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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: Science Review of Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR3535 Against Mosquitoes in Florida

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REFERENCE: Weeks, Emma, Study Director. (2019) Field Evaluation of Two Topically Applied Insect Repellent Products Containing IR3535 Against Mosquitoes in Florida. Unpublished document. February 6, 2019. MRID 507791-01.

ACTION REQUESTED

Conduct a science review of a completed field study testing efficacy of two insect repellent formulations, lotion and wipes, containing 20% w/w of the active ingredient, ethyl butyl acetyl aminopropionate (IR3535), against mosquitoes in Florida. This product performance test is required to establish the median complete protection time (mCPT) against mosquitoes to support registration of the proposed skin-applied repellent products. The protocol used to conduct this study was previously reviewed and accepted with recommendations by the Environmental Protection Agency (EPA) and Human Studies Review Board (HSRB) on July 26, 2017. The protocol used in this study was amended to incorporate EPA and HSRB recommendations.

CONCLUSIONS

The EPA has completed its evaluation of the scientific validity of this study (MRID 507791-01) in relation to recommendations of the HSRB and the Product Performance Test Guidelines, OCSPP 810.3700: Insect Repellents to be Applied to Human Skin[1]. Following EPA evaluation, the EPA concludes that the study provides scientific evidence that supports a complete protection time (CPT) of **3 hours** for both wipe and lotion formulations. This conclusion differs from the applicant's conclusion that the study supports a CPT of 14 hours (Attachment 5). The EPA rationale supporting the conclusion for the 3-hour CPT is described in detail below. The HSRB will be asked to comment on this study.

Supporting Rationale for Conclusion of 3-hour CPT

As background regarding EPA's science policy for the determination of CPT:

- The definition of adequate landing pressure for determination of CPT is defined in OCSPP 810.3700: Insect Repellents to be Applied to Human Skin, pg. 28, Section K (6): Minimum landing pressure to initiate or continue test. The definition states, "*Landing pressure should be measured before treatment and intermittently throughout the course of the test by untreated control subjects. Testing should not be conducted or continued unless landing pressure of the target species is at least one mosquito landing within one minute*." Exposure periods vary in length depending on experimental design. One landing per minute is meant to indicate the standard rate of landings. For a five-minute exposure period, it is expected mosquitoes land on subjects at an approximate rate of one landing per minute, resulting in at least five landings in five minutes.
- Based on the Repellency Awareness Graphic Guidance policy[2] for determining CPT, CPT is estimated for each product at each site. The most conservative CPT at either site rounded down to the nearest integer is selected for product labeling.

- With regard to the subject study MRID 507791-01:
  - Site 1 achieved adequate mosquito landing pressure on control subjects with both products for over 12 hours.
  - Site 2 had adequate landing pressure through the first two exposure periods during test days 5 and 6. This observation is consistent with behavioral tendency of *Culex* spp. (the predominant mosquito species at Site 2) to be most active at dawn and dusk. However, landings were irregularly distributed between the controls and did not consistently reach a rate of 1 landing per

[1] OPPTS 810.3700: Insect Repellents to be Applied to Human Skin. July 7, 2010. https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-810-product-performance-test-guidelines

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

minute on a single individual control subject from the 3rd exposure period for both days to the 20th exposure period on both days. The irregular landing pressure across control subjects at Site 2 suggests low mosquito activity rather than mosquitoes' preference for either subject. Therefore, the maximum CPT supported by the data at this test site is 3.5 hours.

- Based on EPA's consideration of the totality of the data presented, EPA conservatively estimated the CPT at 3 hours for both products.

SCIENCE REVIEW

Study objective: The objectives of this study were to establish the median CPT (mCPT) of two insect repellent formulations, a lotion and a wipe, containing 20 % w/w of the active ingredient IR3535 in the field against populations of wild mosquitoes using human volunteer subjects, and to provide reliable data for product registration and labeling purposes. The tested hypothesis was that the products are expected to prevent mosquito landings on human hosts for a period of up to 16 hours post application.

Compliance with Good Laboratory Practice Standards (GLP), 40 CFR, Part 160: The study is a guideline study designed in conformity with recommendations from OCSPP 810.3700 Product Performance Guideline for Testing of Insect Repellents to be Applied to Human Skin. This study was conducted in accordance with EPA, FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), Good Laboratory Practice Standards (GLP) (40 CFR, Part 160) with the exception of testing to produce the certificate of analysis. A "Statement of Compliance with Good Laboratory Practice Standards" is provided on pg. 3 of the study report. The tests that were completed to produce the Certificate of Analysis of the products were done without following GLP; however, the specific reasons for the testing not being GLP were not provided. A "Quality Assurance Statement", signed and dated on Feb. 6, 2019, is provided on pg. 4 of the study report.

Identification of the test system: In this study, the first confirmed landing of wild mosquitoes on human subjects was used as the endpoint to evaluate the repellency of two insect repellent products (20 % w/w IR3535), one lotion and one wipe formulation, applied to human skin. Mosquitoes are the target insect pest repelled by the products. The test was conducted in two distinct field locations within Gainesville, a city located in Alachua Co., Florida, where predominant mosquito species differ (§7.1, pg. 21 of 2732). One site was a residential area in a suburban neighborhood. The other site was a hardwood pine and shrub/brush park area, owned by University of Florida, adapted to depict a natural ecosystem. These sites are known for high mosquito abundance but low incidence of mosquito-borne diseases.

Risk Minimization:

Site monitoring and mosquito processing for identification and detection of mosquito borne pathogens: Sites were monitored weekly for 24 hours using CDC light traps and BG Sentinel traps (§7.2, pg. 22 of 2732). Testing in the field was not initiated until 2 months of pathogen negative samples were available. In addition, the study director contacted Florida Department of Health (FLDoH) for routine notification of cases of mosquito borne diseases and checked

FLDoH website weekly for emerging reports about most current information on presence or absence of mosquito pathogens in the area. No human cases of mosquito-borne viruses in the County were reported to the study director during the study.

Viral pathogen testing: Field collected mosquitoes from traps were transported to the lab for identification and detection of pathogens (§7.2, pg. 22 of 2732). Mosquitoes from monitored sites were sorted by site, trap type, and species. Pools of RNA from samples of 10 female trapped mosquitoes were analyzed for pathogens using quantitative reverse transcription and PCR (qRT-PCR; §6.3, pg. 20 of 2732). In addition, mosquitoes collected from test participants during efficacy testing were analyzed for pathogens as described above but labeled by collection site/subject/TS (test substance) or treatment/species. All tests were negative for Zika (ZIK V), West Nile virus (WNV), St. Louis Encephalitis (SLEV), and Eastern equine encephalitis (EEEV) (Raw data in Appendix 16.5).

Pre-testing Mosquito species distribution: The majority of mosquitoes collected in traps from monitored sites were identified to species (99.6%) and only 0.4% were identified to genera (Data in Appendix 16.12 of study report). Sampling events on site 1 JH (suburban habitat) lasted from June 2 to August 29, 2018. A total of 1,413 mosquitoes were collected from site 1 during that period. The most common genera were *Aedes* spp. (62.2 %), *Ochlerotatus* spp. (16.7%), *Wyeomyia* spp. (7.6%) and *Psorophora* spp. (7.4%). The most common species on site 1 JH was *Aedes albopictus.* Sampling at site 2 NATL (natural habitat) took place from May 22 to September 12, 2018. A total of 3,664 mosquitoes were collected. Most of the species belonged to the genus *Culex* (45.2%); 28.4% of the species belonged to the genus *Ochlerotatus;* and 20.6% and 3.6% of the species belonged to the genera *Psorophora* and *Aedes,* respectively. The most common species on site 2 NATL was *Culex nigripalpus* (Table 1). Four of 2,754 samples (0.14%) were mislabeled and not tested for pathogens; they were identified to species (§7.2, pg. 23 of 2732).

Table 1. Mosquito abundance and species distribution at 2 field sites

Mosquito Genera	Species by genera	Total collected at Site 1	Total collected at site 2
Aedes	*albopictus*	876	22
	vexans	3	96
	Other species	0	13
Anopheles	*quadrimaculatus*	1	2
Anopheles	*crucians*	0	14
	punctipennis	0	1
	walkeri	0	1
Coquillettidia	*perturbans*	3	14
Culex	*erraticus*	7	79
	quinquefasciatus	6	64
	peccator	2	0
	restuans	2	12
	Tarsalis/coronator	2	282
	salinarius	1	35
	nigripalpus	58	1,171
	Other species	0	15
Ochlerotatus	*infirmatus*	199	900
	Atlanticus/tormentor	17	77
	Fulvus pallens	5	17
	Other species	1	0
Orthopodomyia	*signifera*	0	3
Psorophora	*ferox*	100	750
	ciliata	6	0
	howardii	0	3
	species	0	1
Wyeomyia	*mitchellii*	108	0

Data from Appendix 16.12

Experimental design:

Efficacy testing: This field study was conducted with human subjects at two ecologically distinct mosquito habitats, one a natural habitat and the other a suburban environment, in Alachua County, Florida. At each site, the experimental groups consist of 13 treated subjects and two untreated control subjects, who monitored landing pressure immediately prior to test initiation and throughout the duration of the test (§ 8.2 pg. 34 of 2732 in study report). Adequate landing pressure is five mosquitoes landing within five minutes or less monitored by 2 untreated control subjects throughout the test. Subjects were selected from a pool of informed and consenting volunteers that were tested in a lab setting for their attractiveness to mosquitoes and based on being attractive to mosquitoes, they were trained to catch/handle mosquitoes using aspirators. At each site, testing was conducted for a period of 16 hours.

