EPA-HSRB-06-03

George Gray, Ph.D.
Science Advisor
Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: June 27-30, 2006 EPA Human Studies Review Board Meeting Report

Dear Dr. Gray:

The United States Environmental Protection Agency (EPA or Agency) requested the Human Studies Review Board (HSRB) to review scientific and ethical issues addressing a human toxicity study involving one pesticide active ingredient-chloropicrin; guidelines for conducting insect repellant efficacy testing; protocols for conducting two insect repellent efficacy studies; and protocols for conducting five occupational handler exposure monitoring studies. At the Chair's request, the Board developed scientific and ethics criteria for new protocols. The enclosed HSRB report addresses the Board's response to EPA charge questions for the Board's consideration at its June 27-30, 2006 meeting. A summary of the Board's conclusions is provided below.

**Chloropicrin**

Scientific Consideration

- The chloropicrin acute inhalation, human toxicity study, was scientifically sound for the purpose of estimating a safe level of inhalation exposure to chloropicrin. A LOAEL of 100 ppb was a scientifically justified point of departure (POD).

Ethical Considerations

- There was not clear and convincing evidence that the conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent).

- There was not clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

**Insect Repellent Product Performance Efficacy Guidelines**

Actions to Minimize Risks to Human Subjects

- The consensus of the Board was that studies involving humans are necessary to evaluate the efficacy of products to repel insects and other arthropods.

- Risk identification and minimization is also essential. In their protocols, investigators should adequately identify risk to participants and describe adequate steps they will take to minimize these risks.

Types of Toxicity Data That Should Be Generated

- The consensus of the Board was that the minimum set of toxicity data that should be routinely generated before an investigator conducts repellent efficacy testing on human subjects with a new product is that which will assure that subjects would not be at risk of permanent or irreversible harm.

Self–experimentation

- It may not be *a priori* unethical or problematic from a scientific perspective for a principal investigator to be a subject in his/her own study IF:

  - The study was approved by an IRB in the same manner as was required for most human subjects research;

  - Scientific issues:
    a. Principal investigator met all enrollment criteria;
    b. The study was a well controlled trial with a justified sample size adequate to answer the study question with statistical surety;
    c. The principal investigator was one of many subjects, accounting for normal human variability, and allowing results to be generalized to a broad population; and
    d. The outcome measure is objective and measured by another (blinded, when possible) investigator;

  - A plan is in place to assure integrity and safety of the study while the principal investigator was a subject

  - A plan is in place to ensure for study oversight if principal investigator becomes incapacitated;

  - Participation of other research staff/employees should be allowed only if the above criteria are satisfied and if issues of coercion/undue influence can be addressed, which may or may not be possible;

  - The investigator justifies why he/she should be a research subject in the study.

Negative Controls

- The Agency should modify the guideline to say that negative controls "may be" needed (instead of "are") and that examples be given both for when negative controls are needed and when they are not. The language on positive controls may also benefit from further expansion and clarification.

Design of Studies to Support Assessment of Repellent Efficacy

- The Board consensus was that the time to first confirmed bite, or the time to first confirmed "intent to bite" (if ascertainable), has the advantage of minimizing risk of vector-borne diseases. However, for some studies there is a statistical advantage for the use of relative protection as an appropriate outcome measure. Since relative protection procedures in field studies increases the risk of vector-borne diseases, protocols must: (a) justify the level of risk by the probability and social value of the benefits; (b) adequately identify all risks; (c) present a description of adequate steps to minimize the risks; and (d) provide consent materials that include information about the prevalence and risks of any vector-borne diseases, consequences of contracting disease, and alternative effective repellents outside of the research.

Minimum number of subject to evaluate the level of repellent efficacy

- It is critical that the proposed number of subjects be justified on the basis of good research design. Because experiments to test effectiveness of products to repel insect and tick bites are likely to vary in terms of design, response variable, target population of interest, detectable effect size and other important variables, requiring a specific minimum sample size that guarantees sufficient accuracy in all cases might be impractical. Instead, the guideline might require that registrants present their own sample size calculations and that the methodology used in the calculations be justified relative to the factors noted in the bullet list above.

Compensating Research Subjects For Research-Related Injuries

- It is appropriate that sponsors of repellent efficacy research studies should be required to assure that if a subject is injured as a result of participating in a study, then the subject will not have to assume the costs of medical care needed to treat such injuries.

Special Considerations in Informed Consent Materials

- To comply with the human studies rule, consent information for pesticides studies must include: (a) detailed information on the procedure (e.g., number of insect bites or landings anticipated, nature apparatus or field context, length of time of exposure); (b) a clear statement of the risks involved (e.g., discomfort from bites, risk of vector-borne disease, medical consequences of the disease, treatments available for the disease); (c) the voluntary nature of participation (e.g., statements that eliminate the perception of coercion for students or employees; specific instructions on how to

signal desire to withdraw from the study); (d) the fact that there was no immediate direct benefit to the subject in participating as well as a description of alternative available repellents; and (e) other steps outlined above. In addition, informed consent information should be as detailed for experienced subjects as for naïve subjects.

**HSRB Protocol Criteria**

- Before the Board reviewed the presented human studies proposals, the HSRB developed scientific and ethical criteria as a guide for its evaluation of such studies. Such criteria will be helpful for the Agency, study investigators, and other members of the public to understand the Board's approach for the review of proposed human studies.

**Study EMD-003 from Carroll-Loye Biological Research**

Scientific Considerations

The HSRB recognized three major limitations to the protocol as submitted to the HSRB for review. These limitations included: (1) the lack of a clear rationale underlying the conduct of the study; (2) the lack of identification and characterization of the formulations to be tested and (3) the scientific design of the study. Of these issues, the design of the study was seen as the most significant shortcoming of the proposed work.

Ethical Considerations

The Board concurred with the initial assessment of the Agency that the study submitted for review by the Board failed to meet the ethical requirements established in the Agency's human studies rule (40CFR26).

The Board determined the proposed research described in these studies did not comport with the applicable requirements of 40CFR26, subparts K and L. The study documents submitted for review also failed to comply with the requirements of 40CFR26, subpart M. However, the deficiencies noted, while significant, were not irreparable.

**Study EMD-004 from Carroll-Loye Biological Research**

Scientific Considerations

- It was not clear whether new studies involving human subjects were necessary; however, if the repellency had never been tested with North American mosquitoes, the tests were probably necessary.

- The potential benefits of the study were clear, i.e., that an effective repellent would be available that would have either greater efficacy and/or fewer drawbacks than what was currently approved. However, empirical evidence or procedures to determine risks to subjects (e.g., of vector borne disease) were not adequate.

- It was not clear if the stated numbers of subjects would be repeated in both testing locations. The basis for the dose levels and formulations was not provided. There were no controls with just the formulation matrix without the repellent.
- These issues would need to be addressed before the protocol could be considered acceptable.

Ethical Considerations

- The Board concluded that the proposed research did not comport with the applicable requirements of 40 CFR 26, subpart K.

- The proposed research does comport with 40CFR26 subpart L, as pregnant women and children were excluded.

- Although the ethical concerns identified by the Board could be remedied, there were sufficient questions raised about the adequacy of the research design to cast doubt on whether the proposed research would meet the criteria for IRB approval found under 40 CFR 26.1111(a) (1). In other words, absent a sound research design, any exposure of human subjects to risk would be unnecessary and unjustifiable.

**Occupational Handler Exposure Monitoring Studies**

Scientific Considerations

- The occupational handler exposure monitoring studies were components of a large-scale exercise to create a contemporary database on occupational exposure to agricultural pesticides. The undertaking is in itself likely to be worthwhile in quantifying and improving our understanding of the exposures and risks of pesticide handlers.

- The potential benefits are large and the risks appear to be relatively modest. However, the materials supplied for HSRB review failed to deal adequately with risks and benefits. None of these protocols can be properly evaluated in regard to scientific validity because they lack: (1) a developed rationale documenting the need for new data; (2) a clear and appropriate plan for the handling of the data (including its statistical analysis), and (3) an explanation of the uses to which the data will be put. These points need to be addressed briefly at least in each specific protocol and, more fully, in a separate and new "governing document" that is not simply a generic description of the planned activities.

- Additional validation studies are recommended to determine the extent to which dermal exposure measurements may underestimate true exposure. Laboratory-based removal efficiency studies or field-based biomonitoring studies could be conducted to achieve this goal. Such studies should be published in the peer-reviewed literature. Broader participation of the scientific community and of parties with a direct interest

in the database project, such as the labor community, would likely improve the quality of the database and enhance the credibility of its use in risk assessments.

