June 30, 2009

EPA-HSRB-09-01

Kevin P. Teichman, Ph.D.
Acting Science Advisor
Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: February 17, 2009 EPA Human Studies Review Board Meeting Report

Dear Dr. Teichman:

The United States Environmental Protection Agency (EPA or Agency) requested the Human Studies Review Board (HSRB) to review scientific and ethical issues addressing: a completed Carroll-Loye Biological Research Mosquito Repellent Efficacy Study (SPC-001) and a completed Carroll-Loye Biological Research Tick Repellent Efficacy Study (SPC-002). In addition, the HSRB explored several technical aspects of spatial/area repellent testing, to help familiarize the Board with the technology as it prepares to review spatial repellent protocols at a future HSRB meeting. A summary of the Board's conclusions concerning these topics is provided below.

Assessment of Completed Carroll-Loye Biological Research Study SPC-001: Efficacy Test of Picaridin-Based Personal Repellants with Mosquitoes Under Field Conditions

Science

- The Board concurred with the Agency's assessment that this study provides scientifically valid results to assess the repellent efficacy against mosquitoes of the formulations tested. However, from a statistical perspective, EPA may wish to reevaluate how the data should be used to inform conclusions regarding specific protection times.

Ethics

- The Board concurred with the Agency's assessment that the study submitted for review was conducted in substantial compliance with subparts K and L of 40 CFR 26.

Assessment of Completed Carroll-Loye Biological Research Study SPC-002: Efficacy Test of Picaridin-based Personal Tick Repellents.

Science

- The Board concurred with the Agency's assessment that this study provides scientifically valid results to assess the repellent efficacy against ticks of the formulations tested.

However, from a statistical perspective, EPA may wish to reevaluate how the data should be used to inform conclusions regarding specific protection times.

Ethics

- The Board concurred with the Agency's assessment that the study submitted for review was conducted in substantial compliance with subparts K and L of 40 CFR 26.

Space Insect Repellent Testing

Because the HSRB may be reviewing spatial/area repellent protocols in the future, the Board desired to be familiar with such technology. The HSRB considered four factors as part of its educative process of spatial insect repellents technology: environmental aspects, study design, sample size/statistics, and human subjects. The Board noted the following points:

Environmental aspects

- Environmental and human factors are important aspects to address in spatial insect repellent testing.

Study design

- Setting some type of minimal allowable pest density should be required in the study design.
- Investigators should provide some statement regarding the efficacy they are trying to determine (number of pests, duration, etc.).
- The nature of the spatial dispensing device and the standard use conditions should be carefully described.

Sample size/statistics

- In spatial/area studies, the experimental unit to which repellents are being applied is a particular space and/or area. Thus the sample size questions must address the number of different spaces/areas that should be included, and not the number of subjects or traps within each space.
- Issues such as the allocation of units to groups and the balance of other relevant factors are absolutely critical and greatly affect the statistical analysis of the resulting data.
- Censoring, if unaccounted for, can seriously bias results.
- It is crucial to know enough about the response variable and the factors that affect it so that a reasonable experiment can be designed and the proportion of censored observations can be kept to a minimum.

Human subjects

- Justification for the involvement of humans (as "bait") must provide sufficient information to allow the HSRB to determine that the involvement is appropriate from both scientific and ethical perspectives.

Sincerely,

Sean Philpott, Ph.D.
Chair
EPA Human Studies Review Board

NOTICE

This report has been written as part of the activities of the EPA Human Studies Review Board, a Federal advisory committee providing advice, information and recommendations on issues related to scientific and ethical aspects of human subjects research. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the view and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does the mention of trade names or commercial products constitute a recommendation for use. Further information about the EPA Human Studies Review Board can be obtained from its website at http://www.epa.gov/osa/hsrb/. Interested persons are invited to contact Paul Lewis, Designated Federal Officer, via e-mail at lewis.paul@epa.gov.

In preparing this document, the Board carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented within the structure of the charge by the Agency.

U. S. ENVIRONMENTAL PROTECTION AGENCY
HUMAN STUDIES REVIEW BOARD

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Consultant to the Human Studies Review Board
Daniel Strickman, Ph.D., National Program Leader, Veterinary and Medical Entomology, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD

Human Studies Review Board Staff

Paul I. Lewis, Ph.D., Executive Director, Human Studies Review Board Staff, Office of the Science Advisor, United States Environmental Protection Agency, Washington, DC

1 Membership and Chairpersonship expired effective March 27, 2009
2 Membership and Vice Chairpersonship expired effective March 27, 2009
3 Membership expired effective March 27, 2009
4 Not in attendance
5 Resigned from the HSRB Effective March 9, 2009

INTRODUCTION

On February 17, 2009, United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB) conducted a teleconference meeting via telephone. Advance notice of the meeting was published in the Federal Register "Human Studies Review Board (HSRB): Notice of a Public Teleconference Meeting" (74 Federal Register 19, 5653).