Attractiveness test: The attractiveness test consisted of an arm-in-cage evaluation. Subjects introduced one arm into a 30.5 X 30.5 X 30.5 cm cage containing 24 female *Aedes aegypti* mosquitoes, 7-14 day old (mosquito density: 1 mosquito /1,182 cm^3). Mosquitoes had been taken from a pathogen-free laboratory colony that has been in existence for 10 years (§ 8.1, p. 29 of 2732). Female mosquitoes had not received a blood meal. The mosquitoes used for attractiveness test were tested for absence of pathogens. Criteria for attractiveness was 5 landings/minute. The test could be repeated up to 2 more times. Subjects that did not meet the

attractiveness criteria after 3 trials were disqualified from further testing (§8.1, pg. 30 of 2732). Those that qualified proceeded to training on the use of aspirators for catching and handling mosquitoes.

Aspirator training: Subjects were dressed as for test day to prevent mosquito bites and introduced into a 3 m X 3 m X 3 m cage with 60 free flying *Aedes aegypti* female mosquitoes (1 mosquito / 1,182 cm^3) where they practiced aspirating mosquitoes that landed on a partner's leg. The training lasted approximately 1 hour (§8.1, pg. 30 of 2732). Mosquitoes were from a pathogen-free colony and had not received a blood meal prior to their use in this test.

Test subjects selection and randomization: To meet an approximate 50:50 sex distribution, the first 7 males and 7 females that arrived plus the next person to arrive of either gender were selected for participation in field test. The remaining 5 subjects were kept as alternates. The 15 subjects selected to participate in field test were randomly assigned to either treatment or control groups by drawing from a box containing 13 pieces of paper with the word "treatment," and two with the word "control." Afterword test subjects drew from another box containing 16 pieces of paper; eight with the word "right" and another eight with the word "left" for randomly assigning treatment to either right or left leg. The randomization occurred for each subject on each day in which they participated in testing. There was no blinding of treatments (§8.2, pg. 31 of 2732).

Rate of product application: The two formulations were tested in the field on 13 replicated subjects each using the standard dose of 1 g of product/600 cm^2 (1.67 mg/cm^2) skin surface area (§8.2, pg. 32 of 2732). Prior to product performance testing in the field, individual rate of application, based on standard dose, was estimated by adjusting the standard dose to the surface skin area per subject according to the formula (§8.2, p. 32 of 2732):

Weight of test substance to apply (g) per subject = [surface area of limb[1] cm^2/600 cm^2] X 1 g

[1] lower leg

Two staff members double checked the calculation. Surface area of treated skin was estimated by multiplying length of lower leg (ankle to knee) by average circumference of lower leg. Average leg circumference was estimated from four measurements, taken with a measuring tape from subjects. The top of the leg was denoted by an eye liner pencil line which ran below the knee and around through the bend of the knee (§8.2, pg. 31 of 2732). Length of the leg was measured from the top of the sock to the eye liner pencil line. Four circumference measurements were taken as follows: one was taken around the top of the sock, the second measurement was taken around eye liner pencil line, the third measurement was taken around the widest part of the leg, and the fourth measurement was taken half way between the widest part of the leg and the top of the sock (§8.2, pg. 32 of 2732). Leg measurements were recorded on participants' check-in sheets (Appendix 16.9 of study report).

Test Substance Application Procedure: Prior to applying the test substance, the exposed lower leg of each subject was washed with water and unscented soap, rinsed with a solution of a 70% isopropanol, and allowed to air dry. After drying, subjects replaced their socks and shoes (§8.2, pg. 31 of 2732) and the skin surface area was measured as described above under the "Rate of

product application" section. The target amount of test substance to be applied per subject was weighed using a calibrated balance. For the lotion, a beaker was labeled with subject's ID and initials, the balance was tared with the weight of the beaker, and the amount of test substance per subject was added to the beaker and re-weighed discarding the excess until amount dispensed was within 0.05 g of target dose amount. For wipes, the wipes were squeezed over the beaker, and the target amount to be applied per subject was weighed following the same procedure. On test days, the legs were prepared, washed and measured and the test substance was weighed prior to application. The test substance was applied to the participants at the same time once all the test substance was weighed, and the first exposure to mosquitoes occurred approximately 2 hours post test substance application. Study staff spread the test substance on subjects' lower leg using a gloved finger, and evenly distributed the test substance over the treated area. Beakers were weighed to ensure that amount of test substance remaining in the beaker was less than 0.05 g. Time of application was recorded. The average amount of test substance applied to subjects and average rate of application across 13 subjects (n-13) are found in Table 2.

Table 2. Average individual rate of application for lotion and wipe formulations.

Average values (n=13)		Test days				
	Aug 15/2018	Aug 19/2018	Aug 26/2018	Sept. 1/2018	Sept. 8/ 2018	Sept. 15/2018
Average skin surface (cm^2) (range)	1117.89 (930-1357.13)	979.96 (803.63-110898)	1070.57 (926.13 – 1459.94)	1074.97 (833.25-1316)	1071.93 (718.63-1574)	1013 (721-1505.75)
Application amount (g) (range)	1.85 (1.39-2.28)	1.62 (1.32-1.82)	1.78 (1.54-2.43)	1.78 (1.44-2.58)	1.77 (1.18-2.62)	1.68 (1.17-2.45)
Individual rate of application (mg/ cm^2) (range)	1.65 (1.63-1.66)	1.65 (1.54-1.66)	1.66 (1.65-1.66)	1.66 (1.64-1.68)	1.65 (1.63-1.68)	1.65 (1.64-1.66)

Data from Appendix 16.9

[1] The trials started on the 8th and 15th of Sept. and went over night, ending next day on the 9th and 16th**.**

Field testing, landing pressure and exposure periods: Treated subjects were randomly assigned to pairs of chairs that were numbered. Each subject drew a piece of paper from a box containing 14 pieces of paper. Two pieces of paper had each number (1-7). The treated subject without a corresponding treated subject partner was paired with a staff member (§8.2, pg. 34 of 2732). Control subjects sat in pre-selected chairs. Each pair was 3 m/10 ft apart. Before each exposure period started, adequate landing pressure of at least 5 mosquitoes landing within 5 minutes or less was monitored by 2 untreated subjects throughout the test (§ 8.2, pg. 34-35 of 2732). The protocol originally defined adequate landing pressure as 5 landings in 5 minutes on each control subject. After test days 1 (August 15, 2018) and 2 (August 19, 2018) the protocol was amended to redefine adequate landing pressure as 5 landings in 5 minutes or less on at least one of the control subjects (Appendix 16.6, IRB revision 8 and Appendix 16.3, amendment 7). For a more detailed discussion of this amendment and its impact on the study, see the sections titled "Protocol Deviations and Amendments" and "EPA's Discussions of Results and Conclusions". Time of each landing and time of achieving threshold for each untreated control subject (5 mosquitoes within 5 minutes or less) were recorded. Table 4 summarizes total species

of mosquitoes collected from control and test subjects by day and site. The study report notes that not all landing mosquitoes could be collected as it was recognized that it was more important to prevent bites on subjects. Not collecting all landing mosquitoes constituted a protocol deviation on test days 1, 2, and 3. A protocol amendment was approved by the IRB on August 27, 2018, prior to test day 4 (Appendix 16.6. IRB revision 8 and Appendix16.3 Amendment 7). Mosquitoes collected after landing on exposed skin of test subjects were identified and tested for pathogens. Exposures began 2 hours post product application (§8.2, pg. 34 of 2732). Once each exposure period began, treated subjects exposed their lower leg for 5 minutes at approximately 30-minute intervals until experiencing their first confirmed landing or until end of the trial, whatever occurred sooner (§8.2, pg. 35 of 2732). Some of the intervals between exposure periods were longer than 30 minutes but none exceeded one hour (§9.3, pg. 44 of 2732). This deviation occurred once in each test day except test day 1. On test day 2, one period between exposure periods lasted 42 minutes. On test day 3, one period between exposure periods lasted 52 minutes. On test day 4, one period between exposure periods lasted 53 minutes. On test day 5, one period between exposure periods lasted 52 minutes. On test day 6, one period between exposure periods lasted 42 minutes. Researchers reported these occurrences as not subject-specific deviations in §9.3 Protocol deviations, pp. 42 through 44 of 2732 of study report.