- The HSRB recommended that specific criteria for withdrawal from study participation due to heat stress be included in these worker exposure protocols, and that the protocols included a heat stress management plan. In addition, the length of each study should be truly representative of a full workday, and each protocol should document the basis for the proposed duration of the study.

- The HSRB was gratified to receive the Agency's response to its query regarding the use of diazinon in the AHE37. It is the understanding of the HSRB that the Agency would inform the AHETF that it needs to identify a pesticide other than diazinon in this protocol to evaluate exposures associated with open pour activities and applications using open cabs, and that the Agency would ensure that future protocols comply with the most current risk mitigation measures specified in IREDs and REDs.

Ethical Considerations

- The Board concurred with the initial assessment of the Agency that the studies submitted for review failed to meet the ethical requirements established in the 40CFR26.

- The Board determined the proposed research does not comport with the applicable requirements of §40CFR26, subparts K and L. However, the deficiencies noted, while significant, were not irreparable.

In conclusion, the EPA HSRB appreciated the opportunity to advise the Agency on the scientific and ethical aspects of human studies research and looks forward to future opportunities to continue advising the Agency in this endeavor.

Sincerely,

Celia Fisher, 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 mention of trade names or commercial product 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.

**United States Environmental Protection Agency Human Studies Review Board**

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* Recused from chloropicrin discussion and deliberation
**Not in attendance at the June 27-30, 2006 Public Meeting

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## INTRODUCTION

On June 27-30, 2006, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB) met to address scientific and ethical issues surrounding a human toxicity study involving one pesticide active ingredient-chloropicrin; guidelines for conducting insect repellant efficacy testing; protocols for conducting two insect repellent efficacy studies; and protocols for conducting five occupational handler exposure monitoring studies.

The Pesticide Registration Improvement Act (PRIA) requires that EPA complete its decision-making process on certain types of applications to register a pesticide product within specified amounts of time after receiving the application for registration. In addition, PRIA established deadlines for EPA to complete "reregistration" of pesticide active ingredients that are contained in pesticide products initially registered before 1984. Reregistration involves the systematic reexamination of these older pesticides, applying contemporary scientific and regulatory standards. When a pesticide active ingredient is approved for use on food, EPA combines reregistration with the tolerance reassessment process mandated by the Food Quality Protection Act of 1996 (FQPA).

Chloropicrin is undergoing reevaluation in the reregistration process. As part of the review of the available toxicity data on chloropicrin, EPA had identified a study involving intentional exposure of human subjects which EPA intends to use in its risk assessment. In accordance with 40 CFR 26.1602, EPA sought HSRB review of this study.

EPA regulates pesticides intended for use on skin to repel arthropod pests. As part of the application for registration of a new repellent, EPA requires data to demonstrate that the product is effective. The Agency had developed a guideline for the conduct of such studies, and presented it to the Board for comment. The Agency had also received protocols for two insect repellent efficacy studies, and as required by the recently promulgated regulation, EPA is required to submit the protocols to the HSRB for its review and comment. See 40 CFR 26.1601.

In addition, EPA routinely considers the human health risks of occupational handlers of pesticides in both its reregistration program and as part of its review of an application for registration pending under FIFRA and PRIA. EPA has received five protocols for conducting new research involving human subjects to collect data on the levels of exposure received by people when mixing, loading, and applying pesticides under various conditions. In accordance with 40 CFR 26.1601, EPA sought HSRB review of these proposed protocols.

For the human studies or guidelines under consideration, the Agency provided the Board with the complete study report or associated protocols and any supplements available to the Agency. Similarly, guideline documents were included with appropriate background information. Completed studies were assigned a unique identifier (e.g., the Master Record Identifier-MRID), which the Agency uses to manage documents. When a company submits multiple documents pertaining to a single study, each document is typically assigned a unique tracking number.

In addition, for each study, protocol or guideline to be evaluated, the Agency provided a review of the ethical conduct. Each ethics review identified any deficiencies which were identified compared to appropriate ethical standards. EPA has intentionally deferred making a final determination of whether the chloropicrin study satisfies the ethical standards for acceptability in 40 CFR sections 26.1704 – 26.1706, pending the advice of the Board.

For most studies and protocols, the Agency develops documents, called Data Evaluation Records (DERs), containing a scientific review. The Board was provided with one or more DERs for chloropicrin, the two proposed insect repellent efficacy protocols, and each of the five Agricultural Handlers Exposure Taskforce (AHETF) protocols. DERs contain summaries of the study design, methods and results, describe potential deficiencies, and provide conclusions about the usefulness of the study in risk assessment.

In addition to the DERs, the Agency had prepared or included several other background documents which address various elements of the issues to be reviewed by the HSRB. For example, for the AHETF protocols, a number of types of documents had been provided including transmittal documents and the charge questions, general background information pertaining to the manner in which the Agency completes exposure/risk assessments, the AHETF protocols and various documents that the AHETF had developed related to the manner in which it intends to conduct studies, the background documents related to the AHETF protocol review by the Western Institutional Review Board of Olympia, Washington, and the EPA science and ethics reviews of these protocols.

The Agency asked the HSRB to advise the Agency on a range of scientific and ethics issues and on how proposed and completed studies should be assessed against the provisions in 40 CFR 26 of EPA's final human studies rule**.** This report transmits the HSRB's comments and recommendations from its June 27-30, 2006 meeting.

**REVIEW PROCESS**

On June 27-30, 2006 the Board had a public face-to-face meeting in Arlington, Virginia. Advance notice of the meeting was published in the Federal Register "Human Studies Review Board: Notice of Public Meeting (71 Federal Register 32536 and 71 Federal Register 33747). At the public meeting, following welcoming remarks from Agency officials, Celia B. Fisher, HRSB Chair, proposed a set of scientific and ethics criteria consistent with the language of 71 Federal Register 6137 to guide Board evaluation of completed studies. The Chair's scientific criteria asked the Board to consider the following two questions: (1) did the research design and implementation meet scientific standards and (2) did the data generated by the study have implications for the Agency's Weight of the Evidence (WOE) review and, when applicable, aspects of the risk assessment? The Chair reviewed the Chair's science criteria and the Board's criteria for scientific standards for human dosing studies established at the Board's May 2006 meeting. The Chair's ethics criteria asked the Board to consider three questions: (1) did the study fail to fully meet specific ethical standards prevalent at the time the research was conducted; (2) was the conduct of the study *fundamentally unethical* (i.e., specifically was there clear and convincing evidence that the research was intended to seriously harm participants or failed to obtain informed consent); and (3) was the conduct of the study *significantly deficient*

relative to the ethical standards prevailing at the time (i.e., was there clear and convincing evidence that identified deficiencies that could have resulted in serious harm based on knowledge available at the time the study was conducted *or* the information provided to participants could seriously impair informed consent).

The Board then heard presentations from the Agency on the following topics: scientific and ethical issues addressing a human toxicity study involving one pesticide active ingredient-chloropicrin; guidelines for conducting insect repellant efficacy testing; protocols for conducting two insect repellent efficacy studies; and protocols for conducting five occupational handler exposure monitoring studies. At the Chair's request the Board developed scientific and ethics criteria for new human study protocols.

The Board heard oral public comments from the following individuals:

Chloropicrin

Robert Sielken, Ph.D., of Sielken and Associates and John Butala, Ph.D. of Toxicology Consultants, Inc. on behalf of the Chloropicrin Task Force.

Jennifer Sass, Ph.D. on behalf of the Natural Resources Defense Council.

Guidelines For Conducting Insect Repellant Efficacy Testing

Scott Carroll, Ph.D., on behalf of the University California at Davis and Carroll-Loye Biological Research.

Protocols For Conducting Insect Repellent Efficacy Studies: Study EMD-003 And Study EMD-004

Scott Carroll, Ph.D., on behalf of Carroll-Loye Biological Research.

Mr. Dan Giambattisto on behalf of EMD Chemicals, Inc.

Mr. Niketas Spero on behalf of Insect Control and Research, Inc.

Protocols For Conducting Occupational Handler Exposure Monitoring Studies: Study AHE34, Study AHE36, Study AHE37, Study AHE38 And AHE42

Victor Canez, Ph.D., Elliot Gordon, Ph.D., Mr. Curt Lunchick, and Mr. Larry Smith on behalf of the Agricultural Exposure Handlers Task Force

Ms. Shelly Davis on behalf of Farmworker Justice Fund

In addition, the Board received written public comments from the Agricultural Exposure Handlers Task Force, Carroll-Loye Biological Research, the Farmworker Justice Fund, the FMC

Corporation, Toxicology Consultants, Inc. and the Walter Reed Army Institute of Research, Vector Control/Repellents Program.

