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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: Evaluation of One Topically Applied Insect Repellent Product Containing Oil of Lemon Eucalyptus Against Three Species of Ticks Under Laboratory Conditions

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REFERENCE: Robert T. Jones, Study Director. (2020) A Single Group Trial to Determine the Complete Protection Time of an Insect Repellent Formulation Containing 30% Citriodiol® (Oil of Lemon Eucalyptus) Against Three Species of Ticks. Unpublished Document. April 23, 2020. The original submission MRIDs are 510045-01 through 510045-28. The revised (24 April 2020) submission MRID is 511322-01 (single MRID), with 28 volumes.

ACTION REQUESTED

A science review of a completed laboratory efficacy testing study for a single skin-applied insect repellent formulation was requested. The formulation was an alcohol-based pump spray containing 30% w/w of Oil of Lemon Eucalyptus (OLE). The formulation was tested against three species of ticks: *Ixodes scapularis; Amblyomma americanum;* and either *Dermacentor variabilis, Dermacentor andersoni,* or *Rhipicephalus sanguineus.* Product performance testing is

required to establish the median Complete Protection Time (mCPT) against ticks to support efficacy claims against ticks on the label of the proposed skin-applied repellent product. A draft protocol for this study was reviewed (DP 457664) and accepted with recommendations by the Environmental Protection Agency (EPA) and Human Studies Review Board (HSRB) at a meeting on April 24, 2018. Protocol Version 3.0 was effective after October 5, 2018, and incorporated EPA and HSRB recommendations. The protocol was amended, including 3 times while the study was active. The study was conducted according to OCSPP 810.3700 Guidelines for Testing of Insect Repellents Applied to Human Skin and Protocol 3.0 and subsequent amendments. Protocol amendments are provided in MRID 511322-01 Vol. 4 (App. 16.3). Protocol deviations occurring during the course of the study are provided in MRID 511322-01 Vol. 5 (App. 16.4). Refer to Attachment 1 for a summary of protocol revisions in response to EPA and HSRB comments and recommendations.

CONCLUSIONS

The EPA evaluated the scientific validity of the research in relation to recommendations from the EPA, HSRB, and the Product Performance Test Guidelines OCSPP 810.3700 for testing of Insect Repellents to be Applied to Human Skin. Study MRID 511322-01 Vol. 1 was conducted in accordance with Good Laboratory Practices (GLP) as described in 40 CFR §160 and provides scientific data that are acceptable to support a Complete Protection Time (CPT) of up to 4 hours. CPT is defined as the time between product application and the time point signaling repellency failure. The HSRB will be asked to comment on this study.

SCIENCE REVIEW

Study objective: The primary objective of this study was to establish the mCPT of a single insect repellent formulation in a pump spray: Coleman Botanicals Insect Repellent Oil of Lemon Eucalyptus (EPA Reg. No. 84878-2), containing 30 % w/w of the active ingredient Citriodiol (OLE) (CAS No. 1245629-80-4; PC Code: 040522). The Certificate of Analysis is provided in MRID 51132201 Vol. 26 (App. 16.10). Three species of ticks, *Ixodes scapularis, Amblyomma americanum,* and either *Dermacentor variabilis, Dermacentor andersoni,* or *Rhipicephalus sanguineus,* were used to evaluate repellency using human volunteer subjects.

The second objective was to estimate the average rate of application applied by consumers [a.k.a. standard (typical) consumer dose] measured as ml/cm^2. Data generated from this study (dosimetry) will be used for labeling claims purposes. The tested hypothesis was that the product applied at a "typical consumer" rate would repel ticks from human hosts for a period of up to 10 hours post-application.

Endpoints: Crossing of questing ticks placed on treated forearms of human subjects was used as the endpoint to evaluate the residual performance of the insect repellent product. Complete Protection Time or CPT was defined as the time between application of the repellent product and the time of repellency failure. Repellency failure is measured by 1 tick crosses 3 cm of treated skin without being repelled, followed by a second tick crossing within 30 minutes from the primary crossing. The second crossing was considered the confirmatory crossing. The mCPT was calculated for each species using Kaplan-Meier survival analysis across a sample size of 25

subjects. The endpoint for estimation of average consumer dose (dosimetry) was the grand mean across 25 subjects. The grand mean for determination of standard consumer dose is calculated from each subject's means (n=25). Subject's means are calculated from triplicated applications per subject, measured in g/cm^2. The typical consumer dose used for testing efficacy was converted from mg/cm^2 to volume, ml/cm^2, using the specific gravity of the product (0.900 g/mL). Testing for the calculation of mCPT as a function of residual repellency was conducted using the tick species identified as representative public health pests, *Rhipicephalus sanguineus*, *Amblyomma americanum*, and *Ixodes scapularis* and using a typical consumer dose of 0.793 µL/cm^2. Testing assessment occurred over a period of 10 hours.

Compliance with Good Laboratory Practice Standards (GLP); 40 CFR, Part 160:
The study was designed as a guideline study in conformity with recommendations from OCSPP 810.3700 Guidelines for Testing of Insect Repellents Applied to Human Skin. This study was conducted in accordance with EPA, FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), and (GLP) Standards (40 CFR, Part 160). A Statement of Compliance with Good Laboratory Practice Standards was provided in the Study Protocol Version 3.4 (MRID 511322-01 Vol. 2 (App. 16.1)). A Quality Assurance Statement, signed and dated on November 14, 2019, was provided on pg. 4 of the study report (MRID 511322-01 Vol. 1)

Identification of the test system: Ticks were the target pest for determining repellency by the product. The objective of the study was to evaluate residual efficacy of the product against three tick species, *Ixodes scapularis, Amblyomma americanum,* and either *Dermacentor variabilis, Dermacentor andersoni,* or *Rhipicephalus sanguineus.* The final evaluation occurred using *Ixodes scapularis, Amblyomma americanum* and *Rhipicephalus sanguineus* at Barbara Sawyer Insectaries, London School of Hygiene and Tropical Medicine (MRID 511322-01 Vol. 28 (App. 16.12).

Rearing and Testing Conditions: Ticks were obtained from National Tick Research and Education Resources at Oklahoma State University (OSU) from pathogen-free colonies. After receipt, male and female ticks were separated and kept in labeled pots in cooled incubators at 25 ± 3 °C; RH: 80%, and 16 L:8 D photoperiod. Labeled tick pots were placed on trays and trays were partially filled with 2 L of potassium sulfate solution. Ticks were handled with gloves. Tick accountability and tests for detection of tick pathogens is provided in (MRID 511322-01 Vol. 24 (App. 16.8)).

Experimental design: Determination of Consumer Dose, Application of Standard Consumer Dose and Procedure for Testing Repellency.

