October 16, 2007

MEMORANDUM

SUBJECT: Consultant response to United States Environmental Protection Agencies (US EPA) Human Studies Review Board (HSRB).

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TO: US EPA HSRB
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BACKGROUND:

The HSRB of the EPA has identified several methodological issues related to mosquito repellent testing and has requested inputs from independent consultants[1]. Specifically, the HSRB is considering the outcome measure first confirmed bite (FCB), defined as one bite confirmed by another within a 30 minute period[2], and its correlate, first confirmed landing with intent to bite.

The following responses are specific, that is they are concerned only with the questions posed by the HSRB and not with wider issues such as: the relative merit of using FCB versus other repellent outcome measures like relative protection; or the applicability of regulatory estimates of repellent performance to real-world use scenarios. Further, responses provided herein represent the opinion of the consultant, and do not necessarily reflect those of the Canadian Department of National Defence.

HSRB QUESTIONS/CONSULTANT RESPONSES:

Section 1 – Time intervals between first and second bites.

Q1. What do data show about the variability of the time intervals between first and subsequent landings in mosquito repellent field trials?

A1. FCB is not the most common endpoint used in peer-reviewed and published field studies on repellent efficacy. Further, even where FCB is used, non-confirmed bites are not always reported. Consequently, there is a relative paucity of data available for critical analyses of this question.

In our own recent studies[3,4], the initial bite on a repellent-treated subject was usually confirmed by another bite within 30 min. Is this representative of what normally would be expected? To the extent that bites tend to be temporally aggregated – it likely is. However, the specifics of such clustering are not expected to be constant, but rather should vary based on factors such as the interplay between biting pressure and the relative protection (RP) afforded by the product. In our work, because biting pressure was high (e.g., 10+ bites/min) at the time of the first bite, expectation is for a confirming bite to occur within 30 min (also see A4). Such may not be the case under a different set of experimental conditions.

Q2. 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*
- *Characteristics of test sites*
- *Characteristics of test subjects*
- *Characteristics of test methods*

A2. A variety of factors can influence estimation of repellent efficacy. These include, but are not necessarily limited to, characteristics of: mosquito populations, test sites, test subjects and test methods. A brief discussion of each of the above-listed factors is presented below. If required, additional commentary is available in several recent publications that have tackled this subject area [5,6].

Mosquito populations. Substantial intergeneric, interspecific and intraspecific variability has been demonstrated in the response of mosquitoes to repellents. Certain genera (Anopheles)[7,8] and species (An. albimanus[9]) are less "sensitive" to deet than are others; and variability between species strains has been demonstrated[10,11]. Other mosquito based-factors that might influence outcomes from repellent tests include nutritional status, endogenous activity cycles, age/parity status and mosquito population density[5,6].

Test sites. Variability between test sites can influence repellent performance estimates. For example, differences in mosquito population density or species make-up (as discussed above) may substantially affect outcomes. Further, climatic influences such as wind speed, temperature, humidity and ambient light[5,6] might impact mosquito activity and behaviour and/or the persistence of repellent products on test subjects.

Test subjects. Variability between test subjects can have a profound impact on estimates of repellent performance[3,5,6]. In some of our recent work, despite virtually identical test circumstances, FCB estimates varied by approximately 40% among subjects[3] and moderate-intensity exercise was associated with an approximately 50% reduction in product performance[4]. Although causal relationships remain elusive, subject-based variability likely involves the interplay of factors such as: intrinsic variability in the release of cues (e.g., semiochemicals) that affect mosquito behaviour and/or subject-based differences in evaporative, absorptive or physical loss of repellent.

Test methods. Potential impacts of test method on repellent performance estimates are numerous. They include: simultaneous use of the same subject (or even different subjects[12]) for biting pressure and repellent performance estimates[5,6], variable performance outcome measures (e.g., FCB versus RP), intermittency of exposure, inconsistent data analyses, varied subject-activity patterns, various emphasis on product versus subject based variability, different approaches to product dosing or area treated, etc.