For their deliberations, the Board considered the materials presented at the meeting, written public comments and Agency background documents (e.g. pesticide human study, Agency data evaluation record (DER) of the pesticide human study, weight of evidence review, ethics review, pesticide human study protocol and Agency evaluation of the protocol).

## CHARGE TO THE BOARD AND BOARD RESPONSE

### Chloropicrin

### Charge to the Board

Chloropicrin is a non-selective soil fumigant whose primary toxic effect is sensory irritation in which stimulated free nerve endings mediate sensations and clinical signs in the nose, eyes, throat, and upper respiratory tract. Chloropicrin is a unique soil fumigant in that it is also used as an indicator chemical or warning agent (2% or less by weight in formulations). The Agency is developing an assessment to estimate inhalation risk to bystanders and workers from acute exposures to chloropicrin.

Scientific considerations

The Agency's "Weight of Evidence" (WOE) document and Data Evaluation Records (DER) for chloropicrin described the study design of the acute inhalation, human toxicity study. The Agency had concluded that the human toxicity study was appropriate for developing a point of departure for extrapolation of inhalation risk to bystanders and workers exposed to chloropicrin.

Please comment on whether the study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of inhalation exposure to chloropicrin.

### Board Response to the Charge

Background of Study

To determine a subject's sensitivity for the detection and characterization of feel to the human eye, nose, and/or throat produced by chloropicrin vapors, as well as the odor threshold, healthy volunteers (18 to 35 years of age, average 23 years) were exposed to a range of vapor concentrations and exposure durations in a controlled laboratory setting. The investigation consisted of three phases, very brief exposures (Phase I) and more extensive exposures (Phases II and III). These phases are described in more detail below.

The study report cited Krieger (1996) as a review of the risks to workers from exposure to chloropicrin in agricultural applications. It appeared that this reference was relied upon for basing concentration and duration for the human sensory study. From this reference, a time-

weighted average of 0.1 ppm (100 ppb) was indicated to evoke no response in humans. The report then indicated concentrations of 0.15 to 0.3 ppm would evoke concentration-dependent sensory detection via chemesthesis, as well as reflex tearing and cough. Concentrations above 0.3 ppm would evoke an increasing degree of irritation. Odor was noted as occurring at about 0.9 ppm. The extended phases were focused on concentrations of likely occupational relevance, both below and just above 100 ppb, the ACGIH (American Conference of Governmental Industrial Hygenists) threshold Limit Value (TLV) and OSHA Permissible Exposure Limit (PEL).

Statistical analyses including all subjects for each phase of the study were provided in the study report. The EPA provided a logistical regression when appropriate as well as an analysis for only those subjects positively detecting chloropicrin for Phases I and II of the study.

Phases of Study

Phase I: The objective of Phase I was the identification of chloropicrin by odor (both nostrils, single sniff), eye feel (one eye, 25 seconds), or nasal feel (one nostril, 7 seconds) at 356 ppb, 533 ppb, 800 ppb and 1200 ppb generated from a vapor delivery device. Phase I consisted of 62 subjects (32 male and 30 female) for odor and 63 subjects (32 male and 31 female) for eye feel. The same subjects participated for both odor and eye feel. Confidence of feel was rated 1 to 5, with 1= very low, 3= moderate, and 5= very high confidence. Severity of feel was not rated in Phase I. For Phase I, approximately 10% to 13% of subjects failed to detect either odor or eye feel after momentary exposures to chloropicrin over the range evaluated. Approximately 13% (8 of 62) of subjects (5/30 female and 3/32 male) failed to detect the odor of chloropicrin over the range of concentrations evaluated. Approximately 11% 7/ 63 (11%) of subjects failed to detect eye feel (two male and five female) at any concentration. The feel of chloropicrin in the nose was not a reliable endpoint and was therefore dropped prematurely from the Phase I study by the study director. The median concentration of all subjects for detection of eye feel was 900 ppb, or 790 ppb for males and 1010 ppb for females, although this gender difference was not significant. The median for only those subjects detecting odor was 356 ppb while eye feel was between 356 ppb and 533 ppb.

Phase II: The purpose of Phase II was the detection of chloropicrin in the eyes, nose, and/or throat during exposure to 50 ppb, 75 ppb, 100 ppb, or 150 ppb chloropicrin vapor in a walk-in chamber for 20-30 minutes. (Odor was not studied in Phase II). A total of 62 subjects (32 male and 30 female) participated in Phase II. 12 of 30 female subjects and 14 of 32 male subjects also participated in Phase I of the study. Subjects responded “yes” for a positive feel or “no” for no feel. A level of confidence to each event (eye, nose, throat) was also recorded with 1= not certain, 2= moderately certain, and 3= very certain. The first exposure in a day consisted of a known blank (air). This exposure served to acclimate the subjects to the task in the chamber. The subjects were asked to perform ratings as they would for future blinded exposures. One female subject left the exposure chamber after 16 minutes of chloropicrin at 75 ppb. An explanation for this subject’s premature exit from the chamber was not provided. At 150 ppb, this same subject along with another male in the chamber left the chamber after 15 minutes. On a separate day of testing, one female and one male subject also left the exposure chamber after 15 minutes of exposure to 150 ppb. Again, no explanation was given for these subjects’

premature departure from the chamber. No subjects left the chamber at 50 ppb or 100 ppb. The results of Phase II indicated that eye feel was more sensitive than either nose or throat feel. ANOVA results provided in the study report indicated that concentration and duration were significant ($p<0.0001$) for the eye response only. As a group, subjects differentiated 50 ppb chloropicrin in the eyes from the blank after 20 minutes of exposure. Differentiation from blank occurred after 5 minutes at 75 ppb, 3 minutes at 100 ppb, and 2 minutes at 150 ppb. There were no significant statistical interactions of response with sex for the eyes, nose, or throat responses. On an individual basis, binary detection indicators (yes/no) developed by the Agency were combined by participant across dose levels. Using eye feel as a marker of detection of the chemical, 20 of the 62 participants (32%) could not detect chloropicrin at any concentration:12 of 30 (40%) females and 8 of 32 (25%) males failed to make progress toward eye feeling over a 30 minute period of exposure. In addition, 46/62 (74%) and 48/62 (77%) subjects could not detect the chemical via the nose or throat, respectively at any concentration, again indicating the greater sensitivity of the eye.

Phase III: The goal of Phase III was the detection of chloropicrin vapor as evidenced by irritation to the eyes, nose and/or throat after 1 hour (60 minute) exposures repeated over 4 consecutive days. Concentrations tested included blank (air), 100 ppb, and 150 ppb. This phase included a clinical exam of the eyes, nose and throat, as well as pulmonary function testing with the outcome variable FEV1 (Forced Expiratory Volume) and FVC (Forced Vital Capacity), rhinomanometry, and nasal cytology. In addition, an assessment was performed based on ocular cytology from samples of cells taken from the conjunctival membrane inside the lower eyelid and from the concentration of exhaled nitric oxide sampled from the lung (eNO) and nose (nNO). Subjects participated in 3 cycles [(6 days per cycle) of 6 sessions, each beginning on Friday and ending on the subsequent Friday] (no measurements taken on Saturday or Sunday). Subjects remained in exposure chambers for 1 hour per session on Monday through Thursday (4 consecutive days). The 3 cycles included exposure to 100 ppb, 150 ppb, and just air (blank). The order in which the subject was exposed to these concentrations was random to prevent confounding. At least one week separated the end of one cycle of exposures and the beginning of another for each subject. Subjects rated their symptoms in three setting: (1) severity of effect for eye, nose, and throat while in the chamber (0= no symptom to 3= severe); (2) before and after exposure in the chamber and at the beginning and end of each week of exposures; and (3) at the beginning and end of a cycle of exposure. Symptoms were rated using the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), a series of 28 questions in seven domains, where the subjects used a seven point scale from Not Troubled to Very Troubled. The first two instruments referred to how the subject felt at the time of rating, the RQLQ referred to how the subject felt over the previous week. When in the exposure chamber, subjects rated symptoms (0 to 3) after 30 seconds, at 1 minute, and every minute until the end of the exposure at 60 minutes. Every 10 minutes, study personnel read and recorded the subjects' blood oxygen saturation from a pulse oximeter attached to the finger (data not included in report). A total of 15 males and 17 females participated in Phase III. Two females in Phase III also participated in Phases I and II. One male in Phase III also participated in Phases I and II and one male in Phase III participated in Phase II only.