If the mCPT was not established prior to 12 hours, testing was extended until CPT was established for seven subjects (i.e., minimum required to determine median CPT) or until 16 hours post application (§8.2, p. 35 of 2732). All subjects not achieving confirmed landings at the end of test were considered right censored by Kaplan-Meier analysis. The lotion formulation was tested at site 1 on the 1st day (trial 1), and at site 2 on the 2nd, 3rd days (trials 2, 3), and on the 6th day (trial 6). The lotion was successfully tested only once at each site (trial 1 at site 1 and trial 6 at site 2). Trials 2 and 3, which were testing the lotion at site 2, began early in the morning and were stopped by the researchers according to the conditions of the protocol, due to low mosquito landing pressure toward the middle of the day. Wipes were tested at site 1 the 4th day of testing (trial 4) and at site 2 the 5th day of testing. Trial 4 began early in the morning and trial 5 began at dusk (Table 3).

Table 3. Summary of trials per product and sites

Test substance	Sites	Trials	Dates (2018)
lotion	Site 1	1	Aug 15
	Site 2	2° 3° 6*	Aug. 19 Aug. 26 Sept. 15-16
wipes	Site 1	4	Sept. 1
	Site 2	5*	Sept. 8-9

*Trial 5 for wipes and trial 6 for lotion at site 2 began at dusk on Sept. 8 and 15, respectively, and continued into the early morning of the next day. All other trials began early in the morning.

° Trials 2 and 3 were stopped by the researchers due to low mosquito activity and were not used for determination of CPT.

The mCPT for lotion was calculated with data from trials 1 and 6. The mCPT for wipes was

calculated with data from trials 4 and 5. See Table 5.

Three non-consecutive exposure periods were skipped during test day 1 (periods 8:37; 12:30 and 14:07) due to misinterpretation of protocol provision for skipping exposure periods due to low mosquito landing pressure. A protocol amendment was prepared to clarify the language in the protocol and approved by IRB (Appendix 16.6, IRB revision 8 and Appendix 16.3 amendment 7).

***Study results**:*

Raw data for mosquitoes landing on and collected from test and control subjects on test days are in Appendix 16.12, and summarized in Table 4. Across sites and days, *Aedes albopictus* was the most commonly captured species.

Table 4. Summary of mosquitoes collected from control and test subjects during trial days 1 through 6, quantified by species

Product	Date	Site	Day	Genus and species	Total spp.	Total mosquitoes
Lotion	Aug. 15, 2018	1-JH	1	*Aedes Albopictus*	35	42
				Ochlerotatus atlanticus	7	
	Aug. 19, 2018	2-NATL	2	*Mansonia dyari*	1	5
				Ochlerotatus infirmatus	3	
				Coquillettidia perturbans	1	
	Aug. 26, 2018		3	*Culex tarsalis*	1	10
				Aedes Albopictus	5	
				Ochlerotatus triseriatus	1	
				Psorophora ferox	1	
				Ochlerotatus atlanticus	1	
				Coquillettidia perturbans	1	
Wipe	Sept. 1, 2018	1-JH	4	*Aedes Albopictus*	10	18
				Psorophora ferox	2	
				Ochlerotatus atlanticus	2	
				Mansonia dyari	3	
				Wyeomyia mitchelli	1	
	[1]Sept. 8-9, 2018	2-NATL	5	*Coquillettidia perturbans*	2	7
				Mansonia dyari	5	
Lotion	[1]Sept. 15-16, 2018		6	*Aedes Albopictus*	1	5
				Mansonia dyari	3	
				Anopheles quadrimaculatus	1	

Data from Appendix 16.12

[1] The trials started on the 8th and 15th of Sept. and went over night, ending next day on the 9th and 16th.

Table 4 above reports the number of mosquitoes captured from exposed skin of both test and control subjects, not the total number of all mosquitoes landing on control and test subjects (see (Appendix 16.6. IRB revision 8 and Appendix 16.3 Amendment 7). Specific times of mosquito landings on test subjects and time for reaching threshold landing on control subjects is found in Appendix 16.12 of the study report (MRID 507791-22).

Statistical analysis: Sample size of 13 subjects per treatment is based on the EPA power analysis calculations in Appendix 5: Power/Sample Size Calculation, of revised study protocol, dated January 22, 2018, which were reviewed and accepted by the HSRB. Kaplan-Meier Survival Analysis was used to estimate mCPT. The lower 95% confidence intervals (CI) were calculated and are in Appendix 16.10. Upper 95% CI were not calculated due to lack of information resulting from right-censored data (§11.0, pg. 49 of 2732 in study report). On trial day 1 (lotion tested at site 1), trial day 5 (wipes tested at site 2) and trial day 6 (lotion tested at site 2), mCPT was estimated from 7 subjects that experienced confirmed landings. The remaining 6 subjects that did not experience confirmed landings by the time of test completion at 16 hours post-application were right censored for the analysis. Since it is not possible to estimate mCPT with less than 50% of the subjects in the sample, mCPT was not estimated for trial day 4 (wipes tested at site 1), when 7 out of 13 of the treated subjects were right censored and 6 experienced confirmed landing (Table 5; corresponds to Table 15 pg. 49 of 2732 in study report). As noted earlier, no mCPT was calculated for trials 2 and 3 because the trials were stopped due to lack of mosquito landing pressure and the protocol dictates that the data would not be used.

Table 5. Summary of calculated mCPT values from the Study Report

Test trial days	Site	Product type	Subjects		mCPT in hrs. mts.	Lower 95% CI
			CPT	Right censored		
1	1	20% IR3535 Lotion	7	6	14:08	13:08
4	1	20% IR3535 Wipes	6	7	> 16	12:04
5	2	20% IR3535 Wipes	7	6	14:06	12:18
6	2	20% IR3535 Lotion	7	6	15:10	13:38

The researcher recommended that for purposes of labeling, CPT for both formulations should be established at 14 hours. "*In the case of these results, median CPT for the Akiva lotion is 14 h and 8 minutes and for the Akiva wipe 14 h and 6 minutes. Therefore, the CPT for both TS (test substance) tested in this study are 14 hours*." (§10.0 Statistical Analyses, pg. 49 of 2732 in study report).

Protocol deviations and amendments (Appendices 16.3 & 16.4, pp. 268-280 of 2732)**:**

Deviations (Appendix 16.4, pp. 282-284 of 2732)

- Mosquitoes collected from test and control subjects: Not all mosquitoes landing on test subjects could be aspirated (§ 9.3 pg. 43 of 2732 in study report). All mosquitoes that could be collected from exposed skin of test subjects were retained for identification and pathogen testing. This constituted a protocol deviation for test days 1, 2, and 3. A protocol amendment was approved prior to the start of test day 4, on August 27, 2018 (Appendix 16.6. IRB revision 8 and Appendix16.3 Amendment 7).

- Length of interval between exposure periods: In all trial days, except trial day 1, one of the intervals between exposure periods was longer than 30 minutes and shorter than 1 hour. In test 2 at site 2 for testing lotion, the interval between periods 12:59 and 13:41 was 42 minutes long. In test 3, at site 2 for lotion, the interval between exposure period 10:35 and 11:17 was 52 minutes long. In test 4 at site 1 for testing wipes, the interval between exposure period 16:40 and 17:33 was 53 minutes long. In test 5 at site 2 for wipes, there was an interval of 52 minutes between the exposure periods, 23:50 and 00:42. In test 6 at site 2 for lotion, there was an interval of 42 minutes between exposure periods 00:31 and 01:13 (§ 9.3 pg. 44 of 2732). These time intervals exceeding 30 minutes are deviations from the IRB-approved protocol V5 in, "*Between time points the repellent will be left on the leg and re-tested every 30 minutes up to 12 hours or until CPT has been achieved*." (MRID 502889-01, § 8.5.8 Exposure Duration on pg. 17)

- Use of beaker instead of spatula for weighing dose amount of lotion applied to subjects. This change constitutes a deviation on test days 1, 2, and 3. It was submitted to and approved by the IRB as protocol amendment on August 27, 2018 (IRB revision 8 Amendment 7 Appendix 16.3). The study director explained that changing from spatula to beaker enhances precision and is consistent with the method for weighing the wipe formulation.

- Skipped exposure periods: Three non-consecutive exposure periods were skipped during test day 1 (periods 8:37, 12:30, and 14:07) due to misinterpretation of protocol provision for skipping exposure periods due to low mosquito landing pressure. A protocol amendment was prepared to clarify the language in the protocol and it was approved by IRB in revision 8 (Amendment 7 Appendix 16.3).

Amendments:

After testing began, there were four amendments to the protocol (Appendix 16.3 Amendment 7 pg. 279 of 2732):

- To change the method of weighing dose amount of lotion applied to test subjects. Due to fluidity and volume needed for some subjects, the dose amount was weighed directly on a beaker rather than on a spatula. The beaker is labeled with the participant ID and placed on the balance; the balance is tared. The required

weight of the test substance is then added to the beaker. Then the product is applied to the test subject directly from the beaker using a finger, and the beaker is reweighed until it contains less than 0.05g product.