Summary: Evaluation of how (or whether) all of the above-listed factors would affect estimation of FCB, or more specifically the likelihood of confirmation within a 30 min period has not been systematically reviewed. Certainly, field[13] and laboratory studies[14] have suggested a relationship between mosquito biting pressure or density and time to first bite or FCB, respectively. By extension, similar effects are expected for the likelihood of a confirmatory bite within any 30 min period (also see A4). Likewise, Barnard's[15] observation that parity and age interact to increase the likelihood of biting at the point of FCB suggests that mosquito based factors might also influence these probabilities.

Q3 Can the impact of such factors on the likelihood or timing of an initial and confirming event be predicted? Can it be quantified?

A3. The impact on the above-listed factors on the likelihood and timing of a first and confirming bite can be predicted and quantified. For example, we can reasonably predict that moderate-level exercise will attenuate product performance thereby resulting in a (probabilistic) systematic decrease in the time of the first and subsequent bites. Similarly, we can design a set of protocols to evaluate the phenomena and to quantify (*a posteriori*) the effect for that specific suite of experimental circumstances.

However, given the lack of standardization between studies, the paucity of research specifically directly towards evaluation of the above-mentioned factors and the complexity of the test system, elaboration of anything more than a very basic general model is not possible.

Section 2 – Validity of intermittently sampling to determine FCB

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.

Q4. What is the methodological rationale for the two different designs?

Periodic exposure to mosquito attack is a procedural norm in peer-reviewed and published field studies that use RP as an endpoint. Alternative approaches include continuous exposure to elaborate RP and/or FCB, or various approaches using survivorship analyses. To my knowledge, intermittent exposure of treated subjects to establish FCB has not been used widely in field studies.

From a guidelines perspective, the recent draft US EPA guideline on repellent testing would seem to allow for intermittent exposure in the field even where FCB is the endpoint[2]. However, previous EPA doctrine as well as other relevant guidelines[17,18] indicate exposure in the field is to be continuous or suggest RP as the appropriate endpoint[19].

I can only speculate on the methodological rationale for use of intermittent exposure where FCB is the endpoint. It might provide logistic advantage where multiple subjects are being screened, or could reflect an extension from laboratory approaches where intermittent exposure appears to be more generally accepted[2,16,17].

From an analytic perspective, caution is warranted where FCB is calculated on the basis of non-continuous field exposure because it reduces biting pressure by a factor equivalent to: 1/(1-proportional decrease in exposure). The upshot (where RP < 100%) is a decrease in the probability of receiving a first and confirmatory bite in any 30 min period. In other words, intermittent exposure should systematically overestimate FCB compared against continuous exposure. To illustrate this point, I have modeled the likelihood of FCB failure during any 30 min period under several scenarios:

- continuous exposure of treated subjects to mosquito attack
- intermittent exposure for 10 min/30 min (i.e. two 5-min periods)
- intermittent exposure for 3 min/30 min (i.e. three 1-min periods)

It is assumed that: biting pressure is at the EPA minimum of one bite/min[2,16] or higher, RP is 75% or 95%; the probability of a given mosquito biting is 1-RP; 0 mosquitoes have bitten at time 0; and biting probability is binomially distributed (NOTE: using a Poisson-based approach yields virtually identical results).

Outcomes are shown in the figure (pg. 10) and demonstrate that, within the model construct, intermittent exposure can substantially reduce the probability of seeing a FCB at a given RP. For example, at a RP of 75% (well below the EPA RP standard of 95%[2,16]) and a biting pressure of one bite/min, the probability of a FCB is <20% with intermittent exposure totaling 3 min over 30 min compared to a probability approaching 100% for continuous exposure. Similarly, at the higher RP of 95%, the probability of a FCB can be more than 10 times less during intermittent compared against continuous exposure.