<u>Determination of Consumer Dose</u>

The rate of application for testing repellency was determined by a dosimetry test as indicated in OCSPP 810.3700 Guidelines for Testing of Insect Repellents Applied to Human Skin, across 3 applications per subject and across 25 subjects (12 males and 13 females). The test was conducted outdoors at temperatures ranging from 20 to 25 °C and RH: 35%. Average doses of application per subject (n = 3 applications/person) were used to calculate the grand mean dose of application across 25 subjects (n = 75). Bracelets were used to collect amount of product applied

and calculate typical applied dose by dividing the amount of the product collected on the bracelets by the surface area of the 3 bracelets combined. A single set of bracelets, (3 cm wide) consisted of one bracelet for the lower arm, one for the middle portion of the arm, and one for the upper forearm. Three sets of bracelets (total of 9) were allocated for each subject. Individual sets were placed into a plastic bag and the three sets were placed in a larger bag and labeled for each subject. Prior to dosimetry testing for determination of standard consumer dose, subjects were instructed to read the use instructions on the label and carry out a practice application. Following the practice application, either arm was selected as the test arm and cleansed with unscented soap. The second application was performed on the arm opposite to the one used first, and the third application was performed on either arm randomly selected. Third arm randomization list is provided in MRID 511322-01 Vol. 8 (App. 16.7a). Arms were cleansed with unscented soap between applications. Three bracelets were each evenly placed at lower, middle and upper forearm, and circumferences and width of bracelets were recorded to calculate their surface area. Each bracelet inside its individual plastic bag was individually weighed before and after each application to measure the amount of product applied. Average consumer rate of application (mg/cm^2) was calculated from triplicated amounts of product applied to individual bracelets (difference or increment in bracelets' weight before and after each application) divided by their surface area. The mean for each subject was calculated across 3 replications per subject, and the grand mean was calculated across all subjects' means. The specific gravity of the product (0.900 mg/mL) was used to convert to volumetric dose (mL/cm^2) used for testing. Data on consumer dose (dosimetry) are provided in and MRID 511322-01 Volumes 10 through 23 (Appendixes 16.7c through 16.7p).

Application of Consumer Dose

The surface area of subjects forearms was calculated on their first visit according to the formula: (sum of bracelet set circumferences) X 1/3 (length of the forearm) for those subjects who participated in the dosimetry and repellency phases. Subjects who did not participate in the dosimetry phase did not have bracelet circumferences, so their forearm circumference was measured at 3 points and multiplied by 1/3 to estimate the surface area of their forearms. Hairs from the wrist area of the test arm were clipped 3 cm above the wrist. Boundary and release lines were delineated on treated and control arms in a similar fashion. The boundary line was delineated around both wrists; the release line was placed 3 cm from the boundary line toward the fingers. Average consumer dose of application was used as the standard consumer dose across all subjects for testing repellency. The amount applied to each subject was measured with a micropipette, adjusted to the surface area of each subject's forearm and spread evenly on subject forearm using a gloved finger. The difference in weight of the bottle before and after application was recorded (MRID 511322-01 Vol. 26 (App. 16.10)). A Certificate of Analysis of the test substance is provided in MRID 511322-01 Vol. 26 (App. 16.10). Difference in gloves weight before and after application were recorded in MRID 51132201 Vol. 26 (App. 16.10)). The difference is negligible. Treated arms used for testing repellency were selected according to a randomization schedule (MRID 511322-01 Vol. 8 (App. 16.7a).

Repellency Testing Procedure

Tick species for testing repellency were selected according to randomization schedule (MRID 511322-01 Vol. 24 (App. 16.8). Forty-five ticks of single species, mixed sex were placed in an insectary chamber 30 minutes prior to testing; half were kept into humidity chambers for later use. Tick species, batch number, life stage, number of days at London School of Hygiene and Tropical Medicine (LSHTM), and day of last blood feeding were recorded (MRID 511322-01 Vol. 24 (App. 16.8). The test area was labelled with the participant ID, initials and test date. The test area was equipped with its own thermo-hygrometer, timer and 3 white trays consisting of a single tray for each arm and one tray for holding ticks. Ticks were placed in bowls lined with filter paper and the bowls were placed in one of the 3 trays. The tray holding the bowls with ticks was partially filled with water. Four paint brushes for transfer of ticks, and forceps were provided for handling ticks. Testing began 15 minutes post-product application. Subjects placed their untreated arm at a 30° angle from the bottom of the tray, with their fingers resting flat on the bottom of the tray. Arm support was provided. Ticks were individually screened for active questing, placed on the release line with their mouth parts oriented toward the elbow. A tick qualified as actively questing if it crawled from the release line across the boundary line and upward within 3 minutes of being released. If an active tick was not found in 10 minutes, that time point for testing repellency was missed, and the Protocol Version 3.4 was amended to stop testing when more than 6 time points were missed due to lack of questing behavior as proposed by EPA on July 1, 2019. If a subject missed 6 time points with 1 tick species, he or she was permitted to test with a second and third species; however, if the same subject missed 6 time points with 2 different tick species, that subject was replaced with another subject. Once a questing tick was identified, the tick was placed on the released line of the treated arm which had been placed on a second tray arranged in identical fashion as on the control tray. The movement of the tick was timed. A tick was classified as repelled if the tick did not cross the boundary line or crossed the boundary line but turned back or fell off before 1 minute. A tick was classified as not repelled if it crossed the boundary line into the treated area and remained there for at least 1 minute. Exposure time points were repeated with 1 tick at 15-minute intervals for 10 hours. The CPT was defined by the first non-repelled tick crossing the boundary line followed by a second non-repelled tick crossing within 30 minutes (two time periods) apart from the first. Since time points were 15 minutes apart, a confirmatory second crossing could have been preceded by a repelled tick 15 minutes apart from the first non-repelled tick. One time point was always missed for lunch break and it was not accounted as missed time period. If a confirmatory crossing occurred immediately following a lunch break, the CPT would be considered to have occurred at the period missed during lunch.

Statistical Analysis and Sample Size Determination:

Sample Size Determination: EPA (science, statistics, and ethics) and LSHTM agreed that a sample size of 25 was adequate to ensure that the study included enough subjects to return reliable results without unnecessarily including more subjects than necessary. Sample size determination of 25 subjects per treatment was based on the EPA power analysis calculations in Appendix 3: Power/Sample Size Calculation, within EPA's Science and Ethics Review Memo, dated March 30, 2018, for review of Protocol Version 0.1, dated July 21, 2017.

Median Complete Protection time (mCPT): mCPT was estimated using Kaplan-Meier Survival Analysis. The lower 95% confidence intervals (CI) and Upper 95% CI were calculated for *I. scapularis* and *R. sanguineus* (Tables 5 and 6). Upper 95% CI were not calculated for *A. americanum* due to lack of information resulting from right censored data (Table 4).