- To clarify that while attempts will be made to capture all mosquitoes that land on participants it may not be possible to capture every mosquito, as it is more important that the mosquitoes are not permitted to bite the participants and that the participants do not move from their positions. All mosquitoes captured will be identified and all *Culex* spp. and *Aedes* spp. will be tested for pathogens as described in the protocol.

- To clarify that for the purposes of stopping the study, a test period with low mosquito landing pressure is when both participants have fewer than 5 landings in 5 minutes. If one control subject has fewer than 5 landings, but the other control subject has at least 5 landings in 5 minutes this is considered adequate landing pressure to allow the study to proceed.

- To clarify that test periods when one or both of the control subjects did not have 5 landings in 5 minutes would not be skipped. Only testing during periods where there is rain during the exposure period would be skipped. For purposes of stopping the study, a total of four test periods may be skipped due to weather or having both of the controls receive fewer than 5 landings in 5 minutes. If a fifth test period is skipped or both subjects fail to receive 5 landings in 5 minutes then the study director must stop the test day.

EPA's Discussion of Results and Conclusions:

The amendments and deviations listed above, except for those related to low mosquito landing pressure for control subjects, did not substantively affect the study results (See Appendix 4 EPA Statistical Review for Study MRID 507791-01).

There was no impact on the quality of the data or the results of data analysis for determination of CPT due to the skipping of exposure periods 8:37; 12:30 and 14:07on test day 1, because all CPTs were preceded by periods of adequate landing pressure on both controls. One CPT occurred at period 10:30; another at period 11:30; two CPTs occurred at periods 12:59 and 13:30, respectively, and one last CPT occurred at period 14:01. Landing pressure was adequate for both control subjects on all exposure periods preceding the periods when CPT occurred.

Likewise, sensitive statistical analysis showed that there was no impact on the quality of the data and the results of data analysis due to the long break periods on test days 5 and 6. results (See Attachment 4 EPA Statistical Review for Study MRID 507791-01).

Concerning mosquito landing pressure, EPA agreed with the amendment to revise the criterion to 5 landings on 1 control subject only for the purpose of continuing the study,

i.e., this landing rate would not count as periods with low pressure for the purpose of stopping the study, with the caveat that EPA retained discretion to review the entire dataset and determine whether mosquito landing pressure remained at an acceptable rate throughout the course of the test to allow EPA to calculate a CPT for the product (See Attachment 3: Correspondence between EPA and Dr. Emma Weeks).

1. On test day 1 assessing the lotion product at site 1, landing pressure on control subjects was adequate for the duration of the test. EPA concludes that this is sufficient to support an mCPT of 14 hours for this test, which is the mCPT calculated by the researcher (see Table 5, which corresponds to Table 15 on p. 49 of 2732) and rounded down to the nearest integer according to Repellency Awareness Guidance.

2. While testing the wipe product on test day 4 at site 1, neither control subject experienced a landing during the final 2 exposure periods (periods 28 and 29, 21:00 and 21:19 hrs.). In addition, during exposure period #26 (period 20:00 h), only one landing occurred on each control subject, and during the next exposure period #27 (20:25 period) one control subject experienced 5 landings while the other control subject did not experience any landings (Table 6). Therefore, because landing pressure was inadequate on both subjects for 3 of the final 4 periods # 26, 28 and 29 (20:00, 21:00 and 21:19 hrs.) (Table 6), these exposure periods should not be considered for estimation of mCPT. Because all 6 confirmed landings occurred prior to the drop in control landing pressure and no confirmed landings occurred after the drop in control landing pressure, the last exposure period to be used for estimating CPT is exposure period #25 (period 19:30 h), when landing pressure on control subjects was adequate (See Table 6). After this exposure period, no confirmed landings occurred and all seven subjects who did not experience a confirmed landing would be right censored by Kaplan-Meier analysis and therefore, the mCPT cannot be calculated. Per EPA's guidance on calculating CPT, where CPT should be rounded down to the nearest integer, CPT for this day and site should be considered 13 hours.

Table 6. Test day 4 (Aug. 1, 2018) at Site 1: Wipes.

Number of Exposure Periods	Start time Exposure periods	# landings in 5 minutes or less Control Subject 53	# landings in 5 minutes or less Control Subject 37	CPT in hours.minutes
1	07:20	3	5	
2	07:47	5	5	
3	08:21	5	0	
4	08:52	5	5	
5	09:19	2	4	
6	09:48	5	5	
7	10:20	5	2	
8	10:53	5	5	
9	11:23	5	2	
10	11:54	5	0	
11	12:23	5	4	

Number of Exposure Periods	Start time Exposure periods	# landings in 5 minutes or less Control Subject 53	# landings in 5 minutes or less Control Subject 37	CPT in hours.minutes
12	12:52	5	5	
13	13:25	5	5	
14	13:56	4	5	
15	14:27	5	5	
16	14:56	5	5	
17	15:25	3	5	
18	15:56	5	4	10.51
19	16:32	3	5	
20	17:04	5	5	12.02 12.04
21	17:27	5	5	12.13
22	18:02	5	5	
23	18:31	5	5	13.28
24	18:58	5	4	
25	19:30	5	5	14.19
26	20:00	1	1	
27	20:25	5	0	
28	21:00	0	0	
29	21:19	0	0	

3. During test day 5 at site 2, landing pressure occurred irregularly on both control subjects (Table 7). The lotion formulation broke down (i.e., subjects experienced CPT) at the end of the test; 7 subjects experienced CPTs between periods 16 and 21 (3:54 and 6:48 hrs.). The first 2 CPTs occurred during periods 16 and 17 (3:54 and 4:33 hrs.), when neither control subject experienced enough landings. This suggests that actual CPT might be shorter than reported if control landing pressure was 5 mosquitoes per 5 minutes on both control subjects. The next 3 CPTs occurred between periods 18 and 19 (5:11 and 5:42 hrs.), when only 1 control subject experienced 5 landings in 5 minutes (control subject 6 in period 18, control subject 30 in period 19). The last 2 CPTs occurred between periods 21 and 22 (6:48 and 7:20 hrs.) when both subjects experienced 5 landings in 5 minutes. These last 2 periods when CPTs occurred were preceded by 3 periods, 18, 19 and 20 (5:11; 5:42 and 6:19 hrs.), during which 1 of the control subjects experienced 5 landings in 5 minutes (control subject 6 in period 18, control subject 30 in periods 19 and 20). During this test day, both of the controls experienced 5 landings in 5 minutes during the first 2 periods (18:57 and 19:34), during periods 5 and 11 (21:23 and 00:59 hrs.), and during the last 2 periods 21 and 22 (6:48 and 7:20 hrs.) (Table 7). Only one control subject experienced sufficient landings for the purpose of continuing testing during periods 3 and 4 (20:14 and 20:49), periods 8 to 10 (23:04, 23:39, and 00:32 hrs.), periods 12 to 15 (1:39, 2:12, 2:44, and 3:21 hrs.), and during periods 18 to 20 (5:11, 5:42 and 6:19 hrs.). The 5 landings per minute threshold was not reached consistently for a single control subject; rather, it alternated between the 2 controls. During 5 periods when one control subject

received 5 landings in 5 minutes, the other control subject received zero landings (Table 7).

Table 7. Test day 5 (Sept. 8-9, 2018) at Site 2: Wipes.

Number of exposure Periods	Start time Exposure periods	# landings in 5 minutes or less Control Subject 6	# landings in 5 minutes or less Control Subject 30	CPT by time in hours.minutes
1	18:57	5	5	
2	19:34	5	5	
3	20:14	2	5	
4	20:49	5	2	
5	21:23	5	5	
6	21:56	0	4	
7	22:29	3	2	
8	23:04	0	5	
9	23:39	5	3	
10	00:32	5	1	
11	00:59	5	5	
12	01:39	0	5	
13	02:12	0	5	
14	02:44	3	5	
15	03:21	0	5	
16	03:54	0	2	10.58
17	04:33	0	2	11.38
18	05:11	5	1	12.22 12.21 12.18
19	05:42	0	5	
20	06:19	2	5	
21	06:48	5	5	14.06 14.04
22	07:20	5	5	

Although landing pressure was irregular for much of the test day, the pattern of higher landing pressure in the evening and morning and lower landing pressure in the middle of the night is likely related to the high percentage of *Culex* spp. mosquitoes present at this site. Culex mosquitoes, the predominant spp. at site 2, is most active at dawn and dusk, when landing pressure was adequate for the first 2 periods of the test. This provides EPA with additional confidence that there was adequate landing pressure during the first two periods of the test day. However, following the first 2 test periods, landing pressure was inconsistent. Landing pressure throughout the test day was not adequate to support EPA's agreement with the researcher's calculated

CPT of 14.06 hours for this day (Summary of calculated mCPT values from the study report are presented in Table 5). EPA concludes for test day 5 the 3rd period (20:14 h) should be used as a conservative estimate of CPT for this day, which supports a CPT of 3 hours for the wipe product. This conclusion is based on the following:

- Adequate mosquito landing pressure on both control subjects during the first 2 exposure periods,
- Initial product breakdown (first individual CPT) not occurring until approximately 10 hours post application (including the fifth period during which there was adequate landing pressure on both control subjects),
- Behavioral tendency of *Culex* spp. to be most active at dawn and dusk as observed during the test day, and
- Consideration that under landing pressure of 5 mosquitoes in 5 minutes on both control subjects (periods 20 to 23) (Table 6 on test day 4 (Site 1 test of the wipe product), mCPT for the wipe product was 13 hours, which provides additional support for durability of the product.

