EMD-004.3: Mosquito Repellency with Aerosol Spray Formulations

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Brief Overview of the Studies

The objective of the study EMD-004.3 was to test the repellent characteristics of the test material in aerosol spray formulations against mosquitoes with the efficacy measured as the complete protection time (CPT) which is defined as the time from application of the test material to the First Confirmed "Lite with Intent to Bite (LIBe)" (FCLIBe). A LIBe occurs when a mosquito alights on the treated test skin of a subject and extends its proboscis to the skin surface while ceasing locomotion. A FCLIBe is that which is followed by another within 30 minutes.

Dosimery testing was conducted in the Arthropod Behavior Laboratory at Carroll-Loye Biological Research on several days in late October 2006. Repellency testing of the test material in aerosol spray was conducted in the field in Merced and Butte Counties, California. Testing in Merced County on 15 November 2006 began in a marshland (Mud Slough) known for supporting high densities of *Culex* species had to be shifted to a nearby native grassland (West Bear Creek, San Luis National Wildlife Refuge) at midday due to low biting rates.

Twelve subjects were used in measurements of dosage for the test material preparation. Ten subjects were subject to mosquitoes for efficacy evaluation with the test material, and two subjects were subject to mosquitoes to confirm adequate mosquito LIBing pressure for each of two sites. Six subjects were used in both sites.

In the field test at site #1, no FCLIBe was observed and the CPT was 10+ for all ten subjects. In the field test at site #2, one FCLIBe was observed at 8.75 hr, and all nine others had CPT of 10+. Therefore these two field tests yielded only one event.

Critique of the Study

The studies have strengths as summarized below:

- The experiments were conducted in two different natural settings.
- A dosimetry study preceded the field study and established that dispensing efficiency was strongly correlated with dose received.
- Environmental conditions for the field studies were adequate documented.
- There were two negative control subjects in the field study to document ambient LIBing pressure.

However, there were a number of serious weaknesses in the study report as well as in the study protocol listed below:

- Data from first exposures were recorded as taking place at 3 hr after application for test site #1 and 2 hr after application at test site #2, and subjects were instructed to minimize

abrasion of the treated skin during travel to the site. However, there is no way of knowing how much of the test material was retained for the field exposure.

- The determination of the efficacy endpoint CPT may be inadequate; as a consumer of mosquito repellents, I don't need a confirmed second bite within 30 minutes to confirm the product has ceased to provide protection from mosquito. This definition overestimates the protection time.

- The analysis of CPT is deficient in a number of ways:
    - The analysis presented ignores the right censoring of the time to FCL. The mean and variance cannot be estimated in the presence of censoring. As it ignores right censoring and imputes the time to FCL with the time when a subject withdraws from the experiment plus 0.25 hr, the mean and variance is a biased estimate of the true mean CPT. With the imputation of censored observations, it also underestimates the variability of the CPT.
    - A more appropriate analysis for CPT is survival analysis method such as the Kaplan-Meier method to estimate the median of CPT.

Conclusion

With times to event data such as CPT, the statistical information which is directly related to variability is the number of events. In the field test at site #1 (native grass and shrubland), there were no events observed during the observation period before sunset. Therefore, the field test at site #1 produced no statistical information about the variability of the protection time as all CPTs are censored at 10 hr. Likewise the field test at site #2 (woodland/flooded marsh/picnic area) provides hardly any information about the variability as there was only one FCL and all nine other CPTs were censored at 9.5 hr.

Paraphrasing it statistically, the field test at site #1 is a complete failure, and the field test at site #2 an almost complete failure if the objectives were to estimate the probability distribution of CPT. As an example, in a clinical study of a treatment for prevention of heart failure, if no heart failures are observed during the study period, the investigation learned nothing about the effect of treatment.

It is patently wrong to state that "the data set … gives a rather robust, low variance measure of the minimum duration of performance" as the variance cannot even be estimated (see the bottom of page 12 of 147). The only thing the sponsor can say is that the minimum CPT is longer than 10 hr. The sponsor cannot claim any information about the variance.

In conclusion, the EMD-004.3 study provides an estimate of the minimum CPT, but no estimate of the variability of the minimum CPT. It provides no estimates of the mean or median CPT and certainly no estimates of the variance of these point estimates. Therefore this study is insufficient statistically to be relied upon to assess the repellent efficacy of the formulation tested against mosquitoes.