vector-borne illnesses. The attractiveness test will be conducted as arm-in-cage study, using cages of 45 x 45 x 45 cm capacity, equivalent to a mosquito density of one mosquito per 1,160 cm^3 as recommended in the EPA's guidelines for testing insect repellents applied to human skin.[4] (Protocol, p. 22, §6.2) Subjects not receiving five landings n one minute during screening for attractiveness will be allowed to repeat the test up to three times using fresh mosquitoes. If after three attempts a subject does not receive five landings in one minute, the subject will be deemed unattractive to mosquitoes and disqualified from continuing testing. (Protocol, p. 22, §6.2) Subjects who show attractiveness to mosquitoes "*will be trained in a screened free-flight cage to identify mosquito landing behavior and to use aspirators to collect landing insects before they have time to probe or bite. … Training will continue until the participants are able to skillfully use the aspirator to remove a mosquito before it attempts to probe. If after one hour of training the participant is still unable to complete this activity they will not be permitted to continue with the study*." (Protocol, p. 21-22, §6.3)

Repellency testing for each product will be conducted in the field over the course a single day. Research staff will apply the test substance to subjects at the lab, then the subjects will be transported to the field testing location. Subjects will test the efficacy of each product in the field starting from two hours post application until up to 16 hours post application. Treated subjects will work in pairs, and control subjects will be paired with a member of the research team. Pairs will be situated at locations referred in the study protocol as "collection stations." Pairs of treated subjects will be randomly assigned to eight collection stations, which will be separated from each other by at least 3 m (10 ft.). Throughout the test, control subjects will monitor ambient landing pressure by exposing their untreated leg for up to five minutes prior to each exposure period. Adequate landing pressure is defined as five landings within five minutes or less on both control subjects. (Protocol, p. 25, §6.7) Immediately following the control subject landing pressure assessment, treated subjects will expose their treated limbs for five minutes. Each treated subject will undergo five minute exposures at 30 minute intervals, beginning at the onset of data collection (2 hours post-application)

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

until FCL, signaling the time point of repellent failure and period of complete protection for that subject, or end of study period is reached without FCL, whichever occurs first.

Sample Size and Number of Subjects

The proposed sample size for treated subjects testing repellency is 13 subjects. "*EPA statisticians used a Weibull distribution to simulate the sample size required to estimate the median CPT to a given degree of precision. A power analysis of these outputs resulted in a sample size of 13 participants being chosen. This number allows for over 90% power at a range of expected median CPTs from 2-8 hours (Appendix 2: Sample Size Calculation)*." (Protocol, p. 26, §7.1) Two untreated control subjects will monitor landing pressure throughout the test for a total of 20 individuals involved in test days. Five additional subjects will be enrolled as alternates and required to be present at the start of the test day, ready to replace any subjects who withdraw or are ineligible. Assuming no subject participates in more than one test, a total of 40 subjects could be necessary. (Protocol, p. 26, §7.1)

It is stated in the study protocol that for maintaining "*gender balance among the volunteers, at least 6 volunteers of each sex will be recruited to test each product.*" (Protocol, p. 26, §7.1)

Randomization

The randomization procedure is described in §6.7 of the protocol as follows: *"The participants will be randomized to either control or repellent treatment on the day of testing. The leg that collections will be made from will also be randomised.[sic] A randomisation [sic] schedule will be made for male and female participants for each test (see example in Table 1) and participants will be assigned on order of arrival to the test center. As only 13 participants are required for each treatment the final participant on the randomisation [sic] schedule will not be assigned.*" (Protocol, p. 23) Table 1 on page 23 presents a randomization plan for assigning male and female subjects as either control or treated subjects, maintaining a 50:50 male to female ratio in the sample. Table 1 also presents randomization plan for selecting either right or left leg of subject for testing.

Selection of Field Sites

The researcher is proposing to select a test site from among the following locations in Louisiana: Cameron Parish, Plaquemines Parish, and Grande Isle (Jefferson Parish) to replace data from Florida. The protocol proposes to rely on existing trapping data from Cameron Parish, Plaquemines Parish and Grande Isle Mosquito Control districts for site selection. The site selection will be based on the abundance of salt marsh mosquitoes (i.e., *Aedes taeniorhynchus* and *Aedes sollicitans*) collected at those locations, which are active biters throughout the day. (Protocol, p. 23)

The protocol establishes site suitability based on sufficient numbers of mosquitoes that bite throughout the day (rather than on presence of all target mosquito species that bite during the day) and on absence of vector-borne pathogens. These data will be derived from trapping data from aforementioned mosquito control districts. "*The field site will be selected based on*

preliminary trapping data that demonstrates suitability for the study." (Protocol, p. 23, §6.6) The protocol notes that trapping should confirm the presence of three genera of mosquitoes (*Aedes, Anopheles, Culex*). (Protocol, p. 23)

Rate of Application

The products, AKIVA 20 lotion and AKIVA 20 wipe, will be applied at the EPA standard dose of 1 g/600 cm^2 for testing repellency. (Protocol, p. 19, §5.3) See Attachment 3 for information on the basis for the EPA's recommended standard rate of application.

Estimation of Skin Surface Area

Lower legs will be used for repellency testing. Lower legs surface area will be calculated as the length of lower leg (from ankle to knee) multiplied by its average circumference. The average circumference of each subject's lower leg will be calculated from four points measured around the subject's ankle, knee and two equally spaced points in between. Both of each subject's legs will be measured separately. Both of each subject's legs will be measured during the attractiveness testing and aspirator training visit. (Protocol, p. 22, §6.4)

Test Substance Application

Prior to application, lower legs will be cleansed using unscented soap, rinsed with 70% alcohol solution and dried. (Protocol, p. 24, §6.7) Individual doses will be adjusted to the surface area of each subject's right or left leg and evenly applied on the skin using a single gloved finger. The amount of lotion to be applied will be measured directly by weight. To measure the amount to be applied for testing the efficacy of the wipe, the wipe will be wrung out over a beaker, the liquid will be weighed, and the calculated amount will be applied. (Protocol, p. 24, §6.7)

Application will take place at the test facility approximately 2 hours in advance of the field test initiation. (Protocol, p. 25, §6.7)

Risk Characterization and Margin of Exposure (MOE)

During efficacy testing, subjects will be exposed to two different formulations of repellent products containing IR3535, an EPA-registered pesticide. Subjects will be exposed to mosquitoes from laboratory-reared colonies during assessment of subjects' attraction to mosquitoes and aspirator use training, and to wild mosquito species encountered in the field during efficacy testing.

According to the EPA's risk assessment based on data submitted to the EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classified as category III for acute dermal toxicity (LD_{50} > 3000 mg/kg in rats), category IV for acute oral (LD_{50} > 5000 mg/kg in rats) and inhalation toxicity, and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats (based in a 90-day dermal toxicity study), and for oral toxicity is 600 mg/kg/day in rabbits. Based on the NOAEL of ≥ 3000 mg/kg/day for dermal exposure to IR3535, the Agency has determined that there is no dermal risk of exposure. Although the information in the protocol does not follow the EPA's risk assessment procedures, the

conclusions are the same; the amount of IR3535 applied in these studies does not exceed a level of toxicological concern to test subjects.