Protocol Revisions, Amendments and Deviations:

All protocol revisions and amendments are reported in study report, MRID 511322-01 Vol. 1, Section 10.5 *Protocol Amendments*, and submitted in MRID 511322-01 Vol. 4 (App. 16.3).

The original protocol, Protocol Version 1.0 was created on July 21, 2017 and updated to Protocol Version 2.0 on June 29, 2018, following EPA recommendations on science and ethics and HSRB requirement for GLP compliance. Protocol Version 2.0 was amended 5 times: Protocol Version 3.0, (September 21, 2018), and Protocol Version 3.1 (November 16, 2018), were amended before consumer dose testing began. The protocol was amended again to Version 3.2 (April 26, 2019); Version 3.3 (July 26, 2019), and Version 3.4 (August 23, 2019). A summary of protocol revisions and amendments, in conformity with EPA and HSRB recommendations, is provided in Attachment 1.

List of Protocol Revisions:

Changes to Protocol Version 3.0 included: 1) expanding recruitment beyond London area as recommended by HSRB; 2) addition of comprehension check list to the consent process; 3) removal of 1 week follow-up post-repellency testing; 4) addition of shaving procedure up to 3 cm from wrist; 5) addition of weighing gloves before and after product application; 6) simplified definition of repellency behavior (no requirement for measuring distance traveled by tick); 7) addition of rest period for meal break during efficacy testing; 8) increase compensation to minimum wage; 9) addition of additional conditions to exclusion criteria; 10) addition of additional bracelet for arm circumference measurement; 11) addition of additional detail on non-availability of Epi-Pens and First Aid; 12) change of medical monitor to Dr. Nicky Longley, and 13) administrative changes (removal of Citriodiol proprietary information; change in study dates, and addition of sponsor to signatories).

Changes to Protocol Version 3.1 included: 1) change in study director; 2) change of medical monitor; 3) inclusion of ethics review board and clinicaltrials.gov reference numbers; 4) changes to study dates; 5) addition of certificate of analysis; and 6) change from Chief Investigator to ARTEC Director in compliance with GLP requirements.

The third protocol amendment, Protocol Version 3.2, dated April 26, 2019, was made after consumer dose testing and before repellency testing.

Changes to Protocol Version 3.2 included: 1) Change of study director and addition of new study director and his contact information to Participants Information Sheets; 2) corrections of grammatical and typographical errors; 3) reduction of RH from 90% to 80% following discussion with EPA; and 4) change from ethanol to isopropanol. Protocol Version 3.2 was effective on April 30, 2019.

The fourth protocol amendment, Protocol Version 3.3, dated July 26, 2019, and the fifth protocol amendment, Protocol Version 3.4, dated August 23, 2019, were made during repellency testing.

Changes to Protocol Version 3.3 included: 1) addition of alternative species of ticks, following discussion with EPA; 2) addition of alternative tick supplier; 3) update of general practitioner telephone number; and 4) update of project timelines. Protocol Version 3.3 was effective on July 31, 2019.

Changes to Protocol Version 3.4 included: 1) criteria for missed time points, replacement of subjects, and 2) criteria for use of data from withdrawn subjects. Protocol Version 3.4 was effective on August 23, 2019.

Protocol Deviations:
There were several protocol deviations that occurred during the study. Protocol deviations are listed in Section 10.6 and Section 10.7 of the study report, MRID 511322-01 Vol. 1, and in MRID 511322-01 Vol. 5 (App.16.4). There were 10 subject specific deviations reported in Section 10.6, and 4 not subject specific protocol deviations reported in Section 10.7 of MRID 511322-01 Vol. 1

Subject specific protocol deviations occurring during repellency testing:

> Deviation #1 in Section 10.6 in MRID 511322-01 Vol. 1 and MRID 511322-01 Vol. 5 (App. 16.4):
> Use of outdated Case Report Form, dated Jan. 17, 2019, on subject 593027 for tick repellent test day May 8, 2019. Additional information was added to the form resulting in no loss of data.
>
> Deviation #2 in Section 10.6.2 in MRID 511322-01 Vol. 1 and MRID 511322-01 Vol. 5 (App. 16.4):
> Test substance (TS) was applied to incorrect arm (left arm) of subject 593039 testing *I. scapularis* on May 24, 2019, resulting in 2 µL more product because surface area of left arm was smaller. This amount, 2 µL, was within the margin of standard error and therefore, it was not expected to compromise the validity of the data.
>
> Deviation #3 in Section 10.6.3 in MRID 510045-01 MRID 511322-01 Vol. 1 and in MRID 51132201 Vol. 5 (App. 16.4):
> TS was applied to left rather than right arm of subject 593067 while testing *I. scapularis* on June 4, 2019. The correct dose was applied. The study director required second reading of randomization schedule to avoid further mistakes.
>
> Deviation #4 in Section 10.6.4 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4) :
> Temperature was off range for 1°C on 3 occasions. Temperature was 1°C above 25°C on July 30, 2019, for subjects 593117 and 593016, and 1°C below 25°C on July 30, 2019, for subject 593117. On September 6, 2019, the temperature was 1°C above 25°C for

subject 593101, testing *R. sanguineus*. These small fluctuations are unlikely to compromise test results. Tick species have geographical range that exceeds 30 °C in summer months.

Deviation #5 in Section 10.6.5 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Repellency testing with *A. americanum* was conducted earlier than should have been on subject 593053 (Table 14 in Study report MRID 522322-01 Vol.1). Testing for time point 1h:30 minutes post-application was scheduled for 11:30 but it took place at 10:58 by mistake. No crossing event occurred at that time point and CPT occurred at 12:07, suggesting that this deviation did not impact validity of data.

Deviation # 6 in Section 10.6.6 in MRID 511322-01 Vol. and in MRID 511322-01 Vol. 5 (App. 16.4):
Subject 593092 tested *I. scapularis* on July 1, 2019, *A. americanum* on August 28, 2019, and *R. sanguineus* on September 19, 2019. The consumer dose applied to subject on first visit was 0.801 $\mu L/cm^2$. The consumer dose applied on the 2nd and 3rd visits was 0.793 $\mu L/cm^2$ (see Section 10.7.3 in Study Report, MRID 511322-01 Vol. 1 The dose of 0.801 $\mu L/cm^2$ resulted from miscalculation of average consumer dose across 24 rather than 25 subjects, because data from subject 593075 were excluded from the calculation by mistake. When the average is calculated across 25 subjects, the average consumer dose is 0.793 $\mu L/cm^2$. Efficacy data gathered with 0.801$\mu L/cm^2$ was used for determination of CPT, because the difference of 0.008 $\mu L/cm^2$ is within the margin of standard error of the mean (± 0.217 $\mu L/cm^2$). The average consumer dose of 0.793 $\mu L/cm^2$ was used from this time forward. The tests conducted with 0.801$\mu L/cm^2$ are listed on Table 8 in MRID 511322-01 Vol. 1

Deviation # 7 in Section 10.6.7 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Data from subject 593120 testing *A. americanum* on September 17, 2019, was not used on calculation of CPT because this subject left earlier than 10 hours test duration (this subject was not withdrawn from the study; he tested *R. sanguineus* on September 20, 2019). From this time forward, subjects were reminded of the duration of the test and asked whether they could commit to it.