During the public teleconference meeting, following welcoming remarks from Agency officials, the Board heard presentations from the Agency on the following topics: a completed Carroll-Loye Biological Research Mosquito Repellent Efficacy Study (SPC-001) and a completed Carroll-Loye Biological Research Tick Repellent Efficacy Study (SPC-002). The Board also explored several technical aspects of spatial/area repellent testing. Each of these topics is discussed more fully below.

Oral comments
The following oral comments were presented at the meeting:

Dr. Scott Carroll, Director, Carroll–Loye Biological Research, 711 Oak Avenue, Davis, CA

For their deliberations, the Board considered the materials presented at the meeting, written public comments and Agency background documents (e.g., the published literature, Agency data evaluation record, weight of evidence review, ethics review, pesticide human study protocols and Agency evaluation of the protocol or study). For a comprehensive list of background documents visit www.regulations.gov.

Written comments
No written comments were provided.

CHARGE TO THE BOARD AND BOARD RESPONSE

Assessment of Completed Carroll-Loye Biological Research Study SPC-001: Efficacy Test of Picaridin-Based Personal Repellants with Mosquitoes Under Field Conditions

Overview of the Study

SPC-001 was a field-based study of repellency to mosquitoes of three picardin-based products (7% Picaridin Pump Spray [data will be bridged to 7% Pump Spray and 5.75% Towelette], 15% Picaridin Pump Spray [data will be bridged to 15% Pump Spray and 12% Towelette], and 15% Picaridin Pump Spray formulated with sunscreen). All but one of these products – the formulation with sunscreen – were registered with the EPA prior to testing. Registration of the sunscreen-containing formulation has since been approved.

After HSRB review in October 2007, the protocol was further modified to: (1) clarify the composition of the 15% formulation with sunscreen and to add the two towelette formulations to the dose-determination phase, and (2) to correct minor errors and respond to EPA comments.

The study was conducted by Carroll-Loye Biological Research of Davis, CA between March 15th and June 14th, 2008. The study was sponsored by Spectrum Brands, Inc. of Bridgeton, MO, a division of United Industries Corporation. The study was required by EPA to support registration of these products.

As submitted to the EPA, the completed study consisted of two interdependent analyses: (1) a dosimetry study designed to determine the amount of repellent that users would typically apply (a single dosimetry experiment was used to provide this information both for SPC-001 and a related laboratory-based study of tick repellency, SPC-002 discussed later), and (2) an efficacy study designed to measure the effectiveness of each compound as repellent for those species of mosquitoes likely to be vectors for West Nile Virus (WNV) in the United States. The efficacy study was conducted at two field sites in Butte and Glenn Counties, CA.

Dosimetry was determined by direct measurement of compound application. The dosimetry study enrolled a total of 10 individuals (5 female and 5 male), each of whom tested all three formulations. The dosimetry study was performed at a laboratory site in Davis, CA. Based on the findings of the dosimetry phase, it was determined that the margin of exposure (MOE) for dermal toxicity ranged from a low of 1197 for the 15% spray on legs to a high of 6623 for the 7% spray on arms.

The efficacy of each formulation as a mosquito repellent was determined by measuring the ability of the formulations to prevent mosquito landings (defined as "Landing with Intent to Bite"; LIBe) under field conditions at two environmentally distinct sites in Butte and Glenn Counties, CA. Each efficacy study enrolled 10 participants (5 female and 5 male) for each formulation at each of the two field sites. Two experienced participants (1 male and 1 female) served as untreated controls to measure ambient mosquito pressure. Several volunteers participated in multiple analytic phases, dosimetric and efficacy; a total of 56 volunteers participated in at least one analytic phase of SPC-001. Prior to initiation of the efficacy study, all volunteers were trained in a controlled laboratory setting using lab-reared, pathogen-free insects to recognize a mosquito landing with the intent to bite, and to remove such mosquitoes with an aspirator.

During the field study, treated participants and untreated controls exposed their limbs to mosquitoes for one minute at fifteen-minute intervals, for 10 hours (40 exposure periods) post-treatment or until failure of efficacy, whichever occurred first. Failure of efficacy was defined as the two confirmed LIBes within a single exposure period, or a single LIBe within each of two consecutive exposure periods. Participants worked in pairs to facilitate identification of LIBes and to aspirate mosquitoes during exposure periods. No actual bites were reported, and aspirated mosquitoes were stored for later identification and arboviral testing.

Based on these data, mean complete protection time (CPT) for the 7% pump spray was calculated to be 8.4 ± 2.1 h at the Butte County site and 7.0 ± 2.2 h at the Glenn County site. Mean CPT for the 15% pump spray was 10.1 ± 4.0 h at the Butte County site and 10.7 ± 0.8 h at the Glenn County site. Mean CPT for the 15% pump spray with sunscreen was 12.7 ± 4.9 h at the Butte County site and 10.9 ± 1.3 h at the Glenn County site. Kaplan-Meier survival analyses yielded similar results: median CPT for the 7% pump spray was 9.4 h at the Butte County site

and 7.4 h at the Glenn County site; for the 15% pump spray it was 10.3 h at the Butte County site and 10.4 h at the Glenn County site; for the 15% pump spray with sunscreen it was 13.3 h at the Butte County site and 11.7 h at the Glenn County site.