Pathogen-free mosquitoes will be used for the attraction test and aspirator use training. In the field, exposure to mosquitoes will be limited to five minute periods and subjects will be instructed to aspirate mosquitoes before they probe or bite.

Environmental Monitoring

Environmental conditions such as temperature, relative humidity, wind speed, light intensity, general cloudiness, and precipitation will be monitored at the start and end of test day. Temperature and wind speed will be monitored every half an hour throughout the test. (Protocol, p. 26, §6.8)

Data Collection

Mosquitoes landing on test and control subjects' exposed lower legs will be collected and saved for taxonomical identification and pathogen detection. Mosquitoes collected will be labelled with the participant number, treatment status, and date and time of collection. (Protocol, p. 24-25, §6.7)

Stopping Rules

Under the protocol, the test will be stopped under the following circumstances or conditions:

- Safety reasons
- More than four non-consecutive exposure periods or more than three consecutive exposure periods are missed due to bad weather, rain or wind speed > 10 mph
- Landing pressure is below acceptable levels for more than four non-consecutive exposure periods or more than three consecutive exposure periods
- End of field test period (maximum duration reached or sufficient number of subjects experience FCL)
- At the Study Director's discretion for any reason

The protocol gives the study director discretion to stop the test early if deemed in the best interests of the subjects, the study data, or both. Data from incomplete test days will be recorded but not used for statistical analysis without justification. If a study day is stopped, the rationale must be recorded and the study sponsor must be informed within 24 hours. (Protocol, p. 25, §6.7)

Missing Periods, Periods of Low Landing Pressure and Determination of CPT

If a first landing occurs during an exposure period preceding or followed by a missed period or a period with inadequate landing pressure, then the CPT will be determined by the first landing. If a confirmed landing occurs following a missed period or period of low landing pressure, then the CPT will be determined as occurring at the preceding missed period or period of low landing pressure. "*If a single landing on a test subject during an exposure period is followed by a missed*

exposure period (either through inadequate landing pressure or bad weather) then the first landing will be treated as a confirmed landing. If a confirmed landing occurs during an exposure preceded by a missed exposure period (either through low biting pressure or bad weather) then CPT will be recorded as the earliest time point in that preceding delay." (Protocol, p. 25, §6.7)

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 at the discretion of the Study Director where continued participation may jeopardize the safety of the participant or the integrity of the study. (Protocol, p. 17, §4.4)

Statistical Analysis

The objective of the data analysis will be to estimate the mCPT with 95% CI. "*The times to treatment failure will be analyzed using Kaplan-Meier Survival functions, and from these the median Complete Protection Time and 95% confidence intervals will be calculated*." (Protocol, p. 26, §7.2) The proposed statistical method (Kaplan-Meier Survival Analysis) to analyze the CPT data is appropriate.

Good Laboratory Practice (GLP) Compliance and Quality Assurance

The study will adhere to GLP as defined by 40 CFR part 160 and to the principles outlined in the International Conference on Harmonization Good Clinical Practice. The protocol designates Dr. Kristen Healy as the person performing QA duties, (Protocol, p. 3) and Compliance Services International as the Quality Assurance provider. (Protocol, p. 2) The QA representative will conduct critical phase inspections to ensure study integrity and maintain written and signed records of each inspection. "*All inspection findings will be reported to management and the Study Director. Any problems, amendments or deviations discovered shall be brought to the attention of the sponsor, Study Director and Test Facility Management immediately. The QA representative will review the final reports for accuracy and compliance with GLPs and the protocol. A signed QA statement will be included in the final report that lists the phase inspections that were conducted, their dates, and the dates the findings were reported to management and the Study Director. Auditing activities may be sub-contracted to LSU EHS, in which case copies of the inspection reports will be provided to Kristen Healy in addition to the Study Director and Test Facility Management*." (Protocol, p. 3)

Testing Facility LSU, Department of Entomology, 404 Life Sciences Building, Baton Rouge, LA

Study Site Locations: Repellency testing will be conducted at a potential field site located in Louisiana 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. Kristen Healy

Study Sponsor: Livful Inc. 2972 Webb Bridge Road, Alpharetta, GA 30009 USA

B. Compliance with Applicable Scientific Standards

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

- Experimental design
- Data analysis
- Risk minimization

C. Science Comments

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

1. Objective: The EPA recommends referencing the EPA guidelines[5] in the Objective of the proposed study. The EPA recommends adding testing the product against natural populations of mosquito species of public health importance within the genera *Aedes, Anopheles*, and *Culex* to the objective and purpose of the study. The EPA recommends adding that the purpose of this study is to replace data from one site previously tested in Florida with data from a new site with adequate landing pressure from target mosquito species of public health relevance. (Protocol, p. 11)

2. Biting vs. landing: The EPA recommends changing all references to bites to landings. For example, the Study Synopsis should be revised as follows: "*Subjects will have repellent applied to one lower limb at a standardised [sic] dose rate to account for skin area. They will then expose this area only in a field site where mosquitoes are recorded ~~biting~~* ***landing*** *at a rate of 5 mosquitoes per 5 minute or higher.*" (Protocol, p. 5) Similar revisions must be made throughout the document.

3. Randomization: Provide more detail about how the randomization for assigning test subjects or alternates will occur during each test day.

4. Field site monitoring: A more detailed plan for site monitoring should be described. Field sites should be monitored to ensure that WNV, Zika, dengue, and chikungunya viruses and encephalitis have not been detected for at least one month prior to test initiation. A proposed plan for site monitoring should include description of proposed method for virus detection, the number of traps per site, trapping period, trap collection frequency, and PCR screening of trapped mosquitoes. In addition, the protocol should describe the proposed methodology for species identification and reporting their distribution at the site. The Study Director should communicate at least weekly during the trapping with the local public health service. No vector-borne disease should be detected within 25 miles of the proposed site in the

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

month preceding test initiation. Specify how the Study Director will coordinate with local health departments and mosquito control districts to confirm the absence of reported mosquito-borne disease cases in humans within 25 miles of planned test site a week before each test day is conducted.

5. Field site selection: While it is acceptable to have particular species or genera of mosquitoes be predominant, the EPA stresses the importance of selecting a field test site where representative species of mosquitoes of public health relevance within all three genera (*Culex*, *Anopheles*, and *Aedes*) are present. Trapping data prior to testing should show presence of mosquito species of public health importance within each of these three genera.

6. Rate of application and test substance administration: The EPA recommends converting individual doses to volume using specific gravity of the test substance and describing the method for dispensing dose onto subjects' legs. Indicate how the dose will be dispensed on subjects' legs. For example, will it be dispensed from tuberculin (1 ml) syringes or spatula or directly from beaker? Also specify whether a single technician or multiple technicians will apply the dose. In addition, the time of application should be recorded for each subject in the data collection sheets.