Deviation # 8 in Section 10.6.8 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Clipping of hairs from wrist area was not performed on all participants because it was not needed or possible. Study director observed no difference in results due to clipping or not clipping hair.

Deviation # 9 in Section 10.6.9 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
On July 25, 2019, subject 593107 missed a time point 12:33, between time points 12:18 and 12:48. Lunch break took place after time point 13:48, resuming testing at 14:20.

There were no crossing events until 16:50, when CPT was reached, suggesting that missed time points did not coincide with product failure.

Deviation # 10 in Section 10.6.10 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Humidity was out of range in 4 occasions. On Sept. 5, and Sept. 13, 2019, RH dropped to 31% for subjects 593117 and 593070 testing *R. sanguineus.* RH dropped to 33% on Oct. 2, 2019, for subject 593100 testing *R. sanguineus.* All three subjects experienced CPT. RH dropped to 34% on June 6, 2019, when test subject 593050 tested *A. americanum.* No report of unusual tick behavior or missed time points due to lack of questing.

Not subject specific protocol deviations occurring during repellency testing:

Deviation #1 in Section 10.7.1 MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Thermo-hygrometer placed outside tick storage container (incubator), leading to RH reading below require 80% on April 19, 2019 (pg. 497 of 4553 in Appendix 16.4 MRID 510045-05).

Deviation #2 in Section 10.7.2 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Minimum RH in tick storage container (incubator) was below protocol requirement (RH = 80%) in May 28 (RH=79%) and June 4, (RH=78%). On June 10, 2019, the minimum RH in the incubator was 76% and the maximum was 88%.

Deviation #3 in Section 10.7.3 in MRID 51132201 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Consumer dose miscalculation. The dose 0.801. $\mu L/cm^2$ resulted from miscalculation of average consumer dose across 24 rather than 25 subjects, because data from subject 593075 were excluded from the calculation by mistake. When the average is calculated across 25 subject, the average consumer dose is 0.793 $\mu L/cm^2$. Efficacy data gathered with 0.801$\mu L/cm^2$ was used for determination of CPT, because the difference of 0.008 $\mu L/cm^2$ is within the margin of standard error of the mean ($\pm$ 0.217 $\mu L/cm^2$). This deviation was communicated to EPA on July 5, 2019. EPA recommended to continue the study with the 0.48 g/ 600 cm^2 (0.793 $\mu L/cm^2$). The total of tests conducted with application rate of 0.801$\mu L/cm^2$ are listed on Table 8 on page 45 of 4553 in MRID 511322-01 Vol 1. There was a total of 7 tests conducted on *A. americanum;* 9 with *I. scapularis,* and 4 with *D. variabilis*.

Deviation #4 in Section 10.7.4 in MRID 511322-01 Vol. 1 and in MRID 511322-01 Vol. 5 (App. 16.4):
Excessive missed time points due to lack of tick questioning on May 17, May 28, July 23, Aug. 5, Sept. 3, Sept. 6, Sept. 10, Sept. 14, Sept. 26, and Oct. 7, 2019, for subjects 591 593069, 593027, 593036, 593004, 503067, 593113, 593122, and 593146.

The criteria for repeating a test depended on the number of missing periods due to the lack of tick questing. When no questing tick was found within 10 minutes on the control arm, the time point was missed. When more than 6 time points were missed due to lack of tick questing, the test should have been repeated. If the same subject failed a test with two tick spp., that subject should have been replaced due to possible lack of subject's lack of attractiveness to ticks (Section 10.5, in MRID 511322-01 Vol 1). Test subjects were assigned testing with *I. scapularis*, *A. americanum* and *D. variabilis* according to a randomized schedule (511322-01 Vol. 8 (App. 16.7a). There were several time points missed due to lack of questing with *D. variabilis*. After discussing the matter with EPA on July 1, 2019, it was decided to discontinue testing with this species. While searching for a third species to replace *D. variabilis*, testing continued with *I. scapularis* and *A. americanum* since test days were independent of each other. EPA suggested testing with *D. andersoni* or *R. sanguineus* as replacements for *D. variabilis*. Since *D. andersoni* was not available for purchase, *D. variabilis* was replaced with *R. sanguineus.* Data collected from *D. variabilis* (Table 13 in 511322-01 Vol. 1) was not used for assessment of CPT against ticks.

RESULTS

Dosimetry: Determination of Average Consumer Dose

The standard consumer dose = 0.0007135 g/cm^2, is the overall mean of all subjects means converted to volume, 0.0007928 mL/ cm^2, using the specific gravity = 0.9 g/ml, and expressed as 0.793 µL/cm^2. Table 1 (from Table 1a through Table 1e) summarizes results from dosimetry test used to calculate average consumer dose in MRIDs 511322-01 Vol. 10 (App. 16.7c) through MRID 511322-01 Vol. 23 (App. 16.7p) and presented in Tables 10 to 12 of study report (MRID 511322-01 Vol. 1). The total from all 25 consumer dose means is 0.0007135 g/ cm^2, converted to volume by dividing 0.0007135 g/ cm^2 by specific gravity of the product, 0.9 g/mL. The result is 0.00079278 mL/cm^2, converted to 0.7928 µL/ cm^2, rounded to 0.793 µL/cm^2.

Table 1.a Consumer Applications for Determination of Standard Consumer Dose					
Subject ID	**Replication**	**Amount on bracelet (g)**	**Bracelets' SA (cm^2)**	**Dose (g/cm^2)**	**Average Dose/ subject (g/cm^2)**
593004	1	0.313	162.96	0.0019	
	2	0.363	162.62	0.0022	
	3	0.275	164.86	0.0017	
Total / Subject		**0.952**	**490.44**	**0.0058**	**0.0019**
593006	1	0.049	193.13	0.0003	
	2	0.073	175.55	0.0004	
	3	0.046	174.72	0.0003	
Total / subject		**0.169**	**543.40**	**0.001**	**0.0003**
593011	1	0.156	180.65	0.0009	
	2	0.177	184.54	0.0010	
	3	0.100	168.40	0.0006	
Total / subject		**0.434**	**533.59**	**0.0025**	**0.0008**
593012	1	0.039	180.47	0.0002	
	2	0.121	185.78	0.0007	
	3	0.055	175.42	0.0003	
Total/ subject		**0.215**	**541.67**	**0.0012**	**0.0004**
593013	1	0.024	158.29	0.0002	
	2	0.046	160.94	0.0003	
	3	0.019	155.97	0.0001	
Total/ subject		**0.090**	**475.20**	**0.0006**	**0.0002**

