

US EPA ARCHIVE DOCUMENT

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MEMORANDUM

Date: 16 September 2009

To: Kelly Sherman, Kevin Sweeney, Office of Pesticide Programs, US Environmental Protection Agency

Subject: Response to EPA reviewer concerns about Carroll-Loye Protocol LNX-003

Based on preliminary conversations with the Agency concerning potential deficiencies in Carroll-Loye Protocol LNX-003, we are planning to amend it. The amendment will clarify how procedures as described apply to or include one, both, or either of the two tick species to be used in the test, how subjects are screened for attractiveness to the target tick species, and how stopping rules apply to cessation of tick foraging activity. In addition, this amendment will correct several minor errors and potentially confusing statements. The draft language for the amendment follows this cover page.

We appreciate your observations.

Sincerely,



Dr. Scott P. Carroll, Ph.D.
Study Director

Carroll-Loye Study LNX-003 Proposed Amended Language

Section 1.1 Objective of Research and Endpoints:

Line 139

“For both species, efficacy and duration will be measured as Complete Protection Time, or CPT, defined herein as the time between application of test material and the First Confirmed Crossing of an actively foraging tick from the untreated skin surface of a subject’s hand 3 cm or more into the treated forearm skin area.”

Line 146

“The endpoint will be the time of failure expressed as the time of the FCC for each species for each subject.”

Section 1.3.2 Risks from Exposure to Biting Arthropods

Line 211

“Stopping Rules (§4.7.6) and Medical Management practices (§1.3.6) specify removing any ~~treated limb~~ subject from the study when ~~the repellent begins failing~~ that subject has received confirming crossings for both tick species or the subject shows signs of reacting to a bite or to contact with ticks. ~~Subjects will be exposing small areas of treated and untreated skin for a maximum of 24 minutes per hour.~~”

Line 225

“~~This~~ The training procedure also serves to verify the subject’s attractiveness to ticks in the study. If during subject training any qualifying tick (as defined in §4.7.3) per five exposures of each species fails to cross on the subject, the subject will be excluded from the trial.”

Section 3.1 Candidate Recruitment: Population, Sampling Frame, Representativeness

Line 368

“Nonetheless, while comparable data are not available for ticks, because gender effects seem most plausible, we will enroll similar numbers of males and female subjects.”

Section 3.3.2 Exclusion criteria, all subjects:

Line 447 (*Item number 14*)

“~~Withdraws from testing before receiving a confirmed crossing~~ While we do not anticipate tick avidity as measured by our criteria (§4.8.3.1) will change significantly during the study, should a subject unexpectedly lose attractiveness to either tick species (as gauged on the untreated arm) before a confirming crossing, he or she will be replaced on a later date if it is determined that his or her total exposure duration is less

than 90% of the mean of subjects who did not withdraw, and when not more than 2 of 10 subjects have so withdrawn.”

Line 451

“This exclusion factor is not automatically invoked if the Study Director ends exposures due to other factors, such as ~~darkness~~ subject exhaustion; in such cases the data collected before termination may be sufficient to meet the study goals.”

Line 455 (*Item number 15*)

“Not attractive to target species (during subject training any qualifying tick, as defined in §4.7.3, per five exposures of either species fails to cross on the subject)”

Section 4.1 Number of Subjects

Line 565

“We reduce this risk by confirming subject attractiveness to ticks before they participate in the phase of the test where efficacy data is are collected.”

Section 4.2 Number of Controls

Line 610 reference to §4.8.2.1 is corrected to §4.8.3.1.

Section 4.6 Standard Dose as Determined by Dosimetry

Line 654, table first row second column, the quantity “2.51 $\mu\text{l}/\text{cm}^2$ ” is deleted and replaced with the acronym “TBD”

Line 655, footnote to dosing rates table; the entire footnote is deleted and replaced with the sentence:

“As part of Carroll-Loye study LNX-002 (MRID 47506401), additional dosimetry data will be collected for the Cream 20% product. The augmented data set will be used to determine final dosage.”

Section 4.7.1 Blinding of Study

Line 698

“However, within the treated group, the ~~three~~ two treatments will be indistinguishable to test subjects and staff based on their physical properties.”

Section 4.7.3 Confirming Tick Foraging Activity

Line 728,

“To be included in the test on a treated limb, each tick must first be determined to be a “qualifying tick” (defined as beginning walking on the hand of the subject’s untreated arm within approximately 15 seconds of being placed there). Each tick must then also

meet the crossing criterion on the untreated limb, following the procedure for the treated limb in the same test period (§4.8.2~~3~~.1).

Section 4.7.4 Measuring Repulsion

Line 731

“For each tick species, the number of crossings on each subject’s exposed treated area will be recorded (Appendix 2) as they occur during 3-minute exposure periods commencing once every 15 minutes, beginning at the onset of data collection and ending when the subject receives ~~the First Confirmed Crossing~~ a confirming crossing, a stopping rule is invoked for the subject, or the Study Director stops the test for all subjects.”