7. Determination of CPT, landing pressure and skipping exposure periods: As recommended by the EPA, test subject exposure periods should not be skipped due to low landing pressure. The test should be stopped due to low landing pressure if more than four non-consecutive exposure periods or more than three consecutive exposure periods take place under low landing pressure. The EPA recommends revising the statement as follows: "*If the biting pressure is insufficient test subjects will ~~not~~ carry out their exposures in that time period unless the total number of exposure periods with low landing pressure exceeds three consecutive periods or four non-consecutive periods with inadequate landings on controls, and test should be stopped*." (Protocol, p. 24, §6.7) This statement should be corrected according to the criteria for skipping exposure periods and stopping the test as recommended by the EPA.

   Additionally, the protocol should be amended as follows to clarify that no periods will be missed due to low landing pressure: "*If a confirmed landing occurs during an exposure preceded by a ~~missed exposure period (either through low landing pressure or bad weather)~~ period of low landings or by a missed exposure period due to bad weather then CPT will be recorded as the earliest time point in that preceding ~~delay~~ period*." (Protocol, p. 25, §6.7)

8. Delayed exposures and determination of CPT: EPA recommends that when exposures are delayed, the delay period should be established to ensure that a minimum of three consecutive exposure periods occur before subjects experience an FCL. If the protocol retains the delay between product application and exposure and an FCL occurs during within the first three exposure periods the CPT will be counted as 0 hours. The Study Director may not replace a subject who experiences an FCL at two hours post application or within the first three exposure periods.

9. Data collection: The protocol should include a sample of the data collection forms for treated and control subjects. Data collection forms should include the following for control subjects: time point of landings occurring on control subjects, number of landings occurring per control subject, and time point when threshold of five landings within five minutes is reached per control subject. The forms should record the following for treated subjects: time of product application, time period between time of application and first field exposure, time points of landings occurring on treated subjects, time period from first field exposure to first landing and to FCL, or end of test. In addition, time period between time of application and time to first landing and to FCL should be recorded.

10. Subjects withdrawal and replacement: The protocol should provide explicit criteria for replacing withdrawn subjects and for statistical treatment of their data. EPA recommends that a withdrawn subject whose data are right censored should not be replaced.

11. Kaplan-Meier survival curves: The proposed statistical method (Kaplan-Meier Survival Analysis) to analyze the CPT data is appropriate. However, the protocol should indicate that the 95% CI of the estimated mCPT is calculated with the log-log transformation applied to survival function and Kaplan-Meier survival curves will be presented in the report.

12. Power analysis & statistical design: The proposed sample size of 13 subjects is appropriate (to achieve at least 90% power that the ratio of 95% LCL mCPT/mCPT ≥ 0.6, given that the P5MR (i.e., $CPT_{5th\ percentile}$/mCPT) is assumed to be equal or greater than 0.5. The EPA recommends that the protocol specify the assumption about the variation of CPT data distribution, which is characterized by the value of P5MR, associated with the selected sample size and the result of power. P5MR is an important factor. The P5MR value characterizes the spread of the CPT data distribution. For two products with same mCPT, the product with lower value of P5MR has a wider range of CPT data.

D. Completeness of Protocol Submission

The submitted protocol package and supplementary documentation of review by the LSU Institutional Review Board (IRB) were reviewed for completeness against the required elements listed in 40 CFR §26.1125. The EPA's checklist is appended to this review (see Attachment 2). With EPA's recommendations addressed, the submission will be complete.

E. 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 two topically-applied mosquito repellents (AKIVA 20 lotion and wipe) containing IR3535. As intended, the data resulting from this proposed study will be used to support registration of certain products containing IR3535. 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. These data will be combined and analyzed to determine a median CPT (mCPT), which will be used to develop product labeling. 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. For each product tested, subjects will be randomly assigned to serve as a test subject or untreated control based on their order of arrival on the test day. 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.

   Subjects will be recruited from Baton Rouge, Louisiana and the surrounding area, via advertising posted on bulletin boards and in newspapers, digital advertising, and social media. The advertisement will provide brief information about the study and contact information. The results of testing IR3535 products 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.

   Prior to field testing, subjects will participate in a mosquito attractiveness test and training on how to use an aspirator. To verify a subject's attractiveness to mosquitoes, they will place an arm into a 45 cm x 45 cm x 45 cm cage with 78 mosquitoes. A subject will be deemed sufficiently attractive to mosquitoes and allowed to continue with the testing if they get at least five landings within one minute. A subject who does not get five landings in one minute will be allowed to repeat the same test with fresh mosquitoes two additional times. 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 a screened free-flight cage. Subjects will be dressed as if participating in a test day and wearing a head net and gloves. Study staff will demonstrate how to identify mosquito behavior and then provide subjects with their own aspirators so they may practice aspirating mosquitoes landing on other subjects. Subjects who demonstrate they can use an aspirator effectively will continue in the study. Study staff will place the subjects' aspirators in individual zip lock bags labelled with the participants' identifier numbers.

3. **Risks to Subjects:** The protocol discusses potential hazards associated with these tests including mosquito bites and transmission of vector-borne pathogens; physical risks from being outside in a hot, humid climate; potential reaction to the test substances;

unanticipated loss of confidential information; and psychological risks related to pregnancy testing. The protocol notes that risks will be minimized as follows. To mitigate risks from exposure to mosquitoes and disease vectors, subjects will be trained to aspirate landing mosquitoes before they probe or bite and will wear clothing, gloves and head net to protect untreated areas during the test periods. To minimize the risk of contracting any mosquito-borne diseases during the lab-based mosquito attractiveness test, the cages will be populated with mosquitoes from a colony reared in the laboratory. Mosquitoes from this colony will also be screened for vector-borne illnesses. The field testing sites will be monitored weekly for a month prior to the testing, and all mosquitoes captured during the monitoring phase will be tested for pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified.

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

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

Subjects' privacy and confidentiality will be protected by using identifying numbers, rather than names, on data collection sheets and storing all personally-identifiable information in a secure location. Pregnancy testing will be conducted in private and only a single female member of the research team will discuss the results with the subject.

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

4. **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 IR3535. Registration of effective repellent products could lead to fewer mosquito bites and reduced incidents of vector-borne illnesses.

5. **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.

6. **Independent Ethics Review:** The LSU IRB has reviewed and approved the protocol, informed consent form, and recruitment materials. The IRB is registered with the Office of Human Research Protections (FWA 00003892). 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.

7. **Informed Consent:** During the recruitment period, interested candidates will contact study staff via phone or email to learn more about the study and to self-evaluate whether they meet the eligibility criteria. Those who are interested in continuing with enrollment will be invited to meet with the study staff. Upon arrival at the consent meeting, the candidate's age will be verified. The study staff will provide information about the study orally and to describe the elements of study participation step by step. If the consent meeting occurs in a group setting, each candidate will have the opportunity to meet one-on-one with a member of the study staff to ask questions and to complete the consent form. 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. After all questions are answered, the candidate will answer a series of questions to demonstrate that they comprehend the study procedures. Those who the research determines have an adequate comprehension of the materials and proposed study will be invited to complete the consent process. All individuals will be provided a signed copy of their consent form.

