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Dr. Daniel Strickman's Responses to Discussion Questions

DISCUSSION QUESTIONS FOR MOSQUITO REPELLENT STUDIES

The Human Studies Review Board (HSRB or Board) has discussed and provided advice to EPA on scientific and ethical issues related to the conduct of field studies to evaluate the efficacy of mosquito repellent products. The HSRB has reviewed both proposals for new field studies and the results of completed studies. The HSRB has noted that, although there are many similarities across studies, not all studies employ the same study design. The HSRB has identified several methodological issues for which additional background information would assist the Board in its evaluation of such studies.

BACKGROUND

Currently, EPA requires all pesticide products that claim to repel mosquitoes to provide data on the duration of efficacy under field conditions at two biologically distinct sites. These data are derived from human research with subjects who have been treated with the repellent formulations in the field. The Agency evaluates the duration of repellent efficacy for a subject by calculating the time from application of the repellent to the occurrence of an event indicating an efficacy failure. Historically, for field studies of mosquito repellency, EPA has used the "first confirmed bite" as an indication of efficacy failure on a test subject. Several recent studies have shifted to the "first confirmed landing with intent to bite;" EPA has accepted this alternative endpoint. A "confirmed landing" on a test subject is a mosquito landing followed by a second landing on the same subject within a specified period of time (usually 30 minutes) after the initial landing.

Field studies typically involve 6 – 10 subjects who have been treated with a defined amount of the test material. Each subject is then regularly and repeatedly exposed to ambient mosquito populations for a fixed interval of time until the subject experiences an efficacy failure followed by a confirmation with the specified period of time. Mosquito landing pressure (representing intent to bite) at a site is monitored by concurrently exposing untreated subjects to mosquito landings. A study is considered valid only if there are at least a specified minimum number of mosquito landings on untreated subjects during each exposure interval.

On October 25, 2007, the HSRB will discuss scientific aspects of the design of field studies to assess the efficacy of mosquito repellents. For this meeting the Board has requested consultants to provide specialized information or assistance to the Board. The Board is particularly interested in the frequency, duration and timing of exposure of subjects to potential mosquito landings. The Board requests each consultant to respond briefly to the series of questions below. Please send the responses to the HSRB Chair and Designated Federal Official (DFO) at least one week before the meeting—i.e., by no later than October 18. All responses will subsequently be provided to the other consultants, the HSRB members, and EPA staff for their review, and will be posted on www.regulations.gov under docket ID number, EPA-HQ-ORD-2007-0942. HSRB consultants will be available at the meeting to discuss their responses and address questions from the Board. The questions for Board consultant consideration are provided below:

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DISCUSSION QUESTIONS

- What do data show about the variability of the time intervals between first and subsequent landings in mosquito repellent field trials?

The time between first and second (or subsequent) landings is likely to be very variable. Thirty minutes is usually suggested as a long enough interval to allow for any mosquitoes in the area to land.

- What is the current scientific understanding of how factors other than repellent efficacy could affect the likelihood that an initial event—a mosquito landing or mosquito bite—would be "confirmed" by another similar event within 30 minutes? Please address at least these factors:
  - Characteristics of mosquito populations

The assumption is that the susceptibility of the mosquito population follows a normal distribution, and therefore the first mosquito to land is likely to be at the edge of that distribution. Statistically, the variation and abundance of the population will dictate when the second landing occurs. Presumably, the interval between landings will decrease as the middle of the distribution curve is reached.

The literature documents that particular species of mosquitoes are characteristically more or less susceptible to a given active ingredient. Therefore, the interval between first and second landing will depend on the species. This assumes a normal curve response to repellency, as is observed in the laboratory in dose-response studies.

It is also documented that size of individual mosquitoes affects avidity, generally with larger mosquitoes being more avid. Therefore, a population that had good larval nutrition (and more uniform, larger adults) is probably more likely to have a shorter interval between first and second landings.

Age of mosquitoes affects avidity, with at least some studies showing that older mosquitoes can be more avid. If the population is from a single brood, and therefore of similar age, then two effects will occur with age. First, the older mosquitoes may be more avid and, second, the population will decline in numbers as it ages.

Most species of mosquitoes do not bite when they are gravid or within the first approximately 12-24 hours after oviposition. Especially if the current population of adults developed from a single hatching event (e.g., a rising river hatching a brood of floodwater mosquitoes), there might be an interval when many are gravid or recently parous and the effective biting population is low. The low population would cause a longer interval between first and second landing.

- Characteristics of test sites

Some conditions are known to discourage mosquito biting and host seeking. Low temperatures, higher winds, and small phases of the moon are examples. Presumably, these conditions would lengthen the interval between first and second landings by influencing the avidity of the population as a whole.

- Characteristics of test subjects

People can be more attractive to mosquitoes because of inherent characteristics (skin color, skin chemistry, blood chemistry) or characteristics associated with activity or temporary conditions (apocrine sweat, skin temperature, illness, alcohol consumption). People who are more attractive to biting mosquitoes would experience a shorter interval between first and second landings if we accept the assumption that the normal curve describing attraction to that particular host is narrower.

- Characteristics of test methods

If more of the person were exposed (e.g., only forearms compared to arms and legs), then mosquitoes should find the host more quickly and the interval between first and second landings would be shorter. This is a testable hypothesis and I am not sure it has been addressed in the literature.