It should be pointed out that the above model represents a simple approach to analyzing and representing concerns about FCB and sampling interval. While useful conceptually, it has not been validated (experimentally or statistically) or elaborated (e.g., for periods > 30 min) and hence should not be construed to represent anything more the simple illustrative model intended.

Q5. Which design is used more widely in the field? Why?

A5. As indicated in Q4, these designs have not been widely used in peer-reviewed and published field studies evaluating repellent performance.

Q6. 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?

A6. Outside of theoretical probability models, the present peer-reviewed and published database for repellent testing is not sufficient to allow for elaboration of general quantitative models describing the impact of intermittent exposure on the results of efficacy tests. Specific characterizations, for example by comparing in the same field procedure the estimates of FCB derived through intermittent versus continuous exposure, are possible (but, to my knowledge, have not been done).

Given the absence of a robust framework to characterize the impact of intermittent exposure on FCB-based outcomes, development of specific advice on how to analyze and account for the impact of intermittent exposure on estimates of FCB is not possible. General approaches that might be appropriate include: internal standardization within a given experiment by benchmarking intermittent FCB results against those derived from continuous exposure; adjust upwards minimum biting pressure requirements to offset reduced exposure intervals, and; abandon the approach and instead evaluate performance based on intermittent or continuous exposure for RP and/or continuous exposure for FCB.

Section 3 – Alternative endpoints to FCB

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.

Q7. Does this approach, indeed, increase the precision of estimates of CPT markedly without requiring additional subjects?

A7. The above proposal is difficult to judge in the absence of a more detailed description. Nevertheless, the following comments are provided:

a. The proposal is <u>not</u> directly comparable to FCB unless the suggestion is for an endpoint of 5 versus 2 bites within a 30-min period. This change would actually <u>decrease</u> sensitivity for detecting product differences over at least part of the range of possible biting pressures.

b. Presuming the intent is to have an endpoint of 5 cumulative bites over *x* period, performance might be expressed as: timing of the 5th bite; arithmetic mean time for all 5 bites; or another measure such as mean timing of bites based on survivorship analyses. The latter two approaches likely would improve precision, at least when compared against a measure solely based on time to first bite. Whether or not this in turn would yield <u>useful</u> additional statistical power cannot be determined on the basis on the information provided.

c. If 5 cumulative bites over *x* period is the measure of interest, then the proposed approach seems similar to measuring RP, albeit where outcomes are censored on the basis of a maximum number of bites. In this vein, it is informative that estimates of RP do not appear to be especially sensitive where detection of product-based differences is the goal. Admittedly, most evaluations to date have been limited to conventional statistical analyses. More robust experimental design or analytic techniques might yield greater sensitivity.

d. The above discussion begs the question: what level of difference in protection time is biologically, operationally, or economically meaningful? We[3] have previously argued that substantial advantage might not be gained by designing experiments to be able to detect small variations in repellent performance. Rather, procedures that are adequately powered to detect rather large differences (e.g., 20% or greater) in performance might be sufficient, or even preferable.

Q8. If so, would this increased precision justify the incremental risk to the subjects resulting from their exposure to a greater number of mosquito landings?

A8. This question is difficult to answer because risks and benefits change with experimental, epidemiological and societal context. For example, exposure to several additional mosquito bites during a repellent test in North America likely does not appreciably/meaningfully change the risk that an individual will be infected with an arbovirus. At the same time, a marginal increase in precision in a repellent trial or protection time estimate is unlikely to provide appreciable/meaningful benefit to society.

Q9. Is it practical to test long-lasting repellents to the point of five landings?

A9. Yes. Indeed, multiple bites or landings are the norm where RP is the endpoint.

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<u>Figure</u>: Biting pressure against probability of FCB in a 30 min period. Relative protection (RP) set at 75% (top) or 95% (bottom). Three exposure scenarios considered: continuous, 10 min/30 min or 3 min/30min.