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

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

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

   The protocol notes that subjects will be compensated for their time spent participating in the study as follows: $20 for the participating in the consent meeting, $40 for participating in the attractiveness testing and aspirator training session, and $10 per hour

for taking part in the field test, rounded up to the next hour. Subjects who experience an FCL earlier than others and who study staff cannot return to the test facility at that time will be compensated for their time until they are transported back to the test facility. 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.

F. 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 LSU IRB, the research will likely meet the applicable requirements of 40 CFR part 26, Subparts K and L.

G. Ethics Comments

The study sponsor, ARCTEC, and the researcher met to discuss the EPA's comments on December 14, 2020. The researchers agreed with the EPA's proposed changes. The EPA notified the study team that before the research is conducted the protocol and supporting documents should be revised to address the EPA's comments and recommendations resulting from the review by the HSRB. After all necessary changes have been made, the revised protocol and supporting documents must be resubmitted for review and approval to the overseeing IRB prior to initiating the research.

The EPA's ethics comments are provided below. Minor comments on typographical errors have not been included here. In addition, the EPA has provided ethics-related comments directly on the informed consent document; these are provided to the HSRB as a separate file.

Protocol

1. Testing should extend no longer than 14 hours for the lotion and 13 hours for the wipe, rather than up to 16 hours as proposed. As discussed in the EPA's 2019 review of data generated to support the registration of these products, "Based on the Repellency Awareness Graphic Guidance policy[6] for determining CPT, CPT is estimated for each product at each site. The most conservative CPT at either site rounded down to the

[6] Repellency Awareness Guidance: For Skin-Applied Insect Repellent Producers. https://www.regulations.gov/document?D=EPA-HQ-OPP-2013-0406-0003

nearest integer is selected for product labeling."[7] The EPA concluded that the previously-generated field testing data for the lotion would support a CPT of 14 hours, and for the wipe would support a CPT of 13 hours.[8] Testing the efficacy of these product beyond the CPTs supported by the existing data would be unnecessary to support the product registration. Therefore, testing beyond the limits supported by the existing data would involve unnecessary additional exposure to human subjects and would be unethical to conduct.

2. Revise the protocol 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.

3. Prior to field testing, consistent mosquito landing pressure for the proposed duration of testing should be confirmed in order to avoid engaging subjects in testing and applying repellents unnecessarily. The protocol calls for basing the site selection "on preliminary trapping data that demonstrates suitability for the study." (Protocol, p. 23) Trapping before testing occurs ensures that there are diverse mosquito species and screening for diseases prevents testing where a known vector-borne illness has been identified. Consistent adequate landing pressure allows a test day to proceed to completion, thereby minimizing human exposure to both the test substance and mosquitoes to the greatest extent possible. More specific information needs to be provided about what is meant by "catches should also demonstrate that there could be mosquito host-seeking throughout the collection period." (Protocol, p. 24)

4. Include a more robust discussion of field monitoring prior to testing. (Protocol, pp. 23-24)
   a. In addition to weekly mosquito sampling, the protocol should specify that the Study Director will communicate regularly with the local health departments before and during testing to be aware of any identified vector-borne illnesses. Provide more detail about how this coordination will occur in the protocol.
   b. The EPA and the HSRB recommend that field testing occur in areas with active vector-borne illness monitoring programs.[9]

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[7] EPA. Fuentes and Bohnenblust. Science Review of Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR335 Against Mosquitoes in Florida. October 3, 2019. Presented to the HSRB on October 23, 2019. https://www.epa.gov/sites/production/files/2019-10/documents/1a._science_review_of_ir3535_for_hsrb_10-3-19.pdf, p. 2.

[8] *Id.* p. 13.

[9] Dawson, Liza. April 26, 2017 EPA Human Studies Review Board Meeting Report. June 12, 2017. https://www.epa.gov/sites/production/files/2017-06/documents/hsrb_final_report_april_2017_meeting.pdf

c. Include a statement similar to the following: "*To minimize risks to subjects, field testing will not be conducted where any mosquito-vectored diseases have been detected within the previous four weeks. The site(s) will also not be known Zika virus transmission areas.*"

5. Amend protocol to note the total number of subjects that will be recruited for participation to ensure that a representative sample (age, gender, race/ethnicity) can be selected for the field testing on each day. The protocol will call for 20 subjects per day (15 test/control subjects, 5 alternates). Consider how many additional subjects should be recruited and screened to ensure that there is a sufficiently large pool to choose from. In previously reviewed protocols, researchers screened a pool of participants to determine eligibility that was double the expected number of participants for the study. In this case, that could mean screening up to 80 subjects [(13 test subjects + 2 control subjects + 5 alternates) * 2 test days *2 = 80].

6. Under Section 3.2, revise the protocol to specify that the study will be adhere to "all applicable regulatory requirements, including but not limited to the EPA's Human Studies regulation at 40 CFR 26, Subparts K-L."

7. Provide more detail about the preparation for and timing of the study day. Include details such as whether subjects will be reminded of the restrictions on substances used for washing for the 24 hours before the study (and if so, how and when), how far in advance of the field testing and/or at what time will subjects arrive at the facility to receive treatment, and that a pregnancy test will be conducted for female subjects prior to any treatment. During the initial briefing and test day compliance check, research staff should remind subjects that they are free to withdraw at any time and that they will be compensated for their participation up to the time of their withdrawal.

8. Clarify what clothing subjects will be wearing during the test day. The protocol notes that "all participants will be asked to wear light, loose fitting clothing that fully covers their body." (Protocol, p. 12) However, in the exchange with the IRB, it was noted that subjects would be wearing Tyvek suits to prevent bites during the testing periods. (IRB Minutes and Roster, p. 4)

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

10. Add to the exclusion criteria individuals who:
    a. are sensitive to the product ingredients or any insect repellent products.
    b. have a known or suspected allergy to any insect repellent product.
    c. are not attractive to mosquitoes.

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

12. In the event a mosquito captured during testing is found to carry an arbovirus, provide more information in the protocol about who will make contact with the subjects and how contact will be made (phone, email, both).

13. Clarify in the protocol how email will be employed in the recruitment process. What advertisement will be emailed and to whom will it be emailed? How will the distribution list be established?

14. Please provide the email template and any other documents that will be used at any point during subject recruitment and consent phases.

15. Revise the consent process discussion to reflect the requirement that individuals be provided with a copy of the consent form, and given time and instructed to read it thoroughly before the consent presentation by the Study Director or her delegate begins.

16. Make explicit in the protocol that subjects who withdraw from the study or who are withdrawn by the Study Director will be notified at the time of the withdrawal that their data will be used unless they request otherwise.

17. In Section 6.1, (Protocol, p. 21), delete the first paragraph and replace with "Under federal regulations, female subjects who are pregnant, nursing or lactating may not be enrolled in this study."

18. Regarding mosquitoes used in the attractiveness testing and aspirator training, please clarify whether the mosquitoes will be destroyed after use in each test or whether mosquitoes will be reused in either the attractiveness testing or aspirator use training.