Results of the Study

For Phase II, one female subject left at 75 ppb and again at 150 ppb with another male. On a separate testing day, one female and male left the chamber prematurely at 150 ppb. 38% (8 males and 8 females) of subjects detected chloropicrin initially at 50 ppb and consistently up to 150 ppb. Subjects gave higher ratings to symptoms in the eye than to those in the nose and throat. Subjects gave nominally slightly higher ratings in the nose than in the throat, but expressed no symptoms of consequence at either site. There was no indication of intensification of symptoms based on subject scoring for any parameter on the consecutive days of exposure. For the eye, the study report ANOVA indicated Level of Exposure ($p<0.001$), and interaction of Level of Exposure by Duration of Exposure was significant ($p<0.001$). An ANOVA also revealed an effect of Level of Exposure by Day ($p<0.02$). As a group with all subjects included (even those not feeling), the analysis provided in the study indicated the average rating of eye irritation at 100 ppb reached approximately 0.5 (1=mild) with 30 minutes to reach steady state, which remained until the final minutes and then sometimes regressed. At 150 ppb, the average rating of eye irritation reached 1 (mild, symptom present, but minimal awareness, easily tolerated) with 20 minutes to steady state until fading slightly in the final minutes.

On an individual level, the severity of ocular irritation reported by subjects in Phase III varied from no symptoms to severe at both 100 ppb and 150 ppb. Five of 17 females (29%) and 7 of 15 males (47%) rated no eye irritation at 100 ppb while 3 of 17 females (18%) and 5 of 15 males (33%) rated no eye irritation at 150 ppb. Nasal and throat irritation was never reported above a “2" and mainly consisted of “0" or “1". Scores of severe “3” ocular irritation were sporadic during the first 30 minutes of exposure in 2 females and in 4 males at 100 ppb.

The second half of the exposure to 100 ppb (31-60 minutes) revealed a more consistent response in ocular severity (in 3 females and 5 males). “Severe” (grade 1) was defined as a symptom that was hard to tolerate and that could interfere with activities of daily living or sleeping. At 150 ppb, 4 females and 3 males reported consistent severe eye irritation beginning as early as 8 to 9 minutes of exposure until the end of exposure at 60 minutes. Moderate (grade 2) eye irritation was also reported sporadically during the first 30 minutes by the same individuals reporting severe eye irritation but with a more consistent response in moderate eye irritation during the second half (31-60 minutes) of exposure. Two additional females and two additional males reported moderate eye irritation during the second half of exposure that did not report eye irritation during the first half of exposure. Results for the daily measurements (Cochran Q test) provided in the study indicated the number of times a rating post-exposure exceeded a rating pre-exposure for nasal congestion was not significant ($Q=0.75$) while eye irritation (redness) was significant ($Q=28.8$, $p<0.001$). Nasal congestion and ocular erythema (redness) occurred more than the trivial frequency. However, according to the report, the ocular irritation did not translate into more prominent redness. No biologically significant changes were observed for the lower respiratory variables.

For the lower respiratory variables (FVC, FEV1, eNO), ANOVA analysis from the study report indicated a significant interaction of Level by Order ($p<0.05$) for FVC (Forced Vital Capacity), with only 3% variation in FVC. FEV1 (Forced Expiratory Volume), averaged 93.6% before exposure and 93.7% after exposure. The variation spanned 3% with no statistical

significance achieved. Exhaled nitric oxide by the lungs (eNO) equaled 37.8 before exposure and 39.2 after exposure with no significance achieved. Sex was not significant for any of interactions of the three lower respiratory variables. Two upper respiratory alterations, nNO (nasal nitric oxide) and flow, were observed for one-hour exposures that occurred only day by day. For the upper respiratory variables (nNO, inspiratory flow, expiratory flow), nNO was significant for Level of Exposure by Order of Exposure with 399 ppb before exposure and 425 ppb after exposure ($p=0.012$). Level of Exposure by Order of Exposure by Day was not significant. nNO increased 1% after exposure to blank, 10% after exposure to 100 ppb, and 8% after exposure to 150 ppb. The effect of nNO did not continue from one day to the next. Inspiratory flow and expiratory flow equaled 450 and 415 mL/sec, respectively, before exposure and 435 and 406 mL/sec, respectively, after exposure.

Chloropicrin had a differential effect on flow. Level of Exposure by Order of Exposure was nearly significant ($p=0.087$). However, Level of Exposure by Order of Exposure by Day was not significant. Flow decreased 2% after exposure to blank and increased 2% after exposure to 100 ppb chloropicrin, however, flow decreased by 8% after exposure to 150 ppb. Sex was not significant in any of the relevant interactions for the upper respiratory variables. Physiological effects such as changes in nNO and flow rate may indicate signs of nasal congestion and engorgement.

Cell types and cell numbers from the Rhinoprobe samples were approximately the same at the end of each cycle as at the beginning. For the RQLQ questionnaire results, nasal congestion was the only parameter that reached a level where more than half of the subjects gave a response above zero. 53% of subjects reported a non-zero response to congestion after 4 days of exposure to the blank vs. 41% and 34% after exposures to 150 ppb and 100 ppb, respectively. The average ratings equaled 0.53, 0.34, and 0.41 for the blank, 100 ppb, and 150 ppb, respectively, where a rating of 1 signified hardly troubled at all. Watery eyes, sore eyes, and swollen eyes were scored higher by subjects after exposure to either 100 or 150 ppb chloropicrin than to the blank. The Q test revealed significance for the sore eyes only ($p<0.05$). The highest rating given after exposure to swollen eyes was 0.47.

The LOAEL was determined to be 100 ppb, the lowest concentration tested, based on eye irritation, increased nasal nitric oxide (nNO), and differential effect on inspiratory and expiratory flow. A NOAEL was not established in Phase III.

Critique of the Study

Strengths:

This was an excellent scientific study of eye, upper and lower respiratory irritant effects at various concentrations over various short term (i.e., acute effects) time periods. The objective and subjective measurements and the statistics were reasonable. Most importantly, Phase III of the study provided evidence of upper airway (nasal) respiratory effects and established a LOAEL.

Weaknesses:

Concentrations below 100 ppb were not investigated in Phase III so as to compare with results from Phase II.

HSRB Consensus and Rationale

The chloropicrin acute inhalation, human toxicity study was scientifically sound for the purpose of estimating a safe level of inhalation exposure to chloropicrin. A LOAEL of 100 ppb was a scientifically justified point of departure (POD).

**Charge to the Board**

Ethical considerations

The Agency requests that the Board provide comment on the following:

a. Was there clear and convincing evidence that the conduct of the Cain study was fundamentally unethical?

b. Was there clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted?

**Board Response to the Charge**

The Cain study was conducted from 2002 through 2004. The study was performed in La Jolla, California by researchers at the Chemosensory Perception Laboratory of the University of California, San Diego. The study sponsor was Chloropicrin Manufacturers Task Force, whose mailing address is in care of Steptoe & Johnson, LLP, 1330 Connecticut Avenue, NW, Washington, D.C. The documents provided by the sponsor specifically state that the research was to be conducted with the approval of an Institutional Review Board (IRB) at the University of California, San Diego, and in compliance with the Human Subject's Bill of Rights (a provision of California law). The study was in fact reviewed and approved by an IRB at that university, and the university had provided documentation that it held a Federalwide Assurance with the Department of Health and Human Services. The documentation provided by the university's Human Research Protections Program indicated that it reviewed this study pursuant to the standards of the Common Rule (45 CFR 46, Subpart A) and determined it to be in compliance with that Rule.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the Cain study, as detailed by the EPA (USEPA 2006a). The Board wanted to comment on several specific aspects of the study:

1. The consent forms stated that chloropicrin "is used commonly to fumigate fields for planting and as a warning agent in structural fumigation." It made no mention of prior uses of this compound for the intentional purpose of harming and even killing people. As noted in one of the documents supplied by the EPA (Prentiss 1937), chloropicrin "appears to have been the most widely used combat gas" in World War I. That reference further notes that "as a war gas [it] has a number of desirable offensive properties," and like phosgene gas, "it is a lethal compound." Some members of the Board concluded that in the context of being asked to participate in a study to determine more information about the harmful effects of this gas on human beings, the subjects should have been informed about the historical use of chloropicrin as a war gas. Others concluded that the dose levels and other conditions were significantly distinct from the war use that reference to those usages were not necessary for an informed participation decision.