Science

Charge to the Board

Is study SPC-001 sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy against mosquitoes of the three formulations tested?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment that this study provides scientifically valid results that meet to assess the repellent efficacy against mosquitoes of the formulations tested. However, from a statistical perspective, EPA may wish to reevaluate how the data should be used to inform conclusions regarding specific protection times.

HSRB Detailed Recommendations and Rationale

This study was conducted according to the protocol previously approved by the HSRB, with only one minor deviation. The conduct of the dosimetry study and the field study were very similar to the conduct of previous field mosquito repellent efficacy studies conducted by Carroll-Loye Biological. The study seems to have been carefully conducted with both sexes represented among the subjects and, in the field study, a mixture of 3 genera of mosquitoes was represented. The MOE's were very high and therefore protective of the participating subjects. The report was clearly written and detailed.

The only deviation was the use of historical limb measurements for those subjects who had previously participated and who indicated that they had not changed weight or muscle mass appreciably since the prior limb measurement. This deviation would not have affected the integrity of the resultant data.

There was concern expressed by some Board members regarding EPA's specific conclusions for times of protection afforded by the three repellents. As an example, for site two, the mean complete protection was 10.9 hours for the spray with sunscreen and 15% picaridin. The 12 hours of repellency calculated is not in the 95% confidence interval for the mean protection time, lying above the upper limit of 11.7 hours. The study was not designed to specifically test a CPT of 12 hours or any other time periods, and the results from this study do not support such conclusions. The Board suggests that EPA reevaluate its conclusions on specific protection times in light of the limitations and the variability of these data.

Ethics

Charge to the Board

Does available information support a determination that study SPC-001 was conducted in substantial compliance with subparts K and L 40 CFR Part 26?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment that the study submitted for review was conducted in substantial compliance with subparts K and L of 40 CFR 26.

HSRB Detailed Recommendations and Rationale

The documents provided by Carroll-Loye (Carroll 2008a, 2008b) state that each study was conducted in compliance of the requirements of the US EPA Good Laboratory Practice Regulations for Pesticide Programs (40 CFR 160). Additional regulations – 40 CFR 26 subparts K and L; FIFRA § 12(a)(2)(P); and the California Code of Regulations Title 3, Section 6710 – are also applicable. The study was reviewed and approved by a commercial human subjects review committee, Independent Institutional Review Board Inc. (IIRB, Inc.) of Plantation, FL. Documentation provided to the EPA by IIRB, Inc. indicated that it reviewed these studies pursuant to the standards of the Common Rule (45 CFR Part 46, Subpart A) and found them in compliance.

1. The Board concurred with the conclusions and factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's Science and Ethics Review (Carley 2009a).

2. The Board concluded that this study met all applicable ethical requirements for research involving human participants, in accordance to the following criteria:

    a. Acceptable risk-benefit ratio. The risks to study participants were minimized appropriately and were justified by the potential societal benefits, particularly data on the efficacy of these new formulations as personal insect repellents.

        - Minors and pregnant or lactating women were excluded from participation, with pregnancy confirmed by self-administered pregnancy testing on each "day of study". The potential of stigma resulting from study exclusion was minimized by enrolling three 'alternate' participants, allowing volunteers to withdraw or be excluded without compromising confidentiality.

        - Based on toxicological data currently available for picaridin, study participants were unlikely to be at risk of adverse side effects with exposure.

- The study was designed to minimize the likelihood of insect bites, but when they occur they are usually mild and readily treated with steroidal creams. The study excluded individuals with a history of severe reactions to bites

- Clear stopping rules and medical management procedures were in place, and no adverse events related to product exposure were reported.

- Finally, the field-based trials were conducted only in areas where known vector-borne diseases like WNV had not been detected by county and state health or vector/mosquito control agencies for at least one month. Mosquitoes collected during the field studies also were subjected to molecular analyses to confirm that they were free of known pathogens.

b. Voluntary and informed consent of all participants

- The study protocol included several mechanisms designed to minimize coercive recruitment and enrollment. Monetary compensation was not so high as to unduly influence participation.

- One protocol deviation occurred, as previously reported to the HSRB during its review of completed Carroll-Loye study LNX-001 (EPA HSRB 2008). Contrary to the HSRB-reviewed (EPA HSRB 2007) and IIRB, Inc.-approved protocol, Carroll-Loye researchers used previously recorded limb measurements, rather than collect physical data from all trial participants. This deviation occurred inadvertently when a Carroll-Loye researcher, acting upon an EPA suggestion that use of archival limb measurements was scientifically valid and would minimize study procedure invasiveness, implemented this protocol change without consulting Carroll-Loye management or IIRB, Inc. The result of an error in communication, this deviation again did not place study participants at increased risk or compromise the informed consent process.