19. Clarify what diseases mosquitoes will be screened for and be consistent throughout the protocol. On page 21, the protocol notes that mosquitoes will be tested to confirm the absence of ZIKV, but on page 22, it notes that "mosquitoes will be tested prior to the study to confirm the absence of ZIKV, EEEV, WNV, and SLEV." (Protocol, p. 22) Disease screening is also mentioned on page 12.

20. Clarify the compensation for subjects. The protocol notes that subjects will be compensated $20 for attending the consent meeting, $40 for participating in the mosquito attractiveness testing and aspirator use training, and $10 per hour for their participation in the test day. The consent form notes that subjects will be compensated $20 for participating in the testing and training process and $215 per field test day. If an hourly rate is chosen, the EPA recommends increasing the rate to time and a half for any participation beyond eight hours. Confirm that subjects will be compensated for attending the consent meeting regardless of whether they choose to enroll in the study and for the attractiveness meeting regardless of whether they continue their enrollment in the study.

21. Clarify expectations and compensation for alternates. How long are alternates expected to remain at the test site? What amount will be provided if they show up to the test site on a test day but are not needed?

22. Include in the protocol information about how payment will be made to subjects (cash, check, pre-paid card; mail or in person) and when (end of each event, at the end of the subject's participation, etc.).

23. Delete or provide a rationale for allowing the Study Director or other delegated staff to "end a particular participant's participation on a test day at any time, for any reason." (Protocol, p. 31) Generally, the EPA recommends that the Study Director have limited discretion to withdraw subjects from the study as outlined in the protocol. For example, such discretion could be characterized as: "*Participants' enrollment in the study may be ended at the discretion of the Study Director where continued participation may affect the safety of the participant or where there is a development of any condition that might interfere with study participation.*"

24. Describe how subjects will be transported to the field site. Subjects should be informed in advance (i.e., during the consent process) whether transportation will only be provided at the start and end of the test period. Also describe how subjects who experience failure before the end of the full test day will be handled, e.g., will they remain with the group until all subjects experience product failure, will they be returned to the test facility, or will they be free to leave in their own transportation from the test site.

25. The EPA recommends instructing subjects to wash their treated limb as soon as possible after their participation ends and providing soap, water, and paper towels at the field site or at the facility where subjects will be returning to at the end of the testing day.

26. Under Data Management (Section 8.7), in addition to double entering data and verifying it to ensure accuracy, revise the protocol to note that prior to any data analysis, the Study Director will confirm that all data from all subjects has been entered to avoid analyzing an incomplete data set.

27. Under Ethics and Dissemination:
    a. Include a statement that "This study will be conducted in accordance with the EPA's final regulation at 40 CFR 26 that establishes requirement for the protection of subjects in human research. The protocol, informed consent form, and other required documentation for this study must be approved by an independent institutional review board and submitted to the EPA as required by 40 CFR 26.1125. The report of the completed research is subject to the requirements at 40 CFR 26.1303 to provide documentation related the ethical conduct of the study."
    b. Revise as follows: "All amendments, deviations, and any adverse events will be documented in the final study and reported consistent with Good Laboratory Practices (GLP), 40 CFR 26, Subpart K, and IRB reporting procedures." (Protocol, p. 30)

Consent form

28. The consent form must be revised to include all relevant elements of consent required under 40 CFR 26, Subpart K.
29. The consent form should be revised to provide a simple summary of the study, including describing the test day and test procedures in enough detail to allow subjects to make an informed decision about participating. The current consent form does not include sufficient information about the process for attractiveness testing and aspirator training, how a test day would occur, and what would happen if a voluntary or Study Director-initiated withdrawal occurs. Information on the test site relative to the facility where the test substance will be applied and how subjects will be transported between the two locations should also be included in order to give the subjects a thorough understanding of the procedures to which they are consenting.
30. Add a statement that evaluating adverse effects may require the study personnel to consult with the treating medical personnel, with the subject's consent: "The Sponsor, medical monitor, and the Study Director will determine whether the injury is related to the subject's participation in this study. To do this, they may request to consult with the person/facility that provided medical treatment following an adverse effect, which could require your consent."
31. The consent form should be revised to align with all changes made to the protocol and to address all of the EPA's specific comments on the consent form.

Attachments:

1. EPA Protocol Review (Protocol dated 17 April 2020)
2. Completeness checklists
3. Standard rate of application

Attachment 1 - EPA Protocol Review

Title: *Field Evaluation of two topically applied insect repellent products containing IR3535 against mosquitoes in Louisiana*.

Date: 17 April 2020

Principal Investigator and any sub-investigators: Dr. Kristen Healy

Participating Laboratory:
LSU
Department of Entomology
404 Life Sciences Building
Baton Rouge, LA 70803

Sponsor:
LivFul, Inc.
2972 Webb Bridge Rd.
Alpharetta, GA 30009

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

IRB:
LSU IRB
130 David Boyd Hall
Baton Rouge, LA 70803

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 two skin-applied insect repellent products at preventing mosquito landings on human hosts. The objective of the study is to determine the duration of efficacy of the test materials in repelling mosquitoes when applied at a standard consumer dose of 1 g/600 cm^2. The EPA requires efficacy testing of products claiming efficacy against disease vectors to support efficacy claims on product labels.

The field tests will measure the efficacy of the IR3535 products at repelling mosquitoes in order to establish an mCPT for each formulation of the product.

(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 two personal, skin-applied, tick repellent products, containing the active ingredient 3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester (IR3535) against mosquitoes.

The rationale for testing is to collect data to establish the mCPT for each product formulation. The data supporting currently registered products are not sufficient to establish the mCPT for these specific products. Previously generated data did not have sufficient consistent mosquito landing pressure to evaluate the efficacy of these products.

A standardized protocol will enable the EPA to receive consistent and scientifically reliable data about the complete protection time for each product. Field testing data will provide information about the length of repellency time after treatment before the first confirmed landing by a mosquito occurs. Mosquitoes will not be permitted to bite subjects.

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

The EPA requires product-specific efficacy data for registration of skin applied insect repellent products according to recommendations from the EPA's guideline OCSPP 810.3700. This protocol proposes to generate data to replace previously-generated field testing data from one test site in Florida[10]. The 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 products' label.

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

The EPA requires product-specific efficacy data to support product registration. This protocol proposes to generate data to replace previously-generated field testing data from one test site in Florida, where mosquito landing pressure was insufficient to evaluate efficacy of the proposed products.