4. On test day 6, testing lotion at site 2, the first CPT occurred after 7 hours of testing at exposure period 15 (3:12 h), followed by 6 CPTs occurring at the end of the test period between periods 20 and 23 (6:16 to 7:52 hrs.). Adequate landing pressure on both controls occurred during the first 2 periods (18:51 and 19:24 hrs.), and periods 21 and 22 (6:54 and 7:16 hrs.) at the end of the test. Inadequate landing pressure occurred at periods 6, 8, 11 and 12 (21:42, 23:05, 00:57, and 1:27 hrs.) (Table 8). Only one control subject achieved adequate landing pressure for all other periods, though it was not consistently the same subject. During the periods when only one control subject had 5 landings (periods 3, 4, 5, 7, 9, 10, 13, 14, and 15 to 23), the other control subject received zero landings during 3 of the periods (periods 7, 9, and 19), and a single landing in periods 10 and 16 (Table 8).

Table 8. Landing pressure and CPT data: Test day 6 (Sept. 15-16, 2018) at Site 2: Lotion

Number of exposure Periods	Start time Exposure periods	# landings in 5 minutes or less Control Subject 35	# landings in 5 minutes or less Control Subject 48	CPT by time in hours.minutes
1	18:51	5	5	
2	19:24	5	5	
3	20:05	5	3	
4	20:36	5	3	
5	21:12	4	5	
6	21:42	2	2	
7	22:24	5	0	
8	23:05	1	3	
9	23:41	0	5	
10	00:22	1	5	
11	00:57	3	2	
12	01:27	2	1	

13	02:07	5	2	
14	02:39	5	4	
15	03:12	5	3	10.28
16	03:44	5	1	
17	04:24	5	2	
18	05:00	5	2	
19	05:40	5	0	
20	06:16	5	3	13.37 13.38 13.39
21	06:54	5	5	
22	07:16	5	5	14.36 14.43
23	07:52	5	2	15.01

On test day 6, a similar pattern of landing pressure was observed as on test day 5, which again can be attributed to the behavior of the dominant *Culex* spp. present at this site. Landing data for days 5 and 6 at Site 2 show that landing distribution during the middle of the test period (between the hours of dawn and dusk) was inconsistent across subjects and so the results cannot be attributed to a subject effect or interpreted for calculating a CPT. Therefore, the landing pressure for this test day was not adequate to provide complete confidence in the CPT of 15.10 hours calculated for this test day. EPA concludes that adequate landing pressure on both controls occurred during the first 2 exposure periods for test days 5 and 6 at site 2.

For test day 6, the third period (20:05) should be used as a conservative estimate of CPT for this day, which supports a CPT of 3 hours for the lotion product. This conclusion is based on the following:

- Adequate mosquito landing pressure on both control subjects through the first two exposure periods,
- Behavioral tendency of *Culex* spp. to be most active at dawn and dusk as observed during the test day, and
- Consideration that under landing pressure of 5 mosquitoes in 5 minutes on 2 control subjects on test day 1 (site 1 test of the lotion product), mCPT for the lotion was 14 hours, which provides additional support for durability of the product.

Conformity with Protocol and Amendments:

The protocol was reviewed by EPA and the HSRB. The protocol was revised to address recommendations from both organizations (see Attachment 2) and approved by the IRB on May 10, 2017. The protocol was amended 8 times, on March 21, 2018; April 17, 2018; June 25, 2018; July 17, 2018; July 24, 2018; August 2, 2018; August 10, 2018; and August 27, 2018.

The reported study conformed with the protocol as follows:

- Of the 70 people recruited, 38 took part in field testing. Each test subject took part in between 1 to 6 test days. For each test day, 13 test subjects, 2 control subjects, and 5 alternates were selected from a pool of 20 qualified participants that had consented and were screened for eligibility (54 in total) according to inclusion/exclusion criteria in Section 5.5 of the study report (pg. 16 of 2732). Random selection as test or control subject was accomplished by subjects drawing pieces of paper with written treatment assignments on them as described in section 8.2 (pg. 31 of 2732). Test day procedures. Treatments were randomly assigned to either left or right legs by a similar method.

- The number of prospective subjects for each testing day was 20 – 13 test subjects, 2 control subjects, and 5 alternates. Sample of 15 subjects maintained a close distribution of 50:50 male to female ratio by enrolling in each test day 7 males and 7 females plus one more subject of either sex, and randomly assigning them as test or control subjects (section 8.2 Test day procedures, pg. 31 of 2732).

- Prospective test and control subjects were tested for their attractiveness to mosquitoes. Tested subjects and controls were trained for handling landing mosquitoes and the use of aspirators in the laboratory using pathogen-free, non-blood fed, lab-reared mosquitoes (Section 8.1, pp. 20-30 of 2732).

- For assessment of initial landing pressure prior to study initiation the minimum landing rate is 1 mosquito landing/minute. Control subjects exposed lower leg for up to 5 minutes or until 5 landings occurred. After 5 minutes or 5 landings whatever happened sooner, control subjects' exposed skin was covered (Section 8.2, pg. 34 of 2732).

- Mosquitoes landing on the exposed lower leg of control and treated subjects were collected for identification and pathogen testing. They were labelled with subject number, treatment status, day and time of collection (Section 6.2 Field site mosquitoes, pg. 19 of 2732).

- Five potential sites were monitored at 9 locations for a month and 2 were selected for efficacy testing based on their mosquito populations and low risk of mosquito transmitted diseases. Selected sites were monitored for mosquito populations and detection of pathogens for more than 2 months prior to study initiation. Monitoring was done weekly for 24 hours using BG sentinel and CDC light traps (section 7.1 Location and type of habitat, pg. 21 of 2732).

- Pathogen testing was conducted on *Aedes* and *Culex* species for detection of Zika, West Nile, St. Louis encephalitis and eastern equine encephalitis (Section 6.2 Field site mosquitoes, pg. 19 of 2732, and section 6.3 Viral pathogen testing, pg. 20 of 2732).

- Principal investigator established weekly contact with Florida Department of Health (FLDoH) (Section 7.2 pg. 23 of 2732).

- Individual rate of application was based on standard dose (Section 8.2, pg. 32 of 2732).

- Each product formulation was tested at 2 ecologically distinct sites, one a suburban area and the other, a forest/natural park. Site selection was based on diversity and abundance of mosquito species, and no detection of mosquito borne pathogens (Section 7.0 pp. 21-22 of 2732). One product formulation/site combination trial occurred per day. Test was conducted to coincide with peak mosquito activity (Section 6.2 pg. 19 of 2732). Test duration was extended from 14 to 16 hours (Section 8.2, p. 35 of 2732).

- No more than 6 test subjects who reached the end of test day without experiencing a confirmed landing were right censored for purposes of data analysis (Section 11, pg. 49 of 2732).

- The products, lotion and wipes, containing 20% w/w of active ingredient, IR3535, were tested at the standard dose of 1 g/600 cm^2 (1.67 mg/cm^2) (Section 8.2, pg. 32 of 2732).

Conclusion

The methods used in this study are based on the protocol reviewed and accepted by the EPA and HSRB, Protocol version 7, dated March 28, 2018, as amended to incorporate EPA and HSRB recommendations before testing began. The tests that were completed to produce the Certificate of Analysis of the products were done without following GLP; however, the specific reasons for the testing not being GLP were not provided and they should be provided. EPA's conclusion is based on review of the data and interpretation of test results, and from following standard policy from test guidelines OCSPP 810.3700 and the Repellency Awareness Guidance for determination of CPT. Based on these factors, study results are acceptable to support a CPT of 3.0 hours against mosquitoes for the proposed lotion and wipe products containing 20% w/w of the active ingredient IR3535.

cc: Michelle Arling

Attachment 1: Calculations of Maximum Safe Dosage of IR3535
Attachment 2: Responsiveness to EPA and HSRB Science Comments on Draft Protocol
Attachment 3: Correspondence between EPA and Dr. Emma Weeks
Attachment 4: EPA Statistical Review for Study MRID 507791-01
Attachment 5: Discussion Between EPA and Study Director on Landing Pressure

Attachment 1

Calculations of Maximum Safe Dosage of IR3535:

Accepted exposure level (AEL) and Dermal Absorption are taken from Regulation (EU) No. 528/2012, concerning the making available on the market and use of biocidal products: Evaluation of active substances Assessment Report: Ethyl butylacetylaminopropionate (IR3535). Product-type 19 (insect repellent) (2013) (CA-Dec13-Coc.3.4a – IR3535 draftAR.docx). Weight values are taken from: U.S. Environmental Protection Agency (EPA). (2011) Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F.