2. The consent forms, in describing the likely risks of participating in the study, noted that "[e]xposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure." Some members of the Board believed that this statement was inaccurate in describing the possible risks of exposure to "greater" amounts of choloropicrin (which, as noted in item 1 above, if in a sufficiently high dose, is well known to cause substantial permanent effects, up to and including death). Those members concluded that the consent form should have been more truthful in describing the possible consequences of exposure to high doses of chloropicrin (though it could also have explained why it would not be the case that the subjects could ever end up being exposed to such high doses as a result of participation in the study).

3. The consent form, in describing the purpose of the study, stated that it was "intended to provide information regarding safe levels of exposure." This language might suggest to many prospective subjects that the study was being conducted to see if it is important to create *increased* restrictions on the use of this compound. Some members of the Board believed that the consent form should have explicitly stated that this study was unlikely to lead to increased restrictions and, in fact, its results, if they led to any regulatory changes, would more likely be used to allow greater exposures of people to chloropicrin.

HSRB Consensus and Rationale

The Board concluded that:

There was not clear and convincing evidence that the conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent).

There was not clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board based these two determinations on its conclusion that this study, based on the evidence presented, deviated from, but was not significantly deficient relative to, the ethical standards prevailing when the study was conducted.

**Insect Repellent Product Performance Testing Guideline**

**Charge to the Board**

The U.S. EPA Office of Pesticide Programs requested that the HSRB review and comment on the draft "Product Performance of Skin-Applied Repellents of Insects and Other Arthropods" Testing Guideline in order to determine what changes, if any, are necessary for the guideline to be made consistent with the requirements for protection of human research subjects set forth in 40 CFR part 26. Below is a list of questions that focus on these topics.

a. What actions should an investigator routinely take to minimize the risks to human subjects exposed during laboratory and field research on the efficacy of repellents?

**Board Response to the Charge**

The Board began its review by emphasizing that human studies are essential to assess the efficacy of repelling insects and other arthropods. The only way to determine if these repellents are effective is to test them on or near humans, since animals would not have the same level of attractiveness to the arthropods as humans will.

The following comprehensive list of conditions should be considered by the investigator in order to minimize risks to human subjects. The Board's response to the Agency's charge focused primarily on the ethical considerations and only secondarily on the toxicity data base *per se*.

(1) IRB approval is required before initiating any human exposures.

(2) Initial human tests should be conducted in a laboratory setting, using insects and other arthropods which are known to be disease-free.

(3) Healthy volunteers should be selected who are not sensitive to chemical reactions or drug/cosmetic allergies, and not allergic or overly sensitive to arthropod bites and stings. The subjects selected should not be known to experience any adverse drug reactions or allergies to other substances or toxins, and the selected subjects should not be taking any drugs which might elicit an adverse drug reaction (if the predictions from studies of metabolism of the repellent suggest that drug-chemical interactions might occur at the level of metabolism).

(4) If no known information about exposures in humans is available, testing should begin with the low dose levels and if no adverse reactions are displayed, rise gradually to the level of exposure anticipated to be used in humans. The formulations should be the same as that expected to be applied on human skin. If another formulation is anticipated, such as a coil, then it should also be tested in laboratory experiments.

(5) Although insect repellents would not be expected to yield adverse effects, test subjects should be under close observation by an observer trained to detect, as well as listen to the subject, for any adverse reactions. If such adverse reactions are observed, this would be grounds for terminating the exposure as soon and as completely as possible.

(6) The laboratory results would need to show a substantial likelihood of repellency before field tests should be initiated because of the possibility of subject exposure to disease-carrying arthropods.

(7) The field region for tests should have as low as possible incidence of known disease-agent infested insects or arthropods (prior trapping and microbial assays should document this minimal risk).

(8) The lowest possible number of untreated controls needed to ensure scientific validity should be used in field tests.

(9) The overall toxicity, in terms of both the toxic effects and the levels at which these toxic effects occur, should be determined from the existing animal data base. The search on existing animal data should include acute, chronic, reproductive, eye and skin irritation and dermal sensitization, so that the most reliable information on potential human adverse effects is known.

(10) Any human data from controlled or inadvertent exposures, or from routine uses in the past or in other countries should be accumulated. This information should be analyzed for evidence of direct toxic effects or any adverse side effects, including allergic or sensitization reactions.

(11) The test compound should be compared to data bases on similar chemical classes of compounds so that educated predictions can be made of types of toxicity that might be elicited in humans, the likelihood of allergic or sensitization reactions, the likely disposition and pharmacokinetics of the compound, including absorption, metabolism and clearance. In addition, the metabolism of the compound should be known from *in vitro* tests using human liver samples, in order to predict the toxicity or lack thereof of probable metabolites and to predict the enzymes involved in the compound's major routes of metabolism. This information on metabolism would be useful to predict any likely interactions with drugs that an individual might be taking.

HSRB Consensus and Rationale

The consensus of the Board was that studies involving humans are necessary to evaluate the efficacy of products to repel insects and other arthropods. Risk identification and minimization are also essential. In their protocols, investigators should adequately identify risk to participants and describe adequate steps they would take to minimize these risks.

**Charge to the Board**

b. What types of toxicity data should be routinely generated before an investigator conducts repellent efficacy testing on human subjects with a new product?

**Board Response to the Charge**

In response to the question, the Board proposed a set of data that would meet this requirement :

- The initial evaluation of a compound should include an analysis of chemical structure that emphasizes the detection of possible adverse effects. The analysis can be informed by comparisons with repellents of similar chemotypes for which toxicity data exist. A variety of computer applications and predictive models should be used to predict potential alerts for metabolic activation, target organ toxicity or mutagenesis. This type of evaluation can include the comparison to similar chemotypes of repellents for which toxicity data exist.
- Acute (single dose) toxicity studies should be conducted, with emphasis on the intended route(s) of exposure to the chemical.
- Dermal and ocular irritation should be evaluated. Characteristics of the potential for dermal sensitization and nasal-pharyngeal sensitization or triggering should be considered.
- Absorption of the compound after administration by the route of intended exposure, most likely dermal, should be determined. This can be done in laboratory animals, but may also be done using in vitro assessments of percutaneous absorption in human skin or human skin surrogates. If conducted in animals, the study should include an assessment of the routes of elimination of the compound.
- The mutagenic and clastogenic potential of the compound should be determined. At this stage, this analysis could be an abbreviated battery of *in vitro* genetic toxicology tests.
- Some data on toxicity in a repeat dosing paradigm should be generated. This is particularly important if the compound is available systemically.
- If the compound is available systemically, its metabolic fate should be investigated and it should be determined whether humans are likely to metabolize the compound in a manner that is qualitatively or quantitatively different from laboratory animals is recommended.

It should be noted that, although the Board has made these recommendations for toxicity data, it did not specify the precise methods by which the data set listed above should be generated. The use of animal models, validated in vitro methods or robust predictive tools could be used in combination to generate the recommended data set. Furthermore, if an investigator desires to carry out multiple exposures of a given compound in human subjects, then additional data, with emphasis on subacute and/or subchronic toxicity and the assessment of reproductive hazard, should be included in the toxicology evaluation.

In addition to the toxicity data summarized above, information on the mode of action, potency and projected human dose is useful adjunct information for assessing any potential risk associated with human exposure. Dose selection for efficacy studies in humans should be justified on the basis of animal toxicity studies and/or other relevant data (e.g., from in pharmaco-kinetic computer modeling, *in vitro* studies, and human case series). Present guidelines specify that the amount applied should be up to the typical maximum dose applied by

consumers, with recognition that some clarification or comparison with the toxicology benchmarks from animal studies will aid in dose selection (e.g., lowest NOAEL from sub-chronic studies) and protect human health.

HSRB Consensus and Rationale

The consensus of the Board was that the minimum set of toxicity data (as delinieated above) that should be routinely generated before an investigator conducts repellent efficacy testing on human subjects with a new product is that which will assure that subjects would not be at risk of permanent or irreversible harm.

**Charge to the Board**

c. In private and university research laboratories, investigators themselves have sometimes served as research subjects when assessing chemicals for insect repellent activity. What scientific and ethical issues would such a practice raise? Under what conditions, if any, would such a practice be acceptable?