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

[10] EPA. Fuentes and Bohnenblust. Science Review of Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR335 Against Mosquitoes in Florida. October 3, 2019. Presented to the HSRB on October 23, 2019. https://www.epa.gov/sites/production/files/2019-10/documents/1a._science_review_of_ir3535_for_hsrb_10-3-19.pdf

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 two insect repellent formulations, each containing 20.07% of IR3535 against species of mosquitoes withing the genera *Aedes*, *Anopheles*, and *Culex* at the EPA standard rate of application, 1g/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 subjects is based on a power analysis using Weibull distribution. This analysis was developed by statisticians from the EPA and is presented in Appendix 2 to the protocol beginning on page 34. "*EPA statisticians used a Weibull distribution to simulate the sample size required to estimate the median CPT to a given degree of precision. A power analysis of these outputs resulted in a sample size of 13 participants being chosen. This number allows for over 90% power at a range of expected median CPTs from 2-8 hours*." (Protocol, p. 26)

"*Based on power analysis, a sample size of 13 test subjects for each product in this study design would provide sufficient power (>0.90) to obtain a ratio of the lower limit of 95% CI of the estimated median CPT / estimated median CPT is ≥ 0.6, where the ratio of the lower limit of 95% CI of the estimated median CPT/estimated median CPT expresses the precision of estimated median CPT. Two additional subjects will serve as untreated controls for each field test to monitor the landing rate throughout the study. An additional 5 participants will be enrolled as alternates to replace any test subjects who drop out before testing begins. In total, up to 20 people could be necessary for each test day. Assuming no subjects participate as test, untreated control, or alternate subjects more than once, total of 40 people could be necessary to complete all testing outlined in this protocol*." (Protocol, pp. 5-6)

(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. No positive controls are proposed. 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?

The EPA recommends describing the randomization process in more detail; specifically, the protocol should describe the process for randomly assigning subjects as either treated, control, or alternate 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 for each formulation will be calculated of all test subjects at one test site using the Kaplan-Meier survival analysis. The objective of the data analysis is to estimate the median protection time with a 95% confidence interval. The times to treatment failure will be analyzed using Kaplan-Meier Survival functions for estimation of the mCPT with 95% confidence intervals. The Kaplan-Meier Survival analysis is advantageous since CPTs may not be normally distributed. Kaplan-Meier estimator has been accepted by EPA and the HSRB for the 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. The EPA recommends rounding down mCPT value to the nearest whole number. For example, a mCPT of 3 hours and 45 minutes would be listed on the label as 3 hours.

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

The mCPT will be estimated from the CPT for each participant for each formulation tested at 1field 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. The Kaplan-Meier estimator has been accepted by the EPA and the HSRB for mCPT calculation in past repellent efficacy

studies and is also recommended by the World Health Organization for CPT calculation from these non-parametric data sets.

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

Yes, the design would likely have adequate power to answer the research question.

2.2 How and to what will human subjects be exposed?

Subjects will have the option to test the lotion, wipe, or both formulations of repellent products containing IR3535 (an EPA-registered active ingredient), during repellency testing. Subjects will be exposed to mosquito species encountered in the field during repellency testing, and to mosquitoes from laboratory reared colonies during assessment of subjects' attraction to mosquitoes and aspirator handling training. A single lower leg of each subject will be treated and exposure to the repellent will be continuous throughout the test. Under the current test design, subjects will test a single product on a single day, for a for a maximum of 16 hours from time of product application to the end of the test day.

According to the EPA's risk assessment based on data submitted to the EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classed as category III for acute dermal toxicity (LD_{50} > 3000 mg/kg in rats), category IV for acute oral (LD_{50} > 5000 mg/kg in rats) and inhalation toxicity, and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats (based in a 90-day dermal toxicity study), and for oral toxicity is 600 mg/kg/day in rabbits. Based on the NOAEL ≥ 3000 mg/kg/day for dermal exposure to IR3535, the Agency has determined that there is no dermal risk of exposure. Although the information in the protocol does not follow the EPA's risk assessment procedures, the conclusions are the same; the amount of IR3535 applied in these studies does not exceed a level of toxicological concern to test subjects.

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

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

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

The rationale for testing is to collect product performance data to establish an mCPT for registration of two skin-applied repellent products, AKIVA 20 Lotion and AKIVA

20 Wipes, containing IR3535 as its active ingredient. The data supporting currently registered IR3535 products do not provide this information. The products will be tested at the EPA standard dose (1g/600 cm^2). The standard dose is derived from calculation of a typical consumer dose (Attachment 4).

(c) What duration of exposure is proposed?

Proposed exposure periods consist of exposing treated lower leg from treated subjects to field mosquitoes for five minutes at every 30 minutes 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 be used to monitor landing pressure throughout the test by exposing untreated skin to field mosquitoes for five minutes or until five landings per control subject is achieved, whichever occurs first. 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 FCL. "FCL" is defined as a single landing followed by a second landing within 30 minutes of the first. FCL measures the time point signaling repellency failure. CPT is the measurement for residual repellency or time to product failure from time of product application. CPT is measured by FCL as a single value for each subject. The endpoints are appropriate to the questions being asked. Using the Kaplan-Meier estimator, the mCPT will be calculated for all test subjects for each formulation.

(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.
- Lower legs will be prepared for dose application. Skin surface area will be measured in advance, during pre-test training.
- Pre-test training on how to capture landing mosquitoes using aspirators will be conducted in the lab using pathogen-free mosquitoes from pathogen-free laboratory colonies. Subject screening and subject selection include determination of subject's attractiveness to mosquitoes.
- Two control subjects will be employed to monitor adequate landing pressure throughout the test for determining duration of repellency.
- Pathogen-free and unfed mosquitoes from pathogen-free laboratory colonies will be used for pre-test practice on subjects' screening for attractiveness to mosquitoes and how to use aspirators.
- Efficacy will be conducted at a single site, using 18 treated, 2 untreated and 5 alternate subjects per product formulation.

- 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 treated skin of treated subjects and on exposed untreated skin of controls will be collected and saved for taxonomical identification and pathogen screening.
- Treated and control subjects will work in pairs. Control subjects will not be paired with each other but with a research staff member, who will not collect landings but will work with control subjects assisting them with data recording.
- Alternate subjects (five individuals) 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 a maximum of 1 day of testing per product formulation for a maximum of 16 hours from the time product application.
- Stopping rules and criteria for subject withdrawal are proposed. The EPA recommends revising criteria for skipping exposure periods due to inadequate landing pressure (below five landings in five minutes on either control).
- The EPA recommends adding criteria for replacing withdrawn subjects and use of their data.

(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). The QA representative will conduct critical phase inspections to ensure study integrity and maintain written and signed records of each inspection. (Protocol, p. 3)

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

Sources of variation include mosquito species and activity, and attractiveness of subjects to mosquitoes. Uncertainties relate to mosquito landing pressure throughout the study, and variation in subjects attractiveness to mosquitoes. The uncertainty related to mosquito diversity and landing pressure will be addressed by selecting sites based on pre-test site monitoring data that indicate that relevant species of mosquitoes are present. Control subjects will monitor landing pressure periodically for the duration of the test period. Assuming the EPA recommendations are incorporated, uncertainty will be addressed by establishing a stopping rule based on the number of consecutive and non-consecutive periods of low landing pressure.

The uncertainty related to subjects' attractiveness to mosquitoes will be minimized by pre-testing evaluation of attractiveness according to the EPA guideline on testing repellents to be applied to skin.