Table 1.b Consumer Applications for Determination of Standard Consumer Dose (cont.)					
Subject ID	**Replication**	**Amount on bracelet (g)**	**Bracelets' SA (cm^2)**	**Dose (g/cm^2)**	**Average Dose/ subject (g/cm^2)**
593017	1	0.870	149.68	0.0058	
	2	0.393	149.61	0.0026	
	3	0.619	145.82	0.0042	
Total / Subject		**1.881**	**445.11**	**0.0126**	**0.0042**
593019	1	0.005	174.32	0.0000	
	2	0.003	151.82	0.0000	
	3	0.002	154.75	0.0000	
Total / subject		**0.009**	**480.89**	**0.0000**	**0.0000**
593021	1	0.091	161.20	0.0006	
	2	0.020	168.46	0.0001	
	3	0.089	165.09	0.0005	
Total / subject		**0.20**	**494.75**	**0.0012**	**0.0004**
593022	1	0.056	173.40	0.0003	
	2	0.124	173.40	0.0007	
	3	0.082	175.13	0.0005	
Total/ subject		**0.261**	**521.93**	**0.0015**	**0.0005**
593024	1	0.107	209.11	0.0005	
	2	0.079	183.13	0.0004	
	3	0.169	206.93	0.0008	
Total/ subject		**0.355**	**599.17**	**0.0017**	**0.0006**

Table 1.c Consumer Applications for Determination of Standard Consumer Dose (cont.)					
Subject ID	**Replication**	**Amount on bracelet (g)**	**Bracelets' SA (cm^2)**	**Dose (g/cm^2)**	**Average Dose/ subject (g/cm^2)**
593025	1	0.043	169.35	0.0003	
	2	0.062	157.64	0.0004	
	3	0.093	163.84	0.0006	
Total / Subject		**0.198**	**490.83**	**0.0013**	**0.0004**
593027	1	0.025	184.25	0.0001	
	2	0.022	191.07	0.0001	
	3	0.001	197.78	0.0000	
Total / subject		**0.048**	**573.10**	**0.0002**	**0.0001**
593028	1	0.046	155.50	0.0003	
	2	0.092	157.12	0.0006	
	3	0.031	165.16	0.0002	
Total / subject		**0.169**	**477.78**	**0.0011**	**0.0004**
593032	1	0.228	194.85	0.0012	
	2	0.173	221.12	0.0008	
	3	0.237	203.55	0.0012	
Total/ subject		**0.638**	**619.52**	**0.0032**	**0.0010**
593039	1	0.027	163.73	0.0002	
	2	0.025	166.81	0.0002	
	3	0.037	157.49	0.0002	
Total/ subject		**0.089**	**488.03**	**0.0006**	**0.0002**

Table 1.d Consumer Applications for Determination of Standard Consumer Dose (cont.)					
Subject ID	**Replication**	**Amount on bracelet (g)**	**Bracelets' SA (cm^2)**	**Dose (g/cm^2)**	**Average Dose/ subject (g/cm^2)**
593041	1	0.111	175.55	0.0006	
	2	0.062	173.36	0.0004	
	3	0.110	179.18	0.0006	
Total / Subject		**0.282**	**528.09**	**0.0016**	**0.0005**
593046	1	0.024	168.74	0.0001	
	2	0.016	176.43	0.0001	
	3	0.022	163.71	0.0001	
Total / subject		**0.061**	**508.88**	**0.0003**	**0.0001**
593047	1	0.033	180.24	0.0002	
	2	0.025	170.55	0.0001	
	3	0.038	177.57	0.0002	
Total / subject		**0.095**	**528.36**	**0.0005**	**0.0002**
593050	1	0.040	152.55	0.0003	
	2	0.019	180.56	0.0001	
	3	0.029	162.81	0.0002	
Total/ subject		**0.88**	**495.92**	**0.0006**	**0.0002**
593053	1	0.059	169.56	0.0003	
	2	0.062	191.16	0.0003	
	3	0.090	196.28	0.0005	
Total/ subject		**0.210**	**557.00**	**0.0011**	**0.0004**

Table 1.e Consumer Applications for Determination of Standard Consumer Dose (cont.)					
Subject ID	**Replication**	**Amount on bracelet (g)**	**Bracelets' SA (cm^2)**	**Dose (g/cm^2)**	**Average Dose/ subject (g/cm^2)**
593063	1	0.526	162.65	0.0032	
	2	0.272	154.03	0.0018	
	3	0.647	157.94	0.0041	
Total / Subject		**1.446**	**474.62**	**0.0091**	**0.0030**
593067	1	0.130	175.03	0.0007	
	2	0.016	169.87	0.0001	
	3	0.070	182.10	0.0004	
Total / subject		**0.216**	**527**	**0.0012**	**0.0004**
593070	1	0.056	183.67	0.0003	
	2	0.051	184.86	0.0003	
	3	0.021	184.29	0.0001	
Total / subject		**0.129**	**552.82**	**0.0007**	**0.0002**
593071	1	0.138	157.71	0.0009	
	2	0.120	154.97	0.0008	
	3	0.107	161.86	0.0007	
Total/ subject		**0.365**	**474.54**	**0.0024**	**0.0008**
593075	1	0.060	170.01	0.0003	
	2	0.132	166.40	0.0008	
	3	0.0791	161.83	0.0005	
Total/ subject		**0.271**	**498.24**	**0.0016**	**0.0005**

Application of Standard Consumer Dose for Testing Efficacy

Results from application of standard consumer dose and repellency testing are presented on Tables 2 and 3, respectively. Table 2 presents dose applied to each test subject to achieve the standard consumer dose for all test subjects. Subjects participating in repellency testing were applied standard consumer dose by adjusting amount of applied product to the surface area of each subject forearm (Table 2).