Section 4.7.6 Stop Rules

Line 750

~~“Foraging pressure falls below threshold needed to challenge the Test Material(s)”~~

Line 755

“Subject proves unattractive to target species ~~Three ticks of a species do not cross on subject’s untreated arm during a test interval, in which case subject is retired from testing that species. The second tick species is then tested with the same failure criterion. Since each tick is given 3 minutes to cross on the untreated arm, note that in the unlikely scenario of a full 6 ticks (3 of each species, summing to 6 ticks for both species combined) being tested within an interval, testing of the sixth tick would not begin until after 15 minutes had elapsed, which would normally be the time at which the next exposure interval would begin. If the third tick of the second species crosses the untreated arm at that point, its data from the treated arm will be assigned to the prior exposure interval, and exposures for the next interval will begin immediately.~~”

Section 4.8.3 Test Day

Line 777

“The following test procedures (§4.8.3.1 and §4.8.3.2) are repeated ~~for each species in sequence (not concurrently) by each subject at designated time intervals within each interval, with intervals occurring every 15 minutes,~~ until a stop rule (§4.7.6) is invoked.”

Section 4.8.3.1 Tick screening for active foraging and repellency challenge

Line 790

Subjects screen ticks in two steps. First, whether an individual tick is “qualifying” (sufficiently active in general) is screened as described in §4.7.3. Second, whether an individual tick is “actively questing” is screened by observing whether it walks past the second marker dot in motion toward the elbow of the untreated arm.

Line 792

~~“Every 15 minutes, Each subject selects an unused, qualifying tick and screens it for active questing behavior, repeating with the same tick species until an actively questing tick is identified.”~~

Line 805

“Active questing is verified if a qualified tick travels past the second marker dot in motion towards the elbow on the untreated arm.”

Line 826, a new section is inserted 4.8.4 Additional efficacy data collection.

“In the event that a subject withdraws during the Test Day and invokes exclusion criterion 14 (line 447), his or her data will be replaced by repeating the described procedures (this protocol, especially §4.8.3) on a subsequent day. Subsequent testing groups may consist of as few as one subject, who would work singly with a technician rather than in a subject group.”

Section 4.9 Efficacy – Statistical design and analysis

This section requires numerous corrections as additions (inserts). They are organized in table format for ease of review.

Line #	Resulting sentence
832	Because all subjects use different ticks, all ticks are used only once, and neither organism interacts directly with conspecifics at the level of the skin and the repellent during data collection, we will analyze data <u>for each tick species</u> by subject as independent, replicated values.
837	The objective is to compute, for each test material, a reasonable estimate of mean and standard deviation for the duration between application and sufficient repellency breakdown such that <u>for each tick species</u> there are two ticks crossings on a subject within a half hour period.
840	That pattern is here assessed <u>for each tick species</u> at a resolution of 15 minutes.
843	For each <u>tick species on each</u> treated subject, we will measure (data form Appendix 2):
849	A FCC is a Crossing followed by another <u>“confirming”</u> Crossing within 30 minutes.
855	CPT is measured as a single time value for each subject <u>for each tick species.</u>
855	Based on the requirements for such estimates in the EPA draft repellent efficacy testing guidelines (1999; OPPTS 810.3700), we will calculate mean CPT <u>for each tick species</u> across all 10 subjects, with standard deviation and 95% confidence interval information.
863	To examine the temporal pattern of failure further, we will employ Kaplan-Meier survival analyses by subject <u>within tick species.</u>
867	In addition, we will estimate the Kaplan-Meier median, and the time until 25% failure, for each test product <u>on each tick species.</u>

CLBR Training Manual §1.b. Handling ticks and observing their movement on the skin
Page 51 of the Protocol

Part A. Goals of exercise

An item 3 is added as follows

“Determine if the types of ticks to be used in the study will move up your arm.”

Part D. Learning the methods

First sentence

“Spend about 30 minutes practicing handling two kinds of ticks in the laboratory in preparation for the repellent study.”

Insert after the sixth sentence

Place the tick on the line nearest your wrist, noting the time. “If the tick does not begin walking within approximately 15 seconds, the tick is considered not active enough. Remove this tick and replace it with another, again noting the time. A tick that is active enough will usually as soon as the tick begins to walk toward your elbow. If the tick instead walks toward your hand, elevate your elbow further above the hand and use the brush to gently guide the tick back toward the lines.

Fourth from last sentence

“You will practice these tasks ~~several times~~ five times with each kind of tick in order to familiarize yourself with how to handle the ticks carefully and successfully, and to determine if both kinds of ticks will move up your untreated arm.”