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 Baton Rouge, Louisiana area, and will be representative of the population of concern. Advertisements will be posted through digital, social, and social media to solicit participation from a broad range of individuals.

The EPA recommends that the protocol be amended to add details about the recruitment process. The regulation requires that all recruitment materials used, such as scripts and email templates, be provided to the EPA prior to initiation of the study.

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

Based on the proposed recruitment for this study, participants should be representative of the population of concern: *"Th[e] pool of eligible subjects will be checked to ensure it is representative of age, gender, and ethnicity of the general population.*" (Protocol, p. 22)

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

Yes.

3.2 Equitable Selection of Subjects

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

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

Inclusion criteria

- *Able and willing to give fully informed consent*

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

Exclusion criteria

- *Participated in any other interventional study in the previous three months*
- *Participated in a biting insect test as part of the current study in the previous 72 hours*
- *Employees, managers, and spouses of employees of the LSU and of the study Sponsor (LivFul, Inc.)*
- *Students of the Study Director or any other LSU faculty/researchers involved in the study*
- *Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites*
- *Women who are pregnant, nursing or intending to become pregnant during the course of the study*
- *Individuals with localized skin disorders affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes*
- *~~Allergic~~ Known or suspected allergy or sensitivity to any of the test or reference product ingredients or any insect repellent products*
- *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 LSU and of the study Sponsor (LivFul, Inc.), as well as students of the Study Director or any other LSU faculty/researchers involved in the study are not eligible to participate. Subjects may be students of the LSU, but not working with anyone involved in the administration of the study.

(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 Baton Rouge area in Louisiana and will be representative of the population of concern: "…*eligible subjects the participants will be selected to be representative of age, gender, race/ethnicity of the general population.*" (Protocol, p. 12)

The EPA recommends that the protocol be amended to describe in more detail the recruitment process. For example, where will advertisements be posted and for how long? Additionally, the EPA notes that the Human Studies rule requires the study sponsor to provide all recruitment materials (e.g., phone scripts, email templates) to the Agency prior to the initiation of the study.

Potential subjects will be contacted by someone associated with the study, who will provide more information by phone or email. If the potential subject is interested in participating, he or she will be invited to meet with the study director or other study personnel one on one or in a small group to review the informed consent document. This meeting will cover a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the pesticide to which they will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with the attractiveness test, training on aspirating mosquitoes, and field testing will be described step-by-step to all subjects who participate in the training. The subjects will be shown how the test substances will be applied to their leg for the future testing, will be informed that they will wear gloves to protect their hands and head nets to protect the head, face and neck, and will be shown how to aspirate mosquitoes.

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

Subjects will be recruited through print, digital and social media advertisements. There will be no connection or communication between the researchers and the potential subjects' employers, which minimizes the potential for coercion or undue influence. In addition, students or employees of the study director or other faculty and researchers involved in the study are excluded from participation. Finally, any employees, managers, and spouses of employees of the LSU 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 and consent form have conflicting information about subject remuneration that must be resolved before the research is initiated. The protocol proposes paying subjects $10/hour for the field testing. However, the EPA recommends that for any time beyond eight hours, subjects be compensated at a rate of time and a half (i.e., $15/hour), or at a flat rate for the test day as described in the consent form ($215 per test day). Subjects will also be compensated for the time they spend in the consent meeting, training course, and mosquito attractiveness test. The protocol notes that individuals would receive $20 for attending the consent meeting and $40 for participating in the mosquito attractiveness testing and aspirator use training. The consent form notes that subjects will be compensated $20 for participating in the testing and training process.

The protocol and consent form should be revised to include information about remuneration for alternates who are asked to come to the test site but not enrolled in the study. These documents should also make explicit that everyone who participates in a consent meeting will be compensated regardless of their decision to enroll.

(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?

This is unclear in the protocol and consent form. The EPA has requested that the protocol include more specific information about how and when subjects will be paid.

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?

IR3535, the active ingredient in the two products in the protocol, is an EPA-registered pesticide with an essentially complete supporting toxicity database. It has been tested extensively in animals and is of low toxicity by all routes of exposure. The acute dermal LD_{50} of IR3535 is greater than 3,000 mg/kg body weight. IR3535 is not a skin sensitizer.

Results from toxicity testing:

- A primary eye irritation study on rabbits showed that IR3535 is irritant to the eyes. Irritation was observed for 24-48 hours but was all cleared within 7 days.
- A dermal sensitization study in Guinea pigs showed that IR3535 is not a contact sensitizer.
- A primary skin irritation study on rabbits showed that IR3535 is minimally irritating to the skin. All irritation was cleared by 48 hours.
- The single dose acute dermal LD_{50} of IR3535 is >3,000 mg/kg in rabbits.

According to the EPA's risk assessment based on data submitted to EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classed as category III for acute dermal toxicity (LD_{50} > 3000 mg/kg in rats), category IV for acute oral (LD_{50} > 5000 mg/kg in rats) and inhalation toxicity, and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats (based in a 90-day dermal toxicity study), and for oral toxicity is 600 mg/kg/day in rabbits.

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

Risks to subjects include the risk of exposure to biting mosquitoes, the risk of exposure to disease vectors, the risk of exposure to the test material, risks of being outside in a hot and humid climate, 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 proposed dose and exposure levels are significantly lower than the established NOAELs for the test material. See Section 4.1. Based on the NOAEL ≥ 3000 mg/kg/day for dermal exposure to IR3535, the Agency has determined that there is no dermal risk of exposure.

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

The potential risks have been evaluated by the EPA through a comparison of available toxicity data and the anticipated dermal exposure. The comparison indicates minimal risks. Please see part 4.1(c) (above) for details.

(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 outlines risks and risk minimization measures in table on pp. 12-13. To mitigate risks from exposure to mosquitoes and disease vectors, subjects will be trained to aspirate landing mosquitoes before they probe or bite and will wear clothing, gloves and head net to protect untreated areas from bites during the test periods. To minimize the risk of contracting any mosquito-borne diseases during the lab-based mosquito attractiveness test, the cages will be populated with mosquitoes from a colony reared in the laboratory. Mosquitoes from this colony will also be screened for Zika virus and West Nile virus. The testing sites will be monitored weekly for a month prior to the testing, and all mosquitoes captured during the monitoring phase will be tested for pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified. The Study Director will work with the mosquito control districts to ensure that no vector-borne illnesses have been identified at the field test sites.

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

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

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

Testing will be stopped for an individual when a subject receives an FCL or at the Study Director's discretion "*where continued participation may affect the safety of the participant or where there is a development of any condition that might interfere with study participation.*" (Protocol, p. 17)

The study will also be stopped if certain conditions related to weather delays or mosquito landing pressure are met. "I*f there are 4 non-consecutive exposure periods with low-biting pressure the test day should be stopped. If there are 3 consecutive exposure periods with low-biting pressure the test day should be stopped. …. If there are 4 non-consecutive exposure periods missed due to bad weather the test day should be stopped. If 3 consecutive periods are missed due to bad weather then the test day should be stopped. If a single landing on a test subject during an exposure period is followed by a missed exposure period (either through inadequate landing pressure or bad weather) then the first landing will be treated as a confirmed landing. If a confirmed landing occurs during an exposure preceded by a missed exposure period (either through low biting pressure or bad weather) then CPT will be recorded as the earliest time point in that preceding delay.*" (Protocol, p. 25)

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

A certified first aider will be on site for the duration of the field testing to render emergency aid if necessary. Subjects who experience an adverse event during testing and need professional medical attention will be returned to their own vehicle or taken to a medical facility by a member of the study staff. Any subjects who experience an adverse event after a study day is completed will be advised to visit their medical provider.