Assessment of Completed Carroll-Loye Biological Research Study SPC-002: Efficacy Test of Picaridin-based Personal Tick Repellents

Overview of the Study

SPC-002 was a laboratory-based study of repellency to ticks of three picaridin-based products (7% Picaridin Pump Spray [efficacy data will be bridged to 7% Pump Spray and 5.75% Towelette], 15% Picaridin Pump Spray [efficacy data will be bridged to 15% Pump Spray and 12% Towelette], and 15% Picaridin Pump Spray formulated with sunscreen). All but one of these products – the formulation with sunscreen – were registered with the EPA prior to testing. Registration of the sunscreen-containing formulation has since been approved.

After HSRB review in October 2007, the protocol was further modified to: (1) clarify the composition of the 15% formulation with sunscreen and to add the two towelette formulations to the dose-determination phase, and (2) to correct minor errors and respond to EPA comments.

The study was conducted by Carroll-Loye Biological Research of Davis, CA on March 22nd and 23rd, 2008. The study was sponsored by Spectrum Brands, Inc. of Bridgeton, MO, a division of United Industries Corporation. The study was required by EPA to support registration of these products.

As submitted to the EPA, the completed study consisted of two interdependent analyses: (1) a dosimetry study designed to determine the amount of repellent that users would typically apply (a single dosimetry experiment was used to provide this information both for SPC-002 and a related field-based study of mosquito repellency, SPC-001 discussed previously); and (2) an efficacy study designed to measure the effectiveness of each compound as repellent for two species of ticks: nymphal deer ticks (*Ixodes scapularis*) and nymphal dog ticks (*Dermacentor variabilis*). Both the dosimetry and the efficacy study were conducted at the Carroll-Loye research laboratory in Davis, CA.

Dosimetry was determined by direct measurement of compound application. The dosimetry study enrolled a total of 10 individuals (5 female and 5 male), each of whom tested all three formulations. Based on the findings of the dosimetry phase, it was determined that the margin of exposure (MOE) for dermal toxicity ranged from a low of 1197 for the 15% spray on legs to a high of 6623 for the 7% spray on arms.

The efficacy of each formulation as a tick repellent was determined by placing ticks on picaridin-treated and untreated forearms for 3-minute periods, at 15-minute intervals. Failure of repellency occurred when a particular species of tick "crossed" into the treated area of the forearm, confirmed by another crossing of the same species of tick in either of the subsequent two exposure periods. The untreated forearm of each volunteer served as an untreated control, and was used to establish active questing behavior of each tick used. Prior to starting the efficacy study, participants were trained to handle ticks safely and to monitor their movements and all ticks were removed from participants' arms before biting. Volunteers also worked in groups of three and were attended by technicians at all times.

The efficacy study enrolled 10 participants (5-6 female and 4-5 male) for each formulation. Several volunteers participated in multiple analytic phases, dosimetric and efficacy; a total of 33 volunteers participated in at least one analytic phase of SCI-002.

Based on these data, mean CPT calculated for the 7% pump spray was 5.67 ± 2.09 h (range 3.25 – 9.75 h) for *D. variabilis* and 7.88 ± 1.43 h (range 5.75 – 11.25 h) for *I. scapularis*. Mean CPT calculated for the 15% pump spray was 9.65 ± 4.03 h (range 4.0 – 14.25 h; 3 instances of right-censoring) for *D. variabilis* and 11.80 ± 3.34 h (range 6.5 – 14.25 h; 6 instances of right-censoring) for *I. scapularis*. Mean CPT calculated for the 15% pump spray with sunscreen was 8.17 ± 4.86 h (range 1.0 – 14.25 h; 3 instances of right-censoring) for *D. variabilis* and 8.65 ± 4.31 h (range 3.25 – 14.25 h; 3 instances of right-censoring) for *I. scapularis*. Kaplan-Meier survival analyses yielded similar results: median CPT for the 7% pump spray was 5.50 h for *D. variabilis* and 8.25 h for *I. scapularis*; for the 15% pump spray it was 10.25 h for *D. variabilis* but could not be calculated for *I. scapularis* because of data censoring; for the 15% pump spray with sunscreen it was 7.0 h for *D. variabilis* and 8.25 h for *I. scapularis*.

Science

Charge to the Board

Is study SPC-002 sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy against ticks of the three formulations tested?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment that this study provides scientifically valid results to assess the repellent efficacy against ticks of the formulations tested. However, from a statistical perspective, EPA may wish to reevaluate how the data should be used to inform conclusions regarding specific protection times.