From Appendix 4 in revised protocol: Calculations of Maximum Safe Dosage of IR3535.

Participant	AEL* (mg/kg bw/day)	Weight** (kg)	Max Internal Dose IR3535 (mg/day)	Dermal Absorption* (%)	Max External Dose IR3535® (mg/day)
Adult Male	5	73.80	369	14	2635.71
Adult Female	5	60	300	14	2142.86

*Accepted exposure level (AEL) and Dermal Absorption taken from: Regulation (EU) nº528/2012 concerning the making available on the market and use of biocidal products: Evaluation of active substances Assessment Report: Ethyl butlyacetylaminopropionate Product-type 19 (insect repellent) (2013) (CA-Dec13-Coc.3.4a – IR3535 draftAR.docx).

**Weight taken from: U.S. Environmental Protection Agency Exposure Factors Handbook (2011).

Attachment 2

Responsiveness to EPA and HSRB Science Comments on Draft Protocol

Comment from EPA and/or HSRB	**Action taken by Study Sponsor**
Make editorial revisions and minor edits as recommended in EPA's written comments on Protocol V5 dated April 23, 2017. EPA provided the comments to the study sponsor and the HSRB in a file named Protocol_field_V5_05102017_OPP comments_06-29-2017.pdf	The study sponsor addressed these recommendations. See Appendix 16.1 for revised, IRB-approved protocol. Protocol Version 7, dated March 28, 2018
The exposure of subjects to the test compound will be within acceptable safety margins based on existing toxicology data and based on *Margin of Exposure* calculations as provided in the EPA scientific review	Study sponsor used *Margin of Exposure* calculations as provided in the EPA scientific review to ensure that individual rate of application does not exceed maximum safety dose. Section 10.5.5
Include references to previous studies for justification of employing standard dose for exposure studies	Previous dosimetry studies which identified the amount for a standard dose of product types are referenced in Appendix 4 of the protocol.
Update the protocol to reflect the number of subjects and alternates necessary to ensure statistically-valid results.	The study sponsor revised the protocol to require 13 test subjects, 2 untreated controls, and 5 alternates per test day. Appendix 16.1 for revised, IRB-approved protocol.
Specificity is needed in the confirmatory landing time period	Endpoint is identified as First confirmed landing, and First confirmed landing and CPT are defined in section 5.1 Study endpoint, according to EPA definitions
Specification is needed for timing study to coincide with peak season of mosquito activity	Field testing will be conducted in spring and summer in Fl. when mosquito activity is high. Section 10.1 Field sites of protocol.
Increased duration of test day from 12 to 16 hours since exposures are delayed 2 hours post application	The study sponsor addressed this recommendation in section 10.5.9 Exposure duration.
Pathogen testing should be extended to all relevant vector-borne illnesses. Principal investigator should make contact with FLDoH	*Aedes* and *Culex* species will be tested for Zika virus, West Nile virus, and Eastern equine encephalitis viruses. Principal investigator established connection with FlDoH. Filed test will not occur if cases of diseases are detected within last 4 weeks of monitoring. Section 10.1.1
Skin preparation should be consistent (washing with unscented soap and rinsing with ethanol or isopropyl alcohol) throughout all test subjects including control subjects and instruct treated subjects to avoid disrupting test substance once it is applied.	Section 10.5.2 Subject preparation, in amended protocol includes this recommendation.
Maintaining 50:50 sex ratio in sample	Section 7.2 Randomization in amended protocol describes selection of test and control subjects for each test day. The first 6 males and females to arrive, who have followed pre-test guidelines, will be selected as test subjects.

Comment from EPA and/or HSRB	Action taken by Study Sponsor
Criteria for skipped periods and ending test due to low mosquito landing pressure	Sections 10.5.6 and 10.5.11: the study must be stopped if there is insufficient landing pressure in >4 non-consecutive test periods over the course of 14-hour test duration. The study must also be stopped when 3 consecutive exposure periods are skipped due to weather delays. No more than 4 exposure periods (15% out 28 exposure periods in 14-hour test – 2 exposure periods/hr.) should be skipped due to either weather delay or insufficient landing pressure
No more than 6 test subjects should be right censored	Section 11.2 Data analysis: No more than 6 of 13 subjects should be right censored.
Paired subjects: test and control subjects should be paired	Section 10.5.7 Subject placement: Each treated participant will be paired with another participant. One odd number participant will be paired with a member of staff. Untreated controls will be paired together. Pairs will be located 3m/10 ft apart.
Description of training process and criteria for determining. that a person is sufficiently capable of aspirating mosquitoes. And confirmation that mosquitoes are not blood fed.	Process is described in Section 10.4.3 Insect landing/aspirating training. Training can be repeated up to 1 hour to demonstrate proficiency. Mosquitoes will be used from a pathogen-free lab colony that have been reared for 10 years. Mosquitoes used for training have never had a blood meal and they will be tested for ZIKV, EEEV, and WNV as in section 10.1.3
Intention to comply with GLP and add statement of (entity to be added) independent QAU will perform all QA duties	GLP Compliance and QA as defined by 40 CFR part 160 is included in section 3.
Describe type of products, identify name of active ingredient and specify concentration of active ingredient in the product	Type of Product: Lotion, spray and wipe containing 20% w/w IR3535 is specified section 9. Treatments and Study synopsis
Remove secondary objective for dosimetry testing from primary objective.	Dosimetry as secondary objective is removed. Section 2. Objective and Study Synopsis, Primary objective stated: To determine efficacy duration by estimation of CPT. Definition of First confirmed landing to quantify CPT, and definition of CPT are also included.
List of potential field sites	Potential field sites are included in section 10.1
Description of enrollment and randomization process and randomization of treatment application to either right or left leg.	Included in section 9, Treatments. Treated leg will be randomly chosen.
Mosquitoes collected from control and test subjects should be saved for identification. Record time of mosquito landings on test subjects and record time for reaching threshold.	Recommendation is included in section 10.2 Test insects and section 10.5.6 Continued landing pressure: Time of landing and when threshold number of landings occur will be recorded.

Attachment 3

Correspondence between EPA and Dr. Emma Weeks

From: Fuentes, Clara
To: Weeks,Emma; Bohnenblust, Eric
Cc: Arling, Michelle
Subject: RE: UFL field testing question
Date: Wednesday, August 15, 2018 2:38:58 PM

Eric,
I just talked to Emma and she explained that one of the controls is highly attractive to mosquitoes while the other person is not. So, one of the controls is getting most of the landings while the other is getting less than 1 per minute. Our guidance is that both controls get equal landings, assuming both persons are equally attractive to mosquitoes. In this case the distribution is uneven due to differences in people attractiveness to mosquitoes. That doesn't show that landing pressure is low in the area. It is just being unevenly distributed.
I just want to know if you agree with me that they don't need to miss another exposure period due to insufficient landing pressure. In fact, Emma said that they placed the controls apart from each other and the landing distribution got more evenly disturbed when the controls were placed farther apart from each other.

Clara

From: Weeks,Emma [mailto:eniweeks@ufl.edu]
Sent: Wednesday, August 15, 2018 2:15 PM
To: Arling, Michelle <Arling.Michelle@epa.gov>; Bohnenblust, Eric <Bohnenblust.Eric@epa.gov>; Fuentes, Clara <Fuentes.Clara@epa.gov>
Subject: Re: UFL field testing question
Importance: High

Please let me know what you think. We ar3 assuming it is both people that have to pass the 5 landings per minute and are up at 3 missed exposures so far due to less than 5 on one of the two controls (hour 9 post application). Just want to confirm that is what you mean. Feel free to email, call or text 352 870 4327.
Thanks
Emma

Sent from my T-Mobile 4G LTE device

------ Original message------
From: Arling, Michelle
Date: Wed, Aug 15, 2018 1:59 PM
To: Bohnenblust, Eric;Fuentes, Clara;
Cc: Weeks,Emma;
Subject:UFL field testing question

Michelle Arling
Human Research Ethics Review Officer
Office of Pesticide Programs (S-4248)

1200 Pennsylvania Avenue NW MC 7501P
Washington DC 20460
703-308-5891
arling.michelle@epa.gov

From: Weeks,Emma [mailto:eniweeks@ufl.edu]
Sent: Wednesday, August 15, 2018 12:48 PM
To: Arling, Michelle <Arling.Michelle@epa.gov>
Subject:

Hi Michelle, in the field today!! To clarify both control participants need to get 5 mosquitoes in 5 minutes for that exposure period to go ahead. Correct? Thanks
Emma

Sent from my T-Mobile 4G LTE device

From: Bohnenblust, Eric
To: Fuentes, Clara
Cc: Weeks,Emma; Arling, Michelle
Subject: Re: Clarification about control participants and other lesser points
Date: Friday, August 17, 2018 3:59:48 PM

I've added language after Clara's comments.we can discuss more if need be. I will check my emails this evening and tomorrow from time to time.