Table 2. Dose Applied to Individual Subjects to Achieve Standard Consumer Dose								
Amblyomma americanum			***Ixodes scapularis***			***Rhipicephalus sanguineus***		
Subject ID (R or L) arm	**Arm SA cm^2**	**Dose $\mu L/cm^2$**	**Subject ID (R or L) arm**	**Arm SA cm^2**	**Dose $\mu L/cm^2$**	**Subject ID (R or L) arm**	**Arm SA cm^2**	**Dose $\mu L/cm^2$**
593027 (R)	667.2	534.4	593070 (R)	563.2	451.1	593107 (L)	656.86	520.9
593019 (R)	495.07	397	593027 (R)	667.2	534.4	593117 (L)	548.6	435.04
593022 (L)	544	435.74	593047 (L)	478.6	381.8	593091 (R)	514.4	407.92
593070 (L)	530.5	424.9	593039 (R)	488.3	391.1	593093 (L)	482.856	382.9

Table 2. Dose Applied to Individual Subjects to Achieve Standard Consumer Dose								
Amblyomma americanum			*Ixodes scapularis*			*Rhipicephalus sanguineus*		
Subject ID (R or L) arm	**Arm SA cm^2**	**Dose $\mu L/cm^2$**	**Subject ID (R or L) arm**	**Arm SA cm^2**	**Dose $\mu L/cm^2$**	**Subject ID (R or L) arm**	**Arm SA cm^2**	**Dose $\mu L/cm^2$**
593007 (R)	515.85	413.2	593011 (R)	658	527.1	593039 (R)	488.3	391.1
593028 (R)	485.92	389.2	593024 (L)	581.7	465.9	593054 (L)	732.4	586.6
593050 (R)	516	414.08	593050 (L)	392.84	490.44	593047 (R)	475.96	381.2
593001 (R)	620.4	491.98	593067 (L)	641.72	514	593070 (R)	563.2	446.7
593039 (L)	486.08	389.4	593001 (L)	588.7	466.83	593122 (L)	651.75	516.84
593014 (L)	490	392.49	593069 (R)	624.6	500.3	593092 (L)	478.4	383.13
593047 (L)	476.6	381.8	593016 (L)	455.5	364.9	593087 (L)	524.5	415.9
593026 (L)	506.1	405.39	593036 (R)	665.9	528.06	593120 (L)	487.55	386.63
593036 (L)	681.6	540.51	593054 (L)	732.4	586.6	593116 (L)	409.5	324.7
593054 (R)	682.4	546.6	593007 (L)	508.75	407.5	593119 (L)	577.92	458.29
593002 (R)	498.83	399.56	593092 (R)	448.06	358.9	593124 (L)	546.163	433.11
593069 (R)	624.6	500.3	593086 (R)	637.32	505.39	593100 (R)	382.26	303
593107 (L)	656.86	520.9	593082 (R)	549.2	435.5	593131 (R)	676.7	536.6
593016 (R)	462.9	370.08	593117 (R)	540.9	428.94	593177 (R)	735	582,86
593091 (R)	514.4	407.92	593111 (R)	479	379.9	593127 (R)	578.85	459.02
593111 (L)	479	379.9	593084 (L)	519.49	411.96	593123 (R)	606.51	480.96
593084 (R)	519.96	412.33	593093 (R)	485	384.6	593156 (L)	463	367.2

Table 2. Dose Applied to Individual Subjects to Achieve Standard Consumer Dose								
Amblyomma americanum			***Ixodes scapularis***			***Rhipicephalus sanguineus***		
Subject ID (R or L) arm	**Arm SA cm^2**	**Dose $\mu L/cm^2$**	**Subject ID (R or L) arm**	**Arm SA cm^2**	**Dose $\mu L/cm^2$**	**Subject ID (R or L) arm**	**Arm SA cm^2**	**Dose $\mu L/cm^2$**
593092 (L)	478.4	383.13	593091 (L)	493.93	391.69	593076 (R)	621.98	493.23
593113 (L)	489.06	387.82	593102 (R)	430.05	341.03	593133 (L)	631.68	500.92
593110 (L)	589.58	467.54	593110 (R)	594.63	471.54	593165 (L)	529.5	419.9
593123 (R)	606.51	480.96	593000 (L)	398.45	315.97	593130 (R)	622.89	493.95

Data from MRID 511322-01 Vol.10 (App. 16.7c) through MRID 511322-01 Vol. 23 (App. 16.7p)

Efficacy Testing for calculation of mCPT

Table 3 summarizes results from efficacy testing for determination of mCPT. The product was tested using the standard consumer dose against three tick species, *A. americanum*, *I. scapularis*, and *R. sanguineous,* with sample size of 25 subjects. Testing using *A. americanum* resulted in 10 subjects experiencing CPT. More than half the sample, 15/25 = 60%, of total subjects were censored. CPT for *A. americanum* ranged from a minimum of 156 minutes, ~2:00 hours, to a maximum of 558 minutes, ~9:00 hours for only 40% of the subjects tested. Therefore, the CPT for *A. americanum* could not be calculated but only estimated to be above 10 hours. This estimation is based on 15 subjects out of 25 that were right censored because they did not experience CPT prior to the end of the test day. Refer to Table 4 for results of statistical analysis on *A. americanum* test. Testing using *I. scapularis* resulted in 18 subjects experiencing CPT. CPT for *I. scapularis* ranged from a minimum of 15 minutes, and a maximum of 466 minutes, ~7:00 hours. Testing using *R. sanguineus* resulted in 16 subjects experiencing CPT. CPT for *R. sanguineus* ranged from a minimum of 17 minutes, and a maximum of 570 minutes, ~9:00 hours. Hours were rounded down to the lower whole hour.

Table 3. Recorded CPT or Censored data (CPT or C) by tick species											
Amblyomma americanum				*Ixodes scapularis*				*Rhipicephalus sanguineus*			
Date m/d/19	**Subject ID**	**Time (min)**	**CPT or C**	**Date m/d/19**	**Subject ID**	**Time (min)**	**CPT or C**	**Date m/d/19**	**Subject ID**	**Time (min)**	**CPT or C**
5/28	593019	602	C	5/15	593070	571	C	9/4	593107	349	CPT
5/29	593022	601	C	5/21	593027	346	CPT	9/5	593117	570	CPT
6/4	593070	606	C	5/22	593047	151	CPT	9/6	593091	610	C
6/5	593007	602	C	5/24	593039	15	CPT	9/9	593093	600	C
6/6	593028	480	CPT	5/30	593011	601	C	9/10	593039	495	CPT
6/6	593050	603	C	5/31	593024	576	C	9/11	593054	603	C
6/11	593001	210	CPT	5/31	593050	602	C	9/12	593047	377	CPT
6/11	593039	601	C	6/4	593067	409	CPT	9/13	593070	528	CPT
6/12	593014	604	C	6/5	593001	225	CPT	9/13	593122	540	CPT
6/12	593047	360	CPT	6/10	593069	241	CPT	9/19	593092	601	C
6/13	593026	600	C	6/17	593016	108	CPT	9/20	593187	586	C
6/13	593036	421	CPT	6/19	593036	602	C	9/20	593120	602	C
6/14	593054	211	CPT	6/24	593054	167	CPT	9/24	593116	481	CPT
6/18	593002	602	C	6/27	593007	290	CPT	9/25	593119	512	CPT
6/28	593069	558	CPT	7/1	593092	155	CPT	9/27	593124	602	C
7/25	593107	452	CPT	7/22	593086	608	C	10/2	593100	272	CPT
7/30	593016	600	C	7/24	593082	112	CPT	10/4	593131	601	C
8/9	593091	593	C	7/30	593117	466	CPT	10/8	593177	258	CPT
8/12	593111	603	C	8/2	593111	136	CPT	10/9	593127	197	CPT
8/19	593084	601	C	8/6	593084	17	CPT	10/10	593123	601	C
8/28	593092	601	C	8/13	593093	213	CPT	10/11	593156	137	CPT
9/18	593113	465	CPT	8/23	593091	600	C	10/14	593076	420	CPT
9/19	593110	600	C	9/3	593102	120	CPT	10/16	593133	46	CPT
10/3	593123	156	CPT	9/11	593110	435	CPT	10/16	593165	105	CPT
8/5	593027	525	CPT	9/17	503000	361	CPT	10/18	593130	17	CPT