HSRB Detailed Recommendations and Rationale

This study was conducted according to the protocol previously approved by the HSRB, with only one minor deviation. The conduct of the dosimetry study and the field study were very similar to the conduct of previous laboratory tick repellent efficacy studies conducted by Carroll-Loye Biological. The study seems to have been carefully conducted with both sexes represented among the subjects and with the use of 2 genera of ticks. The MOE's were very high and therefore protective of the participating subjects. The report was clearly written and detailed.

The only deviation was the use of historical limb measurements for those subjects who had previously participated and who indicated that they had not changed weight or muscle mass appreciably since the prior limb measurement. This deviation would not have affected the integrity of the resultant data.

There was concern expressed by some Board members regarding EPA's specific conclusions for times of protection afforded by the three repellents. Two tick species were studied. For one of these, *Dermacentor variabilis*, the mean CPT of 5.7 and 9.7 hours for the 7% and 15% spray, respectively, were below the claims of tick repellency of 7 and 11 hours, respectively. Although the 5.7 and 9.7 hours fall within the 95% confidence interval, mean protection time is not equal to CPT. The study was not designed to specifically test a CPT of 7 or 11 hours, and the results do not support such conclusions. The Board suggests that EPA reevaluate its conclusions on complete protection times for these repellents in light of the limitations and the variability of these data.

Ethics

Charge to the Board

Does available information support a determination that study SPC-002 was conducted in substantial compliance with subparts K and L 40 CFR Part 26?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment that the study submitted for review was conducted in substantial compliance with subparts K and L of 40 CFR 26.

HSRB Detailed Recommendations and Rationale

The documents provided by Carroll-Loye (Carroll 2008c, 2008d) state that each study was conducted in compliance the requirements of the US EPA Good Laboratory Practice Regulations for Pesticide Programs (40 CFR 160). Additional regulations – 40 CFR 26 subparts K and L; FIFRA § 12(a)(2)(P); and the California Code of Regulations Title 3, Section 6710 – are also applicable. The study was reviewed and approved by a commercial human subjects review committee, Independent Institutional Review Board Inc. (IIRB, Inc.) of Plantation, FL. Documentation provided to the EPA by IIRB, Inc. indicated that it reviewed these studies pursuant to the standards of the Common Rule (45 CFR Part 46, Subpart A) and found them in compliance.

1. The Board concurred with the conclusions and factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's Science and Ethics Review (Carley 2009b).

2. The Board concluded that this study met all applicable ethical requirements for research involving human participants, in accordance to the following criteria:

    a. *Acceptable risk-benefit ratio.* The risks to study participants were minimized appropriately and were justified by the potential societal benefits, particularly data on the efficacy of these new formulations as personal insect repellents.

        - Minors and pregnant or lactating women were excluded from participation, with pregnancy confirmed by self-administered pregnancy testing on each "day of study". The potential of stigma resulting from study exclusion was minimized by enrolling three 'alternate' participants, allowing volunteers to withdraw or be excluded without compromising confidentiality.

        - Based on toxicological data currently available for picaridin, study participants were unlikely to be at risk of adverse side effects with exposure.

- Clear stopping rules and medical management procedures were in place, and no adverse events related to product exposure were reported.

- The study was designed to minimize the likelihood of tick bites.

- Finally, the efficacy trial was conducted with laboratory-raised ticks free of known pathogens.

b. *Voluntary and informed consent of all participants*

- The study protocol included several mechanisms designed to minimize coercive recruitment and enrollment. Monetary compensation was not so high as to unduly influence participation.

- One protocol deviation occurred, as previously reported to the HSRB during its review of completed Carroll-Loye study LNX-001 (EPA HSRB 2008). Contrary to the HSRB-reviewed (EPA HSRB 2007) and IIRB, Inc.-approved protocol, Carroll-Loye researchers also used previously recorded limb measurements, rather than collect physical data from all trial participants. This deviation occurred inadvertently when a Carroll-Loye researcher, acting upon an EPA suggestion that use of archival limb measurements was scientifically valid and would minimize study procedure invasiveness, implemented this protocol change without consulting Carroll-Loye management or IIRB, Inc. The result of an error in communication, this deviation again did not place study participants at increased risk or compromise the informed consent process.

Space Insect Repellent Testing

Because the HSRB may be reviewing spatial/area repellent protocols in the future, the Board desired to be familiar with such technology. A HSRB workgroup was created that generated a list of questions to assist the Board when evaluating future protocols. To assist the Board in their understanding of spatial repellents, the Board asked Dr. Dan Strickman to serve as a Consultant to the Board to address such questions. The HSRB considered four factors as part of its educative process of spatial insect repellents technology: environmental aspects, study design, sample size/statistics, and human subjects.