Sent from my iPhone

On Aug 17, 2018, at 3:28 PM, Fuentes, Clara <Fuentes.Clara@epa.gov> wrote:

> My comments inserted in message in red
> Clara
>
> **From:** Weeks,Emma [mailto:eniweeks@ufl.edu]
> **Sent:** Friday, August 17, 2018 2:43 PM
> **To:** Fuentes, Clara <Fuentes.Clara@epa.gov>; Bohnenblust, Eric <Bohnenblust.Eric@epa.gov>
> **Cc:** Arling, Michelle <Arling.Michelle@epa.gov>
> **Subject:** Clarification about control participants and other lesser points
>
> Dear Clara and Eric,
> As discussed over the phone this afternoon:
>
> 1. I will submit a protocol deviation for the change from a spatula to a beaker for weighing out product. The justification for this is that the product is less viscous than expected and in larger volumes could flow from the spatula. (This will be similar to the application method employed for the wipes) agree
> 2. I will **not** submit a protocol deviation for the mosquito landing captures but we will continue to collect, identify and test all mosquitoes that we can catch to the upmost of our abilities. Those that are not caught do not constitute a protocol deviation. (agree) agree
> 3. I have submitted a protocol amendment to the IRB for the fact that we will not have epi-pens on site. As discussed in this meeting and in a prior meeting with Michelle, epi-pens are prescription only. In our eligibility criteria those people that would be entitled to an epi-pen (at risk of serious allergic reaction or anaphylaxis) are excluded, therefore, there is no need for them on-site, neither is it possible for us to hold one without a prescription.
> 4. Finally, no test periods should be skipped for inadequate landing pressure. If both untreated control participants receive less than five landings in five minutes in more than four non-consecutive landing periods then the study should be stopped. If one of the control participants achieves the threshold of five landings in five minutes this is considered adequate landing pressure and it would not count towards the number of periods with low landing pressure. (that is my understanding as well) I think this is the best way to proceed. Although I hesitate

to commit to saying it would not count toward the number of periods with low landing pressure in an absolute sense. I think we will need to consider based on the whole of the data for a situation whether landing pressure is low, say for instance one control subject continuously has no or few landings vs a situation where they are getting 3 or 4 landings in 5 minutes here and there. So To allow for some flexibility, i think it might be better phrased as will not count as periods with low pressure for the purpose of stopping the study.

Additional questions:

1. In the protocol the three consecutive time period cut-off only appears to be applied to weather and not to landing pressure, should they also apply to landing pressure? (Item 4 states that study should be stopped due to low landing pressure in more than 4 non-consecutive landing periods, which makes 16% of skipped exposure periods. Based on that, my interpretation is that 3 consecutive time period cut-off, which is less than 16%, applies to weather; not to landing pressure. Let's hear from Eric). The 3 consecutive periods for stopping should also apply to landing pressure as well as weather.
2. In the protocol it states that the mosquito captures should be placed in vials for identification based on participant number, treatment status, date and time of collection. Is the time of collection important? We pool the samples for testing by genus, date and participant. We are recording which landing mosquitoes are captured on our data sheets. Is it relevant to know which mosquito landed at which exposure period? It would be easier to pool the mosquitoes at the field site just by participant and date if that is acceptable. (I recommend to record the exposure period for mosquitoes collected during each exposure period). You don't need to record the mosquito to the specific landing, the exposure period is sufficient. The time is the time of day which will correspond to a certain exposure period, so 3 pm for instance. This can be helpful to see if there are differences by species over time during the day (which might be helpful if you still see weirdness in the control)

Thanks!
Emma

Emma Weeks
Assistant Research Scientist
Entomology and Nematology Department
University of Florida
970 Natural Area Drive
Gainesville, FL, 32611
Tel: 352-273-3954
Email: eniweeks@ufl.edu

Attachment 4

EPA Statistical Review for Study IR3535 – MRID 50779101

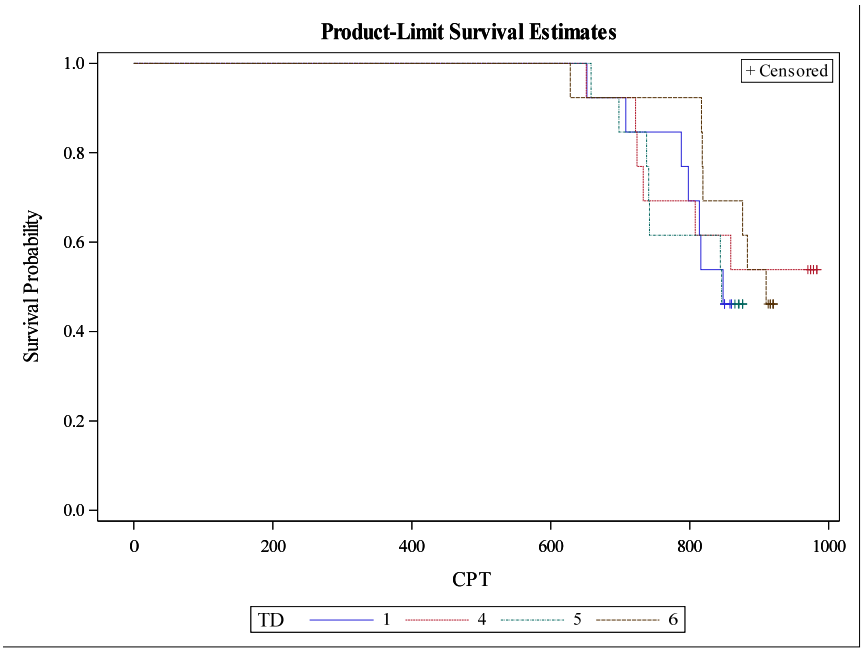
Conclusions

EPA statisticians were asked to review the statistical analysis of the CPT data in the study IR3535. EPA statisticians believe the statistical method (Kaplan-Meier Survival Analysis) used by the registrant to analyze the data CPT of this study was appropriate. EPA statisticians were able to replicate the results of the data analysis of this study. There was no impact on the quality of the data or the results of data analysis due to the skipping of some exposure periods on test day 1, because CPTs were preceded by periods of adequate landing pressure on both controls. Likewise, there was no impact on the quality of the data and the results of data analysis due to break periods longer than 30 minutes on test days 5 and 6, because this did not affect landing pressure. However, two subjects (26 and 35) had CPT during the long break period on test day 4, and the registrant needs to explain how this could happen.

Below are detailed comments from EPA statisticians.

Comment 1: the Kaplan-Meier Survival Analysis used by the registrant to analyze the CPT and estimate the median CPT and its 95% CI was appropriate. EPA statisticians replicated the analysis and obtained the same results as in the report.

Estimated Median CPT from Kaplan-Meier Survival Analysis						
Test Day	**Site**	**Test Substance**	**Estimated Median CPT (minutes)**	**Lower 95% CI (minutes)**	**Estimated Median CPT (hours)**	**Lower 95% CI (hours)**
1	1 (*Aedes)*	Akiva 20 lotion	848	788	14 hrs 8 mins	13 hrs 8 mins
4		Akiva 20 wipe	NC	724	NC	12 hrs 4 mins
5	2 (*Culex*)	Akiva 20 wipe	846	738	14 hrs 6 mins	12 hrs 18 mins
6		Akiva 20 lotion	910	818	15 hrs 10 mins	13 hrs 38 mins
NC = Not calculable. Test days 2 and 3 were unsuccessful due to low mosquito activity.						



Comment 2: skipping exposure periods due to low mosquito pressure on test day 1

The skipping of exposure periods due to the low mosquito pressure on testing day 1 had minimal impact on the median CPT estimate and its 95% CI. The time of skipped exposure periods were 8:37, 12:30, and 14:07. The time of the first subject experienced CPT was at 15:44. Therefore, there were two exposure periods without a CPT after the last skipped exposure period. If the true CPT of a test subject was in the last skipped exposure period, the test subject would have experienced a CPT in the next two exposure periods after the last skipped exposure period.

Comment 3: long break periods on test days 4, 5, and 6

One of the deviations from the protocol was that instead of having a 25-minute break between any two consecutive 5-minute exposure periods, test day 4 had one 53-minute break (16:40 – 17:33), test day 5 had one 52-minute break (23:50 – 00:42), and test day 6 had one 42-minute break (00:31 – 01:13).

- test day 4 had a 53-minute break (16:40 – 17:33). Below is the table of data showing the CPT and the time events. CPT, Break Start, and Break End express the time periods from the time applying the product.