If the person is instructed to walk around between tests, or if s/he moves around even while seated, mosquitoes in the vicinity will have stronger visual cues for orientation and the interval between first and second landings could be shorter. This will be particularly true for mosquitoes that tend to bite larger animals, like floodwater *Aedes, Anopheles,* and some container breeders.

Although we think of most repellents as having very little effect over a distance, almost all of them actually perform in the olfactory phase. Therefore, a volatile repellent (e.g., deet or oil of citronella) will have a greater spatial effect if more volume is used on the individual. A larger volume of a volatile repellent (i.e., if a larger part of the body was treated) might make the degradation curve of effectiveness more shallow and therefore lengthen the time between first and second landings.

When the concentration of less volatile repellents (e.g., Picaridin, PMD) decreases, landing can occur without biting. In this case, the same individual mosquito might land, not bite, then land again. That situation would result in a very short interval between first and second landings.

- Can the impact of such factors on the likelihood or timing of an initial and confirming event be predicted? Can it be quantified?

No.

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At its June 27 - 29, 2007 meeting the Board learned that different designs with different "length-biased" sampling for mosquito repellent field studies are in use. One design exposes subjects to potential mosquito landings for one minute of every 15 minutes; another design exposes subjects to potential mosquito landings for five minutes of every 30 minutes. The DFO is separately providing a CD containing the background materials for the June 27 – 29, 2007 HSRB meeting. The protocols are loaded on the CD. These designs have different "length-biased" sampling.

- What is the methodological rationale for the two different designs?

There is nothing very standard about these timing regimes.

- Which design is used more widely in the field? Why?

The 30 minute design (or a one hour design) is much more common in the literature. One reason is that it is easier to perform. The other reason is that the perceived precision seems to be adequate given the inherent imprecision of the measurement. In other words, you are never going to get consistent protection times on a scale finer than about 30 minutes and even if you did, would it be meaningful in terms of real world variability in performance?.

- Can potential effects of variation in the pattern of intermittent exposure on the results of efficacy testing be isolated from the effects of other variables? If so, can the direction or magnitude of the effects be predicted? How might these influences be analyzed and accounted for in collecting, reporting and analyzing repellent efficacy data?

It might be possible to adjust for wind and temperature, if the studies have been done for that particular area and those species of mosquitoes. That way, you would adjust the result for the conditions measured at the time of the intermittent exposure. A much more serious problem is that the biting activity of mosquitoes varies systematically with time of day. It is never really accurate to do a repellent trial continuously for more than about an hour or two because the avidity of the mosquito population will start to change significantly. The best designs treat people the appropriate number of hours before the peak biting time and the expose all subjects simultaneously.

Dr. Matt Kramer, a USDA statistician who has served as a consultant, has suggested that the precision of estimates of Complete Protection Time (CPT) in repellent testing could be significantly increased by defining a failure of efficacy as the mean time from treatment to a series of several landings or bites. He has stated:

> The precision of CPT increases when it is estimated beyond time to [First Confirmed Bite] FCB or FCLanding. How well CPT can be estimated depends on the distribution of so many bites beyond FCB. The number of

mosquitoes that will bite (*n*) will determine results of the test. Each person in the field should be his/her own control; that way it is possible to know *n* per person, and reduce person-to-person variability.

If using the mean time to the first 5 bites, the SE will decrease proportionally as *n* increases ($n = 5$ in this case). That is equivalent to an increase in the power of the test of 5 times. This method allows for detecting formulation differences near the CPT.

- Does this approach, indeed, increase the precision of estimates of CPT markedly without requiring additional subjects?

Yes. In an extreme case, it might even decrease exposure by requiring less replication.

A statistician needs to look at the problem, but I would advocate that the mean of the times for the 5 bites be used as a single index without concern about the variability between the 5 mosquitoes. The idea would be that the average time for the first 5 landings would be the new statistic used as the CPT.

- If so, would this increased precision justify the incremental risk to the subjects resulting from their exposure to a greater number of mosquito landings?

Yes. If each mosquito is aspirated off before it can bite, using the first five will not increase the risk at all. The exact time of aspiration would be required in order to calculate the statistic, therefore, the volunteer would have to remain exposed for whatever period was necessary following the first landing. In addition, it is possible that the increased precision will actually decrease the exposure of volunteers by reducing the number of replications required.

- **The increase in precision is probably essential if you are going to have any hope of comparing studies performed at different times. The best solution might be to apply Kramer's suggestion of mean landing of five mosquitoes to lab tests with standard strains, completely eliminating risk to the volunteer and greatly increasing the precision of the test. Field tests have so many variables that comparison of products compared in separate field trials is hopelessly inaccurate. Field tests might best be used to build confidence in real performance of a single product, rather than as comparisons between products. It is important to remember that the objective of the trials in question is labeling so that consumers can compare products – not a guarantee or estimate of protection from vector-borne pathogens.**

- Is it practical to test long-lasting repellents to the point of five landings?

Yes. It is just a matter of applying them long enough before the tests at 30 minute intervals on an adequate number of volunteers. All volunteers could be tested

simultaneously in order to minimize the variability of the mosquito population caused by daily variation in biting avidity. Even in the laboratory, avidity varies during the day.

There is a need for systematic evaluation of standardized test methods in order to determine the exact procedures necessary to minimize variability and maximize precision. Standardization will also help prevent attempts by manufacturers to seek favorable results through the use of particular field situations and procedures.