**Board Response to the Charge**

The topic of self-experimentation has been discussed and debated for many years, and the debate is likely to continue. The scientific and ethical issues presented in the assessment of insect repellents are not different from the issues of self-experimentation in clinical research in general. There is not a clear overarching answer; each study may present a different situation.

**Arguments in favor of self-experimentation**

There is a long and noble history of investigators experimenting on themselves. Experiments on yellow fever, pernicious anemia, morphine and cocaine as local anesthetics, *H. pylori* as the causative agent for gastric ulcers, and many others have been instances of researchers using themselves as research subjects.

The Nuremberg Code, written in 1947 as part of the criminal trials of the Nazi doctors, states “5. No experiment should be conducted where there is an *a priori* reason to believe that death or disabling injury will occur; except, perhaps, in those experiments where the experimental physicians also serve as subjects” (USGPO, 1949). If a researcher is not willing to assume the risk of harm from research participation, how can that same researcher ask anyone else to assume that same risk?

Ethical research in compliance with 40 CFR 26 requires voluntary informed consent. Who better understands those risks than the researcher? Who best understands the societal or scientific benefits of the knowledge to be gained from the experiment? There is no chance of misunderstanding information in the consent process.

If the ethical and scientific arguments against self-experimentation can be addressed adequately, then self-experimentation seems quite reasonable.

**Arguments against self-experimentation**

Ethical Considerations

Many have objected to self-experimentation on scientific and ethical bases but their arguments can all be addressed with proper planning and conduct of the research protocol. For this reason, self-experimentation is not *per se* unethical or scientifically flawed *per se*.

One argument against self-experimentation is that researchers may take unreasonable risks with their own health due to a blinding belief in the importance of the research question, as well as and a personal incentives such as of career advancement. Addressing this concern properly requires independent review of the study in order to ensure that the risks are reasonable in relationship to the potential benefits of the research. Therefore, the study must be IRB approved prior to its conduct. As part of its review, the IRB must assure that "risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result" as stated at 40CFR26.1111(a)(2).

Potential coercion of co-investigators and research staff by the principal investigator is another area of concern. For this reason, self-experimentation should be limited to the principal investigator in most circumstances. Co-investigators and research staff (junior members of the research team) should not be enrolled in a study if the principal investigator has power or authority over them in the research setting or in any other setting (e.g., classroom or other work environment). Situations such as these can lead to coercion or undue influence on subordinates to participate in the research, and should be avoided, except when there is an IRB approved protocol that would and allow for truly voluntary participation.

Scientific Considerations

There are many scientific issues that must be addressed in order for self-experimentation to produce scientifically sound data that would be useful and generalizable at the end of the experiment.

One issue involves the type of outcome measure used in the research. If the outcome measure is a subjective one, then the expectation of the self-experimenter is likely to influence the results. This bias may lead to an incorrect study conclusion. To address this problem, self-experimentation should occur only in research protocols with objective outcome measures. The investigator-subject should not assess their own outcomes. The outcome assessor should be blinded to the subject's identity, if possible. In addition, the burden of proof is on the principal investigator to demonstrate how their participation does not introduce bias into the study results.

Oversight of the study is another issue of concern in self-experimentation. Since the principal investigator is responsible for study oversight, this oversight can be compromised during the time that the principal investigator is a research subject. Can the experiment be safely completed, for example, if the investigator became incapacitated while a research subject? This objection can be addressed by identifying the person responsible for study oversight while the

principal investigator is a subject and if the principal investigator becomes unable to resume study responsibilities.

Many of the stories of self-experimentation in the history of medicine have used a sample of one; the researcher was the *only* subject. These studies thus lacked proper controls and did not account for inter-individual variability. Such studies were poorly designed to answer a research study question with rigorous methodology. To address this, a well-written protocol is required, which must have a sample size that is adequate to answer the study question being asked.

Concern also has been raised about whether investigators are thorough in their evaluation of whether they meet all of the study's inclusion and exclusion criteria. If researchers are convinced that they should be and really wants to be subjects, they might not perform all screening tests that are required by the protocol. This issue can be easily addressed by having another investigator perform and assess the screening results. The principal investigator's eligibility to participate in the study should be assessed independently by someone *outside* the research team, to avoid potential coercive influence of the principal investigator on the sub-investigator.

HSRB Consensus and Rationale

It may not be *a priori* unethical or scientifically problematic for a principal investigator to be a subject in his/her own study IF:

1. The study was approved by an by an IRB in the same manner as was required for most human subjects research;

2. The following scientific issues were addressed:

a. Principal investigator met all enrollment criteria;
b. The study was a well controlled trial with a justified sample size adequate to answer the study question with statistical surety (Occasionally a study with a small sample size may be scientifically and ethically appropriate if it is a pilot or feasibility study. However, justification for the sample size chosen is still necessary, although such justification may not be a statistical one. In such a situation, (c) does not apply);
c. The principal investigator is one of many subjects, accounting for normal human variability, and allowing results to be generalized to a broad population; and
d. The outcome measure is objective and measured by another (blinded, when possible) investigator;

3. A plan is in place to ensure the integrity and safety of the study while the principal investigator was a subject.

4. A plan is in place to ensure for study oversight if the principal investigator becomes incapacitated;

5. Participation of other research staff and employees is prohibited except in those cases where issues of coercion/undue influence can be addressed, which may or may not be possible; and

6. The investigator justifies why he/she should be a research subject in the study.

**Charge to the Board**

d. Please comment on the scientific and ethical issues arising from the use of (or decision not to use) negative controls groups in repellent efficacy studies, in both laboratory and field studies.

**Board Response to the Charge**

Scientific Considerations

Negative controls (i.e., untreated/unprotected) are used in repellant studies to show "biting pressure". This can be categorized as sufficient, insufficient, or it can be quantitatively measured (bites/minute over time). Negative controls are also used in field studies to confirm effectiveness that is shown in laboratory studies,

The use of a control group has been an essential characteristic of repellent efficacy studies conducted in the laboratory, because a comparison of the data from the treatment and control groups shows a measure of efficacy. The use of negative control groups in laboratory studies appears to be a safe practice, since the insects involved are known to be disease-free. In contrast, the uncontrolled nature of field studies means that the same assurances cannot be provided to participants. Because negative controls are not exposed to the pesticidal active ingredient, there is no risk of toxicity from the chemical. The risk of harm and discomfort for subjects is primarily of two types. In laboratory and field studies, there is the discomfort of the bite itself, which might include minor pain, itching and swelling. The discomfort experienced by humans is variable, some having negligible reaction, others having a definite allergic response. In addition to this risk, field trials have the added risk of subjects acquiring a vector-borne infection. Fortunately, field procedures, such as capture of insects just prior to biting can reduce such risk substantially. In addition, because negative controls are not exposed to the pesticidal active ingredient, there is no risk of toxicity from the chemical.

Nonetheless, the Board failed to reach consensus regarding negative control groups in field studies of repellent effectiveness. The basic scientific justification for such controls is to confirm that "biting pressure" exists. If that is the only purpose, a single negative control may suffice. As some Board members suggested, biting pressure might even be established through trapping or other methods that did not involve an unprotected human subject. Other Board members recognized, however, that it may be important to establish a particular level of biting pressure in order to compare the extent and duration of repellency from trial to trial and compound to compound. In fact, that condition appears to be essential for accurate product labeling. In any case, since even one unprotected human subject could be at risk of vector-borne disease, the use of negative control groups should not be a default component in the design of repellency studies. Instead it should be justified in each protocol in which it is proposed.

Ethical Considerations

By minimizing risk in the laboratory studies (e.g. screening for past sensitive reactions, captive breeding of infection-free insects, and mechanical aspiration at bite), the use of negative controls in the laboratory should not be considered ethically problematic as long as it is scientifically justified. Steps can, and should be taken to minimize risk in field studies. However, the risk of a significant life altering infection can never be reduced to zero. Thus, the potential benefit from such studies must justify this risk. The science must be sound and alternative approaches - such as live trapping or laboratory studies - must be shown to be inadequate. The consent process must be truly informed and subjects must be volunteers with the full right of withdrawal. These issues must be specifically and completely addressed in the study protocol.

HSRB Consensus and Rationale

The HSRB suggested that the Agency modify the guideline to say that negative controls "may be" needed (instead of "are") and that examples be given both for when negative controls are needed and when they are not. The language on positive controls may also benefit from further expansion and clarification.