ID	Time Applied	End Time	CPTHM	censor	CPT	Break Start Time	Break End Time	Break Duration	Break Start	Break End
5	5:16	16:07	10.51	N	651	16:40	17:33	53	684	737
35	5:11	17:13	12.02	N	722	16:40	17:33	53	689	742
26	5:09	17:13	12.04	N	724	16:40	17:33	53	691	744
14	5:20	17:33	12.13	N	733	16:40	17:33	53	680	733
30	5:09	18:37	13.28	N	808	16:40	17:33	53	691	744
61	5:19	19:38	14.19	N	859	16:40	17:33	53	681	734
6	5:22	21:32	16.10	Y	970	16:40	17:33	53	678	731

ID	Time Applied	End Time	CPTHM	censor	CPT	Break Start Time	Break End Time	Break Duration	Break Start	Break End
33	5:18	21:32	16.14	Y	974	16:40	17:33	53	682	735
59	5:18	21:32	16.14	Y	974	16:40	17:33	53	682	735
31	5:14	21:32	16.18	Y	978	16:40	17:33	53	686	739
32	5:14	21:32	16.18	Y	978	16:40	17:33	53	686	739
15	5:09	21:32	16.23	Y	983	16:40	17:33	53	691	744
57	5:09	21:32	16.23	Y	983	16:40	17:33	53	691	744
Units of CPT, Break Duration, Break Start, Break End are minutes										

- Test Subjects 26 and 35 had a CPT during the long break period. This may be an error in the recording and the registrant needs to explain how this could happen.
- Test subject 14 had CPT on the exposure period right after the long break. This subject might experience a CPT earlier (CPT = 680 + 25 = 705 minutes) if the break was only 25 minutes instead of 53 minutes. EPA performed a sensitivity analysis where the CPT of test subject was assumed to be 705 minutes. The results were similar to that of the original analysis (the estimated median CPT was unable to estimate due to low number of test subjects experiencing CPT, the lower bound of 95% CI of estimated median CPT was 722 vs. 724 minutes in the original analysis).

- Test day 5 had a 52-minute break (23:50 – 0:42). Below is the table of data showing the CPT and the time events. CPT, Break Start, and Break End express the time periods from the time applying the product.
-

ID	Time Applied	End Time	CPTHM	censor	CPT	Break Start Time	Break End Time	Break Duration	Break Start	Break End
48	17:08	4:06	10.58	N	658	23:50	0:42	52	402	454
59	17:10	4:48	11.38	N	698	23:50	0:42	52	400	452
44	17:06	5:24	12.18	N	738	23:50	0:42	52	404	456
35	17:03	5:24	12.21	N	741	23:50	0:42	52	407	459
32	17:03	5:25	12.22	N	742	23:50	0:42	52	407	459
46	16:56	7:00	14.04	N	844	23:50	0:42	52	414	466
5	16:56	7:02	14.06	N	846	23:50	0:42	52	414	466
65	17:07	7:32	14.25	Y	865	23:50	0:42	52	403	455
60	17:02	7:32	14.30	Y	870	23:50	0:42	52	408	460
31	17:01	7:32	14.31	Y	871	23:50	0:42	52	409	461
15	16:56	7:32	14.36	Y	876	23:50	0:42	52	414	466
33	16:56	7:32	14.36	Y	876	23:50	0:42	52	414	466
62	16:56	7:32	14.36	Y	876	23:50	0:42	52	414	466
Units of CPT, Break Duration, Break Start, Break End are minutes										

- There were many exposure periods after the long break on test day 5 before the first CPT occurring. Therefore, the long break period on test day 5 would not affect the quality of the data in the study.

- test day 6 had a 42-minute break (00:31 – 01:13). Below is the table of data showing the CPT and the time events. CPT, Break Start, and Break End express the time periods from the time applying the product.
-

ID	Time Applied	End Time	CPTHM	censor	CPT	Break Start Time	Break End Time	Break Duration	Break Start	Break End
68	16:55	3:23	10.28	N	628	0:31	1:13	42	456	498
5	16:55	6:32	13.37	N	817	0:31	1:13	42	456	498
26	16:52	6:30	13.38	N	818	0:31	1:13	42	459	501
30	16:50	6:29	13.39	N	819	0:31	1:13	42	461	503
44	16:57	7:33	14.36	N	876	0:31	1:13	42	454	496
66	16:50	7:33	14.43	N	883	0:31	1:13	42	461	503
65	16:55	8:05	15.10	N	910	0:31	1:13	42	456	498
59	16:57	8:10	15.13	Y	913	0:31	1:13	42	454	496
33	16:54	8:10	15.16	Y	916	0:31	1:13	42	457	499
57	16:54	8:10	15.16	Y	916	0:31	1:13	42	457	499
15	16:50	8:10	15.20	Y	920	0:31	1:13	42	461	503
50	16:50	8:10	15.20	Y	920	0:31	1:13	42	461	503
62	16:50	8:10	15.20	Y	920	0:31	1:13	42	461	503
Units of CPT, Break Duration, Break Start, Break End are minutes										

    - There were many exposure periods after the long break on test day 6 before the first CPT occurring. Therefore, the long break period on test day 6 would not affect the quality of the data in the study.

  Given the time of starting and ending a long break period on test day 1, the registrant needs to explain why two test subjects (26 and 35) had CPT during the break.

Comment 4: rounding CPT data

Compared to other mosquito repellency studies, there is a difference in the way the data were entered into the analysis in this study. In other previous studies and in the power analysis, the data were rounded to the nearest half hours. For example, a CPT of 35 mins will be rounded as 30 mins (or 0.5 hrs), a CPT of 123 mins will be rounded as 120 mins (or 2 hrs), etc. The registrant did not round the CPT data to the nearest lower half hour before the data analysis. EPA statisticians concluded that this does not have any substantial impact on the results of the analyses.

Attachment 5: Discussion Between EPA and Study Director on Landing Pressure

- On May 15, 2017, the sponsor, LivFul Inc., submitted an Independent Review Board (IRB) approved draft protocol (Version 5, dated April 23, 2017) of a study to determine the CPT or duration of efficacy of skin-applied repellents containing 20% IR3535, designed to repel mosquitoes in outdoor settings. The protocol and EPA's review, dated June 29, 2017, were discussed at a public meeting by the HSRB on July 26, 2017. The HSRB supported moving forward with testing and made recommendations for the protocol in a report dated October 26, 2017.

- Following the July 2017 HSRB meeting, the researchers revised the protocol and related materials to address the EPA and HSRB comments and submitted the revised documents to the University of Florida (UFL) Independent Review Board (IRB) for review and approval prior to initiating the study. The IRB approved the protocol (Version 7) on March 21, 2018.

- The study was initiated on May 18, 2018, and completed on February 6, 2019. Field testing was initiated on August 15, 2018, and continued on August 19, August 26, September 1, September 8-9, and September 15-16.

- On August 15, 2018, in an email and a phone conversation between the study director (SD) and EPA, the issue of landing pressure was discussed (Attachment 3).

- On August 17, 2018, EPA and the SD discussed, in a phone conversation, options for continuing testing considering problems achieving adequate landing pressure on both control subjects at one site during two prior attempts. EPA suggested switching to a different site; however, SD explained that changing sites at that point was impractical and chose to continue testing at the same site. An option suggested by the SD was the possibility of allowing testing to continue based on adequate landing pressure on at least one control subject. EPA indicated data under this scenario could be reviewed, but that EPA would need to assess whether landing pressure was adequate or not for determining CPT under this scenario.

- On August 17, 2018 (See Attachment 3), SD confirmed via e-mail that no test periods should be skipped for inadequate landing pressure. If both untreated control participants received less than five landings in five minutes in more than four non-consecutive landing periods then the study should be stopped. If one control achieves threshold of five landings in 5 minutes this is considered adequate landing pressure and it would not count towards the number of periods with low landing pressure for the purpose of stopping the study

- The complete IRB approved protocol Version 7, dated March 28, 2018 (pp. 55 thru 149 of 2732), was amended on August 21, 2018, (Amendment 7 on pg. 279 of 2732 of study report) as follows: "*to clarify that a test period with low landing pressure is when both participants have less than 5 landings in 5 minutes. If one of the participants has less but one has 5 landings in 5 minutes or more this is considered adequate landing pressure for the purpose of stopping the study as described in point 4. Point 4: To clarify that test periods that have low landing pressure will not be skipped only those with rain events will be skipped. A total*

of four test periods may be skipped or have low landing pressure. If a fifth test period is skipped or has low landing pressure then the study must stop (pg. 279 of 2732 of study report).

- The agreement between SD and EPA (See Attachment 3) was based on the following condition as stated in EPA email from Dr. Bohnenblust to Dr. Weeks: *"I think this is the best way to proceed. Although I hesitate to commit to saying it would not count toward the number of periods with low landing pressure in an absolute sense. I think we will need to consider based on the whole of the data for a situation whether landing pressure is low, say for instance one control subject continuously has no or few landings vs a situation where they are getting 3 or 4 landings in 5 minutes here and there. So, to allow for some flexibility, i think it might be better phrased as will not count as periods with low pressure for the purpose of stopping the study."* (See Attachment 3: Correspondence between EPA to SD, Dr. Emma Weeks, dated August 17, 2018).

- Upon review of the landing data generated at site 2 for test days 5 and 6, it became evident that the distribution of landings between the 2 control subjects for the majority of the exposure periods occurring between 3rd and 20th period was inconsistent between the 2 control subjects (Refer to Tables 7 and 8, respectively, in this review), indicating that insufficient landings on one control was not due to subject effect (meaning inherent subject's differences in their attractiveness to mosquitoes), but most likely due to low landing pressure for most of the test day.