HSRB Workgroup Questions:

Environmental Aspects

1. *What are the environmental (temperature, wind, time of day, humidity, proximity to water/plants, size and type of space) and human factors (height/weight; gender, age, ethnicity, density of humans in space) that can affect insect behavior and repellent efficacy relevant to space treatment studies?*

Dr. Strickman's reply discussed the strong effect that environmental factors, especially wind (i.e. dispersal), may have on the avidity of the insects, while acknowledging such differences can be species specific. As many of these repellents are released in gaseous or particulate form, temperature and humidity will affect the dispersal behavior in any outdoor space as well. This may alter the size and clumping of the dry or droplet particulate, producing greater deposition, all of which might affect their repellent properties (see EPA air quality criteria documents; Fradin and Day 2002). The presence of other particulates and gases may also affect repellent and insect behavior. Spatial factors and human density within the space may be a factor affecting dispersion and deposition as well. Finally, the effect of the environment on repellent characteristics may have an impact on human inhalation and adsorption as well (see EPA air quality criteria documents for physical-chemical changes due to environmental and spatial factors on human health). Thus, environmental factors, including the characteristics of the test spaces, need to be rigorously controlled.

Dr. Strickman also highlighted the strong effect of human factors on insect attractiveness, noting the four-fold variation in attractiveness due to diet, gender, age, etc. (see, e.g., Qiu et al. 2006), and the overall relevance of using human subjects for repellent testing. He believes such factors could affect the performance of active ingredients. The density and behavior of humans will also affect insect behavior and thus efficacy of the spatial repellents. What has not been considered herein is the impact of human activity behavior on repellent dispersion (Fradin and Day 2002), the impact of deposition of the repellents on clothes, or the reduction of repellents gaseous or particulate repellents.

2. *What factors need to be considered for test spaces with respect to size of area in which the test is conducted? How is the most appropriate test area determined?*

Dr. Strickman commented that "The test area should exceed the label claim for area protected. An ideal test would evaluate the area protected by measuring the occurrence of pests [sic] across a transect." It is assumed that the specifications on space protected for each type of repellent would be different.

The test area would need to be free of other factors, as mentioned above. One can also speculate that odors due to other chemicals might interfere or enhance the odiferous actions of the repellents on insect behavior.

EPA indicated that the products will probably be volatile pyrethroids, and that past tests using them have involved test areas of 100-700 square feet. EPA goes on to state in their response: "Ideally, testing of a spatial repellent would generate results that could be used modularly to generate directions for use—i.e., if efficacy testing shows the size and shape of the area of protection relative to the location of an emitting device and the direction and strength of the wind, then an array of similar shapes covering the entirety of a larger area to be protected would show where multiple emitters should be placed." They also stated: "Most consumer use of spatial repellents is expected to be in relatively small areas containing at least several people. It is thus appropriate to test them with subjects placed closer together than might be acceptable in a test of a topically applied repellent, for which separation of subjects is needed to minimize interaction." EPA states in its Product Performance Test Guidelines (4.v) that the test area size

and its preparation are discussed, and (in 4.vi) that the number and placement of the candle, coil or mat should be consistent with label directions. "Test subjects should be located at the maximum distance from the candle, coil, or mat that the label recommends. If the label states that the candle, coil, or mat should be placed upwind, then test subjects should remain downwind. Otherwise, test subjects should move around the circumference of the test area periodically. Report this time interval with study results." These are important additions to Dr. Strickman's response.

3. *Does the number of human subjects within testing environments of different sizes affect insect activity? Does the number of subjects in a given area affect product efficacy or the measurement of product efficacy?*

Dr. Strickman responded by stating: "In general, results from field tests are unaffected by the number of subjects as long as the density of pests is sufficient. Often, the local population of pests is so high that additional traps or subjects do not affect results from each device or person. In the laboratory or large cage trials, where populations are limited and controlled, additional traps or human bait subjects might reduce the number collected by each trap or person." This statement may be a sufficient answer to the question, though one could add that the density of subjects will affect their behavior (if not still), and such behavior will affect the repellent dispersion. One could speculate, probably inadequately, that sufficient human density might affect insect activity as well.

The EPA response states: "If testing were to show that efficacy varies with the number of people in a test area, this would probably be reflected on the label. But because most testing uses only one number of people—i.e., one sample size for all replicates—this kind of information is unlikely to be available. EPA does not require it." EPA states in its Product Performance Test Guidelines (4.iv) that the number of test subjects is discussed, and if more that one test subject are exposed to the space repellent that the number of bites should be averaged.

4. *Are there any other special considerations regarding insect behavior in such studies that require inclusion in protocols?*

No additional Board comments on either Dr. Strickman's or EPA's response.

Study Design

1. *How is the location of open spaces typically selected? How many different or similar sites are appropriate to assess generalizability?*

Issues relevant to location and generalizability include the availability of the desired species and the abundance of the pest. For consistency in experimental design and interpretation, setting some type of minimal allowable pest density should be required in the study design (this level can be determined after expert consultation). The locations and the number of different kinds of pests should be typical of the attended use so as to make any results more generalizable.