**Charge to the Board**

e. Please comment on the scientific and ethical issues raised by the design of studies to collect data sufficient to support assessment of repellent efficacy using the two different efficacy metrics: time to first confirmed bite (TFCB), and time providing x% protection of treated subjects from bites relative to untreated controls (RP).

**Board Response to the Charge**

The distinction between efficacy and effectiveness is useful in answering the questions about the Insect Repellent Product Performance Testing Guideline. Although the *efficacy* of a repellent can be established using laboratory techniques, the *effectiveness* of a repellent can only be established in the field under actual use conditions.

Scientific Considerations

A particular study design can either minimize risk to all subjects enrolled in a study (for example by using only laboratory mosquitoes or ticks to eliminate the possibility of vector-borne diseases, excluding those who might adversely react to the insect bites), and/or minimize overall risks by reducing the number of "at risk" subjects to the lowest number possible while maintaining scientific integrity, pretesting insects to confirm probable absence of vector borne diseases, and/or utilizing techniques to remove insects prior to bite when feasible. Risk minimization strategies will depend upon knowledge of variability in subject attractiveness, the effectiveness of the repellent, the interaction of biting pressure to insect hunger and subject attractiveness, characteristics of the test environment, and the scientific reliability of generalizing insect performance from the lab to the field.

A question was raised about the provision of prophylactic antibiotics or use of a preventative vaccine in order to minimize risks. The difficulty with this approach is that the effectiveness of these interventions would need to be established, and the dangers associated with such treatments would become part of the overall assessment of whether the risks of the research are offset by the importance of the knowledge to be achieved. The measurement of pre-exposure and post exposure antibodies, unless done in a context to only include subjects who are immune to the vector borne disease of concern, does not minimize risk other than documenting the presence of the subject's immune response. Overall, the best approach is to choose a study design that either eliminates or minimizes the risk of vector-borne diseases.

Ethical Considerations

The time to first confirmed bite, or the time to first confirmed "intent to bite" (if ascertainable), has the ethical advantage of minimizing the risk of exposure to vector-borne diseases. However, based on the background materials for the meeting, the use of relative protection can be an appropriate outcome measure based on statistical advantage. Relative protection could thus would be an appropriate outcome measure for a laboratory based efficacy study. As long as there was a sufficient "biting pressure", one could then do a measure of the duration of relative protection for protected subjects in a field study and compare it to laboratory based measurements. This could also be done using time to first confirmed bite.

Participation in insect repellent research offers no direct benefit to subjects when their exposure to insects or arthropods is for the purpose of the study given the presence of existing insect repellents on the market. As such, the sponsors of repellent efficacy research are obligated to provide insurance to cover possible future medical costs that result from injury or illness experienced by the subjects as a consequence of their participation in the research. It is less clear whether sponsors would have an obligation to provide for lost income in such instances. As noted previously protocols must justify the level of risk by the probability and social value of the benefits, adequately identify all risks, and present a description of adequate steps to minimize the risks.

The informed consent materials also must provide information about the prevalence and risks of any vector borne diseases (if applicable), the consequences of acquiring such a disease as a result of the research, and the availability of effective insect repellents outside of the research.

HSRB Consensus and Rationale

The Board consensus was that the time to first confirmed bite, or the time to first confirmed "intent to bite" (if ascertainable), has the advantage of minimizing risk of vector-borne diseases. However, for some studies there is a statistical advantage for the use of relative protection as an appropriate outcome measure. Since relative protection procedures in field studies increases the risk of vector-borne diseases, protocols must: (a) justify the level of risk by the probability and social value of the benefits; (b) adequately identify all risks; (c) present a description of adequate steps to minimize the risks; and (d) provide consent materials that

include information about the prevalence and risks of any vector-borne diseases, consequences of contracting disease, and alternative effective repellents outside of the research.

**Charge to the Board**

f. Please comment on appropriate approaches for estimating the minimum number of subjects needed to evaluate the level of efficacy of a repellent in laboratory and field studies.

**Board Response to the Charge**

Introduction

As written, the current draft of the Guidelines suggests that six should be the minimum number of research subjects in laboratory or field experiments where the efficacy of an insect repellent is investigated. It is not clear from the Guidelines whether the number refers to the entire experiment or to the number of subjects allocated to each treatment under consideration. The Board argues below that establishing a single sample size for all types of experimental designs and objectives is not the most appropriate approach.

Critique

Correctly estimating the sample size that is needed in an experiment (conducted either in a laboratory or in the field) is important to ensure reliable inferences about the treatment under study. Sample size calculations can be carried out using several approaches, but the two most common ones (at least in terms of usage) are based on:

- Power calculations: sample size is chosen to guarantee that tests of hypotheses reach a pre-determined power. Power is defined as one minus the probability of incorrectly failing to reject the null hypothesis of no treatment effect. In other words, power is the probability of finding a difference if such a difference is "true". That is, in under-powered experiments, investigators have a high chance of not detecting a "true" treatment effect.

- Confidence interval calculations: sample size is chosen so that the $100(1-\alpha)\%$ (for $\alpha$ typically chosen to be 0.05) confidence interval around a treatment effect estimate is sufficiently small. The narrower the confidence interval, the more reliable the point estimate of the treatment effect size.

While smaller than needed sample sizes result in under-powered studies and wide confidence intervals for true effect sizes, excessively large samples are not desirable either. First, resources are wasted when samples are larger than they need to be. While it is always possible to increase the power of a study by increasing the sample size, at some point the cost of obtaining an additional observation outweighs the potential gains in power. Second, very large sample sizes may result in statistically significant results that have no practical implication. Finally, including more subjects in an experiment than is required for statistical reasons may unnecessarily place subjects at risk.

Both the power of a test and the width of the confidence interval around a point estimate depend on various design and data attributes, including:

- Sample size: power increases as sample size increases; the width of confidence intervals decreases as sample size increases.

- Variance across experimental subjects: the smaller the variability in the response across experimental subjects, the larger the power and narrower the confidence interval for a given sample size.

- The size of the effect that needs to be detected: in experiments in which detecting a very small difference between two treatments or between a treatment and a control, the minimum required sample size for achieving a certain power or for keeping confidence intervals to a desired width will be larger than when the difference to be detected is larger. In other words, the smaller the difference sought between two groups, the larger the required sample size, all other factors being held constant.

- Whether the design calls for replicate measurements obtained from the same individuals in the study (which induces correlation across measurements) or for "true" replication involving different individuals observed under the same conditions: correlation across measurements (repeat measures in the same individual) in general decreases the power of an experiment.

The appropriate approach for estimating the minimum required sample size in insect repellency studies in the laboratory or in the field will depend greatly on the design of the study. Factors to be considered when estimating sample size include the following:

- Whether the experiment was conducted in the laboratory or in the field: a larger sample size will typically be required for experiments conducted in the field because uncontrollable factors that may affect the response increase the variance across test subjects.

- The number of treatments (e.g., potency formulations or modes of application of an insect repellent) included in the study.

- The presence of control subjects, and whether the same volunteers will serve as both controls and experimental test subjects (as in experiments in which one arm of each subject is treated with a repellent while the other one is not). Experiments in which the same subjects act simultaneously as controls and as tests require smaller sample sizes (all other factors being equal) than those studies in which different individuals act as controls and tests.

- Whether the design calls for repeated measurements on experimental subjects.

- The outcome variable of interest: this relates to the between-subject variability mentioned earlier. The variance across subjects might be larger for some outcome variables than others. For example, the between-subject variance might be expected to be larger when the outcome variable is repellency of a product over a long period than when the product's repellency over a shorter period is of interest. Thus, the minimum sample size for adequate study

reliability (either in the power or the width of confidence interval senses) would be larger in long-term studies than in short-term ones.

- The presence and potential effect of confounders that cannot be easily controlled via the experimental design. For repellency studies, for example the intensity of a person's odor from carbon dioxide emissions contribute to the attractiveness of the person to blood-seeking mosquitoes. The sample must be large enough to ensure that the variability in the general population of consumers of the product is represented in the study.

- The heterogeneity of the target population from which the sample is drawn: if the product is meant to protect all individuals (e.g., all ages and both genders) then the minimum sample size might need to be computed *within* population strata, to ensure that each population sub-group is adequately represented in the sample and that inferences about the effectiveness of the product can be reliably drawn for the entire population.