Data from MRID 511322-01 Vol. 10 (App. 16.7c) through MRID 511322-01 Vol. 23 (App. 16.7p) and Tables 13 to 16 in MRID 511322-01 Vol. 1

Results from Statistical Analysis

Results from statistical analysis and calculation of mCPT (MRID 511322-01 Vol. 27 (App.16.11)) are summarized in Tables 4, 5, and 6 for each tick species. For *A. americanum* the estimated mCPT > 600 minutes (10 hours.) (Table 4). For *I. scapularis* the calculated mCPT = 290.00 minutes (approximately 4 hours, rounded to the lower value) (Table 5). For *R. sanguineus* the mCPT = 512 minutes (approximately 8 hours, rounded to the lower value) (Table 6).

Table 4. Results from Statistical Analysis: mCPT for *A. americanum*
Estimated mCPT > 600 minutes. Means and Medians for Survival Analysis

MEAN[a]				MEDIAN			
		95% Confidence Interval				95% Confidence Interval	
Estimate	Std Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
517.12	27.74	462.75	571.49				

[a] Estimation is limited to the largest survival time if it is censored.

Table 5. Results from Statistical Analysis: mCPT for *I. scapularis*
mCPT = 290.00 minutes. Means and Medians for Survival Analysis

MEAN[a]				MEDIAN			
		95% Confidence Interval				95% Confidence Interval	
Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
328.920	41.514	247.552	410.288	290.000	100.753	92.525	478.475

[a] Estimation is limited to the largest survival time if it is censored.

Table 6. Results from Statistical Analysis: mCPT for *R. sanguineus*
mCPT = 512.00 minutes. Means and Medians for Survival Analysis

MEAN[a]				MEDIAN			
		95% Confidence Interval				95% Confidence Interval	
Estimate	Std Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
431.760	39.480	354.379	509.141	512.000	39.135	435.295	588.705

[a] Estimation is limited to the largest survival time if it is censored.

EPA's Discussion and Conclusions:

Among the 3 species tested, *I. scapularis* was recorded with the lowest CPT of ~4 hours protection time. For labeling purposes, the Agency chooses the most conservative mCPT of the 3 species. Therefore, the product has been tested on 3 species of ticks and the results of the test support a residual efficacy claim of 4 hours against ticks on the product label.

Conformity with Protocol and Amendments: The protocol was reviewed by EPA and the HSRB. The protocol was revised to Protocol Version 3.0 address recommendations from both organizations and was approved by the Western Institutional Review Board on October 5, 2018. Overall, the protocol was amended 5 times.

The reported study conformed with the protocol, specifically on the following: 1) residual efficacy test to calculate mCPT on 3 species of ticks, *Ixodes scapularis; Amblyomma*

americanum; and either *Dermacentor variabilis, Dermacentor andersoni,* or *Rhipicephalus sanguineus* with a sample size of 25 subjects, and 2) dosimetry test to determine standard consumer dose of application with a sample size of 25 subjects for testing both, dosimetry and repellency under laboratory conditions. Reported deviations from the protocol are not likely to compromise the validity of the data.

Conclusion

The methods used in this study are based on the protocol (DP 457664) reviewed by the EPA and HSRB, as amended to incorporate EPA and HSRB recommendations before testing began and as amended during the study. Study results are acceptable to support a CPT of 4 hours against ticks for the proposed pump spray product containing 30% w/w of the active ingredient, Citriodiol (OLE). Data support the label claim that the product "repels ticks for 4 hours," based on the data from the tick species with the lowest CPT.

cc: Michelle Arling

REFERENCES

MRID 511322-01, contains 28 volumes:
Vol. 1 Study Report
Vol. 2 (App. 16.1) Study Protocol Version 3.4
Vol. 3 (App. 16.2) Recruitment Data Sheets
Vol. 4 (App. 16.3) Protocol Amendments
Vol. 5 (App. 16.4) Report of all Deviations from Protocol
Vol. 6 (App. 16.5) Adverse Events
Vol. 7 (App. 16.6) Documentation of Ethical Conduct
Vol. 8 (App. 16.7a) Consent Forms; Randomization list; Treated arms Randomization List.
Vol. 9 (App. 16.7b) Comprehension Checks and Questionnaires
Vol. 10 through Vol. 23 ((App. 16. 7c through App.16.p) Subjects Case Report Forms for Consumer Dose and Repellency test.
Vol. 24 (App. 16.8) Tick Pathogen Tests; Tick Accountability and Tick Randomization Logs.
Vol. 25 (App. 16.9) Product Storage Log and Tick Storage Logs
Vol. 26 (App. 16.10) Test Substance Certificate of Analysis; Difference in weight of bottle (Dosimetry)
Vol. 27 (App. 16.11)Statistical Analysis
Vol. 28 (App. 16.12) Blueprint of Testing lab facility

The listed MRID 511322-01, which contains 28 volumes, supersedes the original submission in MRID 510045-01 through 510045-28, which contains 27 volumes of raw data identified as Appendices 16.1 through 16.12, excluding study report (MRID 511322-01 Vol. 1).

Attachment 1: Responsiveness to EPA and HSRB Science Comments

Attachment 1

Responsiveness to EPA and HSRB Science Comments

Revise the estimated maximum number of subjects needed for the study to reflect the revised number of subjects for the dosimetry and repellent efficacy phases.
A minimum of 25 and a maximum of 100 will be required to complete testing (pg. 5 Study Synopsis, Study Protocol Version 3.0 (MRID 511322-01 Vol. 4 (App. 16.3)).

Expand recruitment to the London area – limiting recruitment to the London School of Hygiene and Tropical Medicine is too narrow and could result in a skewed pool of subjects.
Recruitment was expanded to the London area (Section 6.1. Recruiting and Enrolment statistics, pg. 18 in study report, MRID 511322-01 Vol. 1, and Section 4.1 Recruitment, pg. 13 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Include a randomization process to avoid biasing the pool towards those who respond immediately.
The protocol was revised to include a section entitled "Randomization." (MRID 511322-01 Vol. 2 (App. 16.1)), Sec. 5.2. This section explained that "the total number of qualified subjects will each be assigned a unique and consecutive number, starting at 593001 based on the order of their enrolment. The numbers will then be randomized by placing the list of volunteer numbers into a STATA dataset, generating a new random variable, and sorting according to that variable. The first 25 subjects in the generated randomized list will be invited to consent for the dosimetry phase and will be offered the opportunity to consent for one or more repellent efficacy trials.".