The investigators should provide some description of the efficacy endpoints they are planning to measure (i.e. number of pests, duration, etc). That will help to assess whether the study design, including location and sites, are adequate.

Finally the Board suggested that there should be some standard conditions set for allowable wind speed, minimum pest density, and pest diversity.

2. *What are common spatial dispensing devices? How are they related to the nature of the product dispenses (e.g. gas, suspended liquid, smoke)? What are the design or measurement challenges for different dispensing devices and products?*

Regarding study design aspects, some type of data should be collected to ensure proper operation and/or dispersion. These might include: (1) determination of ambient concentrations of active product over time and area; and (2) operation of physical devices--to ensure that they are emitting levels of agent as specified in the protocol. Environmental conditions (discussed previously) including wind, temperature and humidity would also need to be accurately recorded for all types of spatial repellents.

3. *What type of dosimetry data is required to determine amount of product application used in the testing? How is discharge time determined? What are the relative design merits of the experimenter or subject discharging the repellent?*

For these products, the physicochemical characteristics of the active ingredient in the specified formulation need to be determined. This would include measures of volatility, lipid solubility and vapor pressure of the active ingredient and determination of whether any of these properties are altered by the formulation. The characteristics would determine the extent to which an ingredient may saturate the air.

To address this question requires specifying what is meant by dosimetry data. If ambient levels of compound are at issue, then these should be determined empirically. If by dosimetry one means exposure in humans, then the physicochemical properties coupled with some constants for skin penetration and inhalation would be required.

If the discharge rate is not adjustable, then general issues could be addressed in a laboratory setting prior to field testing. This could include: (1) typical time of discharge under controlled conditions as related to product design (i.e. how long does a candle burn?); (2) effect of environmental conditions on discharge (practical matters like failure of candles to burn if wind speed exceeds some maximum); and (3) duration of time in the environment after completion of discharge. Protocols should specify if discharge is a single or repeated event, and if repeated, some standardization to when discharge is repeated should be specified.

It is likely that discharge directed by the experimenter would be more consistent than discharge by the subject. If one or more subjects are allowed to discharge a product, then all variables relative to dosimetry would be extremely difficult to control.

For physical devices, the number of knockdowns seems like a reasonable endpoint.

4. *How are outcomes measured in these studies? How are insect knockdown and mortality effects measured? Are both knockdown and landings/bites usually measured in the same study? What is the difference between knockdowns and bites in terms of information regarding product efficacy/effectiveness?*

The ability of a product to disrupt the pest on its way to the host is what should be measured, suggesting that assessment of insect landings should be sufficient. This would seem to require subjects determining number of landings in a specified time period, with criteria for "failure" established. For physical devices, it would seem that the same criteria would apply, and the number of knockdowns can be determined as an associated finding (i.e. do higher knockdowns correlate to decreased landings?).

5. *What is the difference with respect to measurement in assessing efficacy of the active ingredient and effectiveness of the formulation?*

These tests must focus on the intended formulation. Characteristics of the active ingredient serve to identify those compounds that might work for repellency, but the final test must be on the formulation. Data relating to physicochemical properties, dosimetry, etc. should be collected using the formulation. For physical agents, how the device functions should also be evaluated.

Sample Size and Statistics

1. Depending on the outcome measure, what are best practices with respect to human sample size? What is the sample size norm in the field? How is determination of sample size related to square feet of test area? What is the best way to determine the power of these studies?

The general design of experiments to test efficacy of space or area repellents depends on a number of factors, and thus it is a challenge to provide specific answers to the questions focusing on sample size and statistics. The Board attempted to provide some general best practice principles that will require more specific definitions in different situations.

It has been noted that it is possible to test the efficacy of space repellents without using humans as bait. This appears to be the case for repellents such as coils, candles, lamps and others which are designed to either create a chemical barrier that repels flying insects from an area, or which are designed to have a knockdown or even lethal effect on those insects. In this case, efficacy can be tested using devices such as traps and require no human exposure. Every effort should be made to design and implement these types of studies that do not expose humans to potentially harmful bites. Since these studies would no longer be subject to review by the HSRB, no further comments on this matter are relevant.

In spatial/area studies, the experimental unit to which repellents are being applied is a particular space and/or area. Thus the sample size questions must address the number of different spaces/areas that should be included, and not the number of subjects or traps within

each space. For a given response variable, determination of sample size can be addressed using many different approaches. Two of the common ones include:

- Selecting a sample size that will ensure adequate statistical power (probability of detection of an effect of a desired size).
- Selecting a sample size that will ensure estimation of an effect of a desired size with desired accuracy.