(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?

The protocol notes that "Participants will be followed up within 24-72 hours after any exposure to mosquitoes or test product to assess any possible adverse events. Three attempts will be made to contact the participant. Initial contact will be by email, second and third contacts will be by phone and email." (Protocol, p. 28) This period is long enough to identify subjects who have an adverse reaction to the test substance or who develop a reaction in the event they experienced a mosquito bite in the field.

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

According to the protocol, "If a subject is injured or becomes ill as a direct result of his or her participation in this study, the Sponsor will pay for all reasonable and necessary medical expenses required to treat the injury…" (Protocol, p. 30) This is subject to two conditions – the injury must have occurred during the subject's

participation in the study and the injury must be a direct result of exposure to the test substance or study-related procedures. (Protocol, p. 30)

5. **Benefits**

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

   There are no benefits to the subjects of 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?

   The protocol outlines how this study design has been amended from a previously-submitted study that had insufficient landing pressure on the untreated control subjects, which resulted in little data that the EPA could use in a regulatory decision. 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?

The LSU 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?

No.

(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?

Following an individual's review of the consent form and the consent presentation by research staff, a member of the research staff will the ask potential subject a series of questions about the study. Only after the individual can answer the questions satisfactorily will they be invited to consent to enroll in the study.

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

The protocol outlines the consent process as follows:
"*Individuals who express an interest in participating in response to the recruitment materials will be contacted by telephone or e-mail by the Study Director or appropriately trained and delegated study staff to inform them of the basic inclusion criteria and study procedures. At this stage, volunteers will determine their own eligibility and self-exclude if they are aware of being ineligible. If they believe they are eligible and are interested in enrolling in the study, they will be given an appointment to meet with the Study Director or appropriately trained and delegated study staff to learn more about the study and their potential role in it. They will be*

provided with the participant information sheet (PIS) by email or post to review before the consent meeting.

"*Before the start of the consent meeting, volunteers will be asked to show a valid driver's licence, [sic] passport, or other valid identification to verify their age and name. The staff member leading the discussion will briefly outline the study, its purpose, the volunteer's potential role in the study, the potential duration of testing, the identity and function of the repellent to be used, potential hazards associated with the study, and steps taken to mitigate these hazards, the inclusion/exclusion criteria, and the procedures for reporting adverse events. Test procedures including the aspirator training, product application and field landing catches will be described step-by-step. Female subjects will additionally be informed of the requirement for pregnancy testing on each test day, as well as the procedure for testing and how the participant's privacy will be respected. The participant will then be asked if they have any questions regarding the information presented. It will be made clear that the participant does not have to consent and does not need to give any reason for non-consent or withdrawal. While the trial briefing may take place as a group, all participants will have a one-to-one session with an appropriate member of study staff during which they can ask questions and will sign the consent form. If an individual still wishes to enrol [sic] in the study, he or she will be asked a number of questions to ensure they have fully understood the information given. These questions are given in the Table 1 below. Only if the participant can demonstrate their comprehension of the study procedures, will they then be asked to sign the Informed Consent Form (ICF), which will be witnessed by the staff member who led the consent discussion. The participant will then be given a photocopy of the signed Informed Consent Document. The Test facility will retain the original of the ICF."* (Protocol, p. 15)

(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 from enrollment employees, managers, and spouses of employees of the LSU and of the study Sponsor (LivFul, Inc.), as well as students of the Study Director or any other LSU faculty/researchers involved in the study.

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 9.4.

"*Participants' identification data will be required for the enrollment process. This data will be linked on a single form (participant enrollment log) to their unique identification number. The participant identification number will be used on all other data collection forms, which will not contain any other identifiable data. The participants' names will not appear anywhere on the data sheet, or in the reports. The Test Facility will preserve the confidentiality of participants taking part in the study.*

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

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

"*The study records will be maintained at the Test Facility in locked cabinets, and electronic files kept on a password protected computer server. No one outside researchers, Sponsor, IRB, or certain governmental agencies will have access to participants' personal information*." (Protocol, p. 30)

(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 will be compensated for their time and inconvenience for the amount of time they participated, e.g., attending a consent meeting.

According to the protocol, "*Data collected to the point of withdrawal will be used in the analysis of the study if possible, unless the participant requests that their data is not used. In this case it will be removed from the database.*

"Participants also may be removed at the discretion of the Study Director, where continued participation may affect the safety of the participant or where there is a development of any condition that might interfere with study participation.

"If a subject withdraws during the test day, the Study Director or appropriately trained study staff member will be responsible for ensuring that they are safely returned to their own vehicle or other transport by the end of the test day. If the subject withdraws because they require medical treatment, the Study Director or appropriately trained study staff member will be responsible for ensuring that they are safely returned to their own vehicle, other transport or a medical facility as soon as possible." (Protocol, p. 17)

Attachment 2 - Completeness Checklists

The following checklists are public documents. They are used by EPA in reviewing proposed protocols for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to EPA under FIFRA. These checklists only address ethical requirements and do not address the scientific integrity of the proposed study.

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	
(2) Minutes of IRB meetings . . . in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; and a written summary of the discussion of controverted issues and their resolution.	Y	
(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	
(5) A list of IRB members in the same detail as described in §26.1108(a)(2).	Y	
(6) Written procedures for the IRB in the same detail as described in §26.1108(a)(3) and (4).	Y	
(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	
(a)(2) The measures proposed to minimize risks to the human subjects	Y	
(a)(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	
(a)(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	
(a)(5) The balance of risks and benefits of the proposed research.	Y	
(b) All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.	Y	
(c) Information about how subjects will be recruited, including any advertisements proposed to be used.	Y	All recruitment tools and scripts will be provided to EPA before the research is initiated.

(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	
(e) All correspondence between the IRB and the investigators or sponsors.	Y	
(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	

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.	N	EPA provided comments and suggested revisions.
(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	EPA provided comments and suggested revisions.
(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.	N	
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;	N	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	

Attachment 3 – EPA's Power Analysis

Attachment 4 - EPA Standard Rate of Application

Test products should be applied at 1 g/600 cm^2 for aerosols and wipes. For pump sprays the Agency recommends applying 0.5 g product/600 cm^2. These application rates are based on dosimetry tests used in previous studies since 2006, which have been reviewed by EPA and HSRB.
In April 2015, the HSRB reviewed a protocol conducted by SC Johnson and agreed to the use of the EPA's standard application rate to replace dosimetry testing.[11]

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

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

[11] 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.