Number of alternate subjects, including distribution of their sex ratio, should be updated according to amended sample size of 25 test subjects per tick species. In addition, consider recruiting alternate subjects during recruitment process, and describe how participants will be randomly assigned as either test subjects or alternates.
This recommendation was followed. See Section 5.2 pg. 17 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Separate the discussion of the interventions into a description of the test substance, randomization, dosimetry phase, and repellent efficacy phase.
These sections are discussed in separated sections each from Sections 5.1 through 5.4 on pp. 16 to 17 of Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Revise instructions to participants for dosimetry phase. Subjects should be provided the label and instructed to read the label and apply according to the instructions. Researchers should not influence the amount of product applied during the dosimetry phase.
The statement, "*Each subject will be asked to read the product label and apply the product according to label instructions "Directions for Use*" appears in Section 3, pg. 10 and Section 5.3, pg. 17 of Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Prior to initiating the dosimetry phase, both forearms of each participant should be washed with unscented soap and water and dried with paper towels.
The statement, "*Unscented soap will be provided to participants at the consent meeting*" appears in Section 4.3 on pg. 15, and in Section 7.1 pg. 19 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Include the following language: "*All deviations (including minor corrections) will be documented by the Study director, reported to the IRB as required, and included in the final study report provided to EPA."*
The statement is included in Section 10.2 pg. 25 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

EPA (science, statistics, and ethics) and LSHTM agreed that a sample size of 25 would be adequate to ensure that the efficacy study includes enough subjects to return reliable results without including more subjects than necessary. The same sample size and the same subjects should also be employed for dosimetry.
Dosimetry for consumer dose determination and product performance for calculation of mCPT were conducted with a sample size of 25 subjects. A minimum of 25 subject per tick species and a maximum of 100 subjects will be required to complete testing (Study Synopsis, pg. 6 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Hairs from forearms should be clipped for efficacy testing.
The statement "*Electric hair clippers will be used to clip 1/8" hairs from forearm*" appears in Section 7.2 on pg. 19 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

The endpoint for estimation of CPT should be identified as the time to first confirmed crossing, signaling the time point of repellency failure or complete protection time (CPT).
Endpoint for determination of repellency failure is defined in Section 3.1 pg. 11 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1) .

Estimation of skin surface area: Describe in more detail how to measure the forearm surface area. For example, the narrative could include the use of 4 or 3 equidistant bracelets (i.e., 4 or 6 cm wide) placed above the wrist to determine the average circumference of the forearm at more than 2 points, wrist and elbow as proposed, and multiply the average circumference by the length of the forearm. Wrist and elbow are not enough to estimate the average circumference of the forearm. (OCSPP 810.3700 Guidelines).
Three equidistant bracelets (3 cm wide) evenly spaced will be placed along the forearm from wrist to elbow will be used to measure the average circumference of the forearm (Section 7.1 pg. 19 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Clarify whether exposure of ticks to treated skin will start 20 minutes post-application, or whether exposure of ticks to treated skin will begin immediately after application.
Exposure to ticks will start 15 minutes after product application when product has dried. Participants will be asked not to disturb the product during that time (Section 5.2 on pg. 17 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

If the crossing line is eliminated from the design then, the criteria for no repellency should be amended as "a tick is classified as not repelled when it crosses the boundary line and spends 1 minute on treated skin."
This recommendation is addressed in Section 7.2, specifically on the last paragraph on pg. 20 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Ensure that release and boundary lines reference the correct locations (e.g., boundary line will be drawn at the wrist, and release line will be located 3 cm below boundary line.)
This recommendation is addressed in Section 7.2, on pg. 20 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Describe the criteria for determination of first confirmed crossing: Breakdown of the product occurs when a tick crossing into treated skin is not repelled, and it is followed by a second tick that is also not repelled, or it is followed by a second tick that is repelled, but if the second tick is followed by the next tick (the third tick), and that third tick is not repelled then, the third tick is considered a confirmatory crossing within a 30 minute period for the first tick that was repelled.
This recommendation is addressed in Section 7.2, specifically on the first paragraph on pg. 21 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Tick behavior on the control arm should be described as "*a tick that moves steadily from the release line across boundary line and upward*."
This statement appears in Section 7.2 pg. 20 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1) .

If a subject withdraws after a full day of testing with one tick species, they will be replaced for testing with the next tick species. The data for the completed test day will be used. However, if subjects withdraw before completing a test day their data will not be used and they will be replaced with an alternate subject.
Data will be used when withdrawal occurs after completing testing or not earlier than 9 hours into test, provided that number of missed exposure periods do not exceed 6 (Section 4.4 pg. 16 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

The protocol should describe the method employed for quantification of applied product, including how the average consumer dose is calculated. For example, the study protocol should show the calculations that will be employed for quantification of applied product, the mean dose applied by each subject to each limb, and the grand mean across all subjects' means.
The procedure for calculation of average consumer dose is described in detail in Section 7.1 pg. 19 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

Add that the typical consumer dose in (mg/cm^2) will be converted to volume using the specific gravity of the test material."
This recommendation is included in Section 7.1 pg. 19 of Study Protocol Version 3.4 in MRID 511322-01 Vol. 2 (App. 16.1).

The Board discussed the choice of tick species for testing. The sponsor has chosen *Rhipicephalus sanguineus* as a test species. EPA guidelines state that sponsors should use test species for which a label claim is sought, therefore the choice of *R. sanguineus* suggests that the sponsor is seeking labeling for this species. The Board questions the choice of this species over *D. variabilis*. *D. variabilis* is the primary vector for Rocky Mountain Spotted Fever (RMSF) in the US and while *R. sanguineus* is an important tick vector in Europe, it is less important in the US and is mostly associated with dogs and kennels. It is not a frequent biter of humans as

compared to *D. variabilis*. From a scientific perspective and an ultimate label claim, the Board considered *D. variabilis* to be much more relevant.

Testing 3 species of ticks is required for supporting a general efficacy claim against ticks on product labels. On July 1, 2019, EPA agreed on the use of *Rhipicephalus sanguineus* to satisfy testing on 3 tick species due to lack of questing behavior of *Dermacentor variabilis*.

The statement, "*This will be repeated so that five actively questing ticks will be exposed to the treated arm one at a time, at 30 minute intervals (timed from product application) for 10 hours or until treatment failure*" should be changed if the exposure model is changed to 1 tick every 15 minutes.

This statement was deleted from the study Protocol Version 3.4 and replaced with 1 tick every 15 minutes (Section 7.2 pp. 10 to 21 in MRID 511322-01 Vol. 2 (App. 16.1)).