In either case, sample size calculations will depend on:

- The response variable: in efficacy studies, several different response variables can be plausibly justified. These include, for example, number of bites during a given period, time to first bite, or proportional reduction in number of bites. Each of these response variables corresponds to a different probabilistic model and thus the actual size calculations would need to be modified accordingly.
- The size of the effect we wish to detect: Detection of a very small effect requires a much larger sample than detection of a bigger effect. If, for example, we wish to be able to detect a reduction as small as 20% in the number of bites of individuals exposed to a repellent relative to those exposed to a placebo, we will need to design a larger experiment than if we wish to detect reductions of at least 80%.
- The heterogeneity (in terms of relevant differences) in the target population and other factors: if we know that the efficacy of a product depends on gender, ethnicity, and other individual attributes, but also on wind speed, humidity and other environmental factors, then the sample size needs to be large enough to permit randomizing over these attributes or designing an experiment in which these factors can be used, e.g., to define blocks. In any event, it is very important to account for differences across experimental units that are known to affect the response; these may confound results if not appropriately addressed. The possibility of generalizing results from the trial to public usage depends greatly on whether systematic effects on the response (other than the treatment itself) have been accounted for either through randomization or through blocking.
- The anticipated uncertainty associated with the estimated effect size: once every known confounder has been accounted for, all other sources of uncertainty in the estimated model parameters must be known or approximated. The size of this uncertainty is directly related to the required sample size in the experiment.

2. What are best practices with respect to statistical analysis? How is censored data handled?

Implementing an analysis that is technically sound and consistent with the design of the study (which in turn must be consistent with the **stated goals** of the study) is critical. For example, the standard assumption of normality typically is not appropriate when the response variable is time to an event or when it is a count variable (such as the number of bites in a given period).

In the case of space repellents, experiments are typically designed so that a treatment group can be compared to a control group and *relative efficacy* can be estimated. Issues such as

the allocation of units (humans) to groups and of balance of other relevant factors become absolutely critical and greatly affect the statistical analysis of the resulting data.

3. *What are the pros and cons of various endpoints (e.g., ending the study after a set number of hours, waiting until the first landing/bite, other) to assess product efficacy (e.g., to meet assumptions for appropriate statistical analyses)?*

Censoring, if unaccounted for, can seriously bias results. Most statistical methods can be extended to account for some proportion of censored observations, but they tend to fail when the proportion of unobserved responses is large. Thus, it is crucial to know enough about the response variable and the factors that affect it so that a reasonable experiment can be designed and the proportion of censored observations can be kept to a minimum. Minimizing the proportion of censored responses should be an element in the design of the experiment.

Human Subjects

1. *Why are human subjects necessary for such studies if the outcome measures are knockdowns or mortality?*

As with other areas of human subject research, environmental factors and study design features play a large role in determining the ethical considerations and safety of human subjects. The Belmont Report principles of respect for persons, beneficence and justice will serve well as a guide in determining the ethical acceptability of any study that may presented to the HSRB.

Justification for the involvement of humans (as "bait") must provide sufficient information to allow the HSRB to determine that the involvement is appropriate from both scientific and ethical perspectives. Why the study design (including outcome measures) dictates the need for human exposure should be explained. Information provided should include alternative study designs, including a justification why those alternative approaches were ruled out. The types of population(s) to be tested should be explained and justified. While it is presumed that it is unlikely that issues relating to vulnerable populations will arise in this type of testing, consent issue should be adequately presented in the proposals.

2. *What are the potential risks to treated subjects (e.g. inhalation, dermal effects)? What are exclusion criteria in subject selection to avoid such risks? How is the degree of risk related to dosage, ingredient, formulations, and aerosol pressure?*

Details about possible risks from inhalation and contact routes will be important, as will the steps taken to mitigate or minimize those risks. Inclusion and exclusion criteria for the study need to be included. General steps such as not exposing persons with asthma, other breathing problems and product or class sensitivity would be appropriate, but each product protocol would have to adequately answer questions such as these before the test (Note: this raises a similar "justice/beneficence" issue that FDA faces; namely, if products are tested in a study population that excludes persons likely to react negatively to the product, but the product will be used by those persons or groups after approval, then they may be exposed to risks that are unquantified).

If protective clothing is proposed, a description of the protection afforded and the impact on the study design (e.g., decrease in ability to attract insects) should be included. Duration of exposure needs to be described and the potential risks – including fatigue and heat-related illness, agent exposure (dosage, ingredient, etc.) and vector exposure (lab-raised versus wild) – adequately addressed. Prior testing (e.g., laboratory, animal, human, computer modeling) information may be required to adequately assess welfare concerns. References to EPA agent approval and other known agent information will be important for determining safety and assessing risk. This risk information will be considered in relation to the societal benefit, hence scientific validity and the soundness of study design will be important in these studies as they have been in other studies considered by the HSRB.

3. *What is the methodological rationale for continuous versus intermittent exposure? How do human risk differ for these types of exposures? Will exposure start at the beginning of the test period immediately after release of the product?*

No additional Board comments on either Dr. Strickman's or EPA response.

4. *If the test agent has properties to repel or destroy an insect, what is the relationship (if any) to a related mechanism of action to humans?*

No additional Board comments on either Dr. Strickman's or EPA response.

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