- The heterogeneity of environments in which the product is expected to be used: if the product is to be used in a variety of environments (e.g., open fields, forests, marshes, and the typical backyard) where a different concentration of insects and ticks can be expected, the environment must be included as a factor in the experimental design. In laboratory conditions, field insect and tick concentrations can be mimicked by varying the density of insects and ticks in experimental cages. The larger the number of environments in the study design, the larger the minimum sample size needed to achieve the desired level of inferential accuracy.

Actual calculation of minimum required sample size typically requires estimating the sample variance of the point estimate of interest. Point estimates, in turn, follow different sampling distributions depending on the quantity that is being estimated. In repellency studies, various outcomes are of interest and these differ in the distributional assumptions that can be justified:

- When the outcome or response variable is the time to first confirmed bite (TFCB), an appropriate distribution for the response might be the exponential distribution (or the more general gamma family). A point estimate of the mean response is given by the sample mean of the response variable, but construction of a confidence interval for the true mean response must be based on the correct sampling variance calculation.

- When the outcome variable is relative protection, the product passes the efficacy test if treated subjects receive 95% fewer bites than control subjects. Because the number of bites can be best modeled as a Poisson random variable, a point estimate of the number of bites under different treatments and a standard deviation around that point estimate must be estimated under that Poisson model. A normal approximation to the Poisson would be reasonable only when the number of bites anticipated for each subject is large, a situation not likely to be encountered in practice.

HSRB Consensus and Rationale

It is critical that the proposed number of subjects be justified on the basis of good research design. Because experiments to test effectiveness of products to repel insect and tick bites are likely to vary in terms of design, response variable, target population of interest, detectable effect size and other important variables, requiring a specific minimum sample size that guarantees sufficient accuracy in all cases might be impractical. Instead, the guideline might require that registrants present their own sample size calculations and that the methodology used in the calculations be justified relative to the factors noted in the bullet list above.

**Charge to the Board**

g. Please comment on whether or not investigators should have an ethical obligation to provide subjects of repellent efficacy research with insurance to cover possible future medical costs or other losses that result from injury or illness experienced by the subjects as a consequence of their participation in the research.

**Board Response to the Charge**

The broad issue of compensating research subjects for research-related injuries, together with the somewhat narrower one of paying for the costs of medical care for such injuries, has received substantial analysis. The report by the National Academy of Sciences on intentional dosing studies (NAS 2004), the principles of which Congress specifically required to be reflected in the EPA regulations on such studies, directly addressed this issue.

As the NAS Report notes:

Debate continues in the United States about whether compensation should be provided for research-related injuries. The Common Rule requires only that when research involves more than minimal risk, information should be disclosed regarding whether medical treatment and other compensation will be provided for research-related injuries. Many critics of the U.S. policy believe there should be more than disclosure of information about compensation and call for the provision of medical care for research-related injuries without cost to the participants and, in addition, for compensation for lost wages, disabilities, and death. These claims are based on the belief that research participants, whatever their motivations, accept risk on behalf of society. When participants are injured, justice, fairness, and gratitude mandate, at a minimum, the provision of needed medical treatment without cost to the participant. Further study is needed regarding the provision of other types of compensation. (NAS 2004.)

Based on this analysis, the NAS Panel examined the ethical issues associated with intentional human exposure studies, adopting the following as one of its Recommendations:

Recommendation 5-5: Compensation for Research-Related Injuries

At a minimum, sponsors of or institutions conducting intentional human dosing studies should ensure that participants receive needed medical care for injuries incurred in the study,

without cost to the participants. In addition, EPA should study whether broader compensation for research-related injuries should be required. (NAS 2004)

The Board agreed with the reasoning and recommendations listed in the NAS Report with regard to a research subject not being required to bear the costs of medical care needed to treat injuries incurred as a result of participating in a research study.

Indeed, the conclusions of the NAS Report reflect a growing consensus that the provision of such free medical care should be adopted as a requirement for many categories of research studies. For example, in Volume 1 of its 1982 Report, Compensating for Research Injuries, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, concluded that "compensation of injured subjects is appropriate to the research enterprise. A program to assure compensation is thus a desirable policy goal for a just and compassionate government, both as the sponsor of most biomedical and behavioral research and as the means through which society acts on matters of common interest, such as the search for new biomedical discoveries" (at page 64). That Report did not specifically call for the adoption for such a program, since it concluded that it did not have enough information about whether subjects were indeed already receiving such compensation, and about the costs and other practicalities relating to adopting a program.

More recently, in its 2001 report on Ethical and Policy Issues in Research Involving Human Participants, the National Bioethics Advisory Commission (NBAC 2001) reviewed the literature on this issue, concluding that a "comprehensive system of oversight of human research should include a mechanism to compensate participants for medical and rehabilitative costs resulting from research-related industries. The inclusion of this mechanism has long been justified on ethical grounds" (at page 123). It echoed the President's Commission's call for a study of the need for a compensation program.

Similarly, in 2003, the Institute of Medicine, in Responsible Research: A Systems Approach to Protecting Research Participants, commenting that "[b]ecause the contributions of science benefit society as a whole, it seems indisputable that society is obligated to assure that the few who are harmed in government-sponsored scientific research are appropriately compensated for study-related injuries. . . . . The same argument applies to privately funded research, perhaps even to a greater extent, as the economic survival of a company depends largely on the availability of participants to test new therapies, drugs, and other products. Because the participants are ultimately contributing to the profits of the company, any costs that result from the research should be the responsibility of the sponsor" (at pages 188, 190). The Institute of Medicine report also reviewed international standards relating to this issue, pointing out that Guideline 13 of the Council for International Organizations of Medical Sciences (CIOMS) requires that subjects be equitably compensated for "any temporary or permanent impairment or disability." The report concluded that although laws vary, "most [nations] make some provision for compensation" (at page 189).

These arguments have special import in the context of the intentional dosing studies that this Board will be reviewing, including repellent efficacy research. These studies will almost never produce any direct benefits for study participants. On the other hand, there is frequently

the possibility that subjects will suffer significant injuries as a result of their participation. In the repellent efficacy studies, for example, subjects may be at risk or contracting a serious vector-born illness as a result from insect bites received during the course of the study. Given the lack of direct benefits to subjects, and the possibilities of very significant harm, the justification for requiring sponsors to cover the costs of medical care for research-related injuries is heightened.

Three important points also need to be mentioned regarding issues raised by the wording of the charge to the Board. First, the Agency asked for comments regarding whether "investigators" should be required to pay for the costs of such medical care. In most cases, it would be most appropriate for that obligation to be imposed upon the sponsors of research, who are usually the most immediate beneficiaries of the research, rather than the investigator. The investigators should only have this obligation when there is no external study sponsor (i.e., when they are effectively acting as the sponsor of their own study).

Second, the Agency's charge spoke of requiring that subjects be provided with "insurance" to cover the relevant medical costs. The Board believed that sponsors should be provided with some degree of flexibility in demonstrating how they will cover the medical costs of subjects. A sponsor that has sufficient assets, for example, might be able to contractually commit itself to pay for these costs. Given the possible substantial administrative costs of having a sponsor purchase a special type of insurance for subjects, it does not appear appropriate to rule out other ways for assuring that a subject's medical costs are covered.

Third, the Agency's charge raised the possibility of requiring payment for "other losses" beyond the costs of medical care. Payment for such "other losses" (for example, the cost of lost wages when a subject is not able to work for a period of time) is a more complicated and controversial issue than covering medical expenses. With regard to this issue, the Board agreed with the conclusions of the NAS Report that further study should be required in order to better evaluate whether requirements to cover such "other losses" should be imposed.

HSRB Consensus and Rationale

For the reasons discussed above (including justice, fairness and gratitude), the Board concluded that it is appropriate that sponsors of repellent efficacy research studies should be required to ensure that if a subject is injured as a result of their participation, then the subject will not have to assume the costs of medical care needed to treat such injuries.

**Charge to the Board**

h. Please comment on any special considerations that should be addressed in the informed consent materials provided people who are candidates to become subjects in insect repellent efficacy research.

**Board Response to the Charge**

The general requirements for informed consent are outlined in 40 CFR 26.116 of the Agency's final human studies rule. A basic element in seeking informed consent is that the

subject should be told that the study involved research and given an explanation of the purposes of the research, the expected duration of the research, a description of the procedures to be followed, and an identification of any procedures that are experimental.

The informed consent should begin with a clear statement that this study is research, and a statement as to whether the product being tested is approved and marketed in the test formulation or still in the experimental stage. Information about the potential efficacy of the product against the test insect should also be provided.

For the insect repellent studies, it is especially important to be very clear about the experimental set-up, either the laboratory or the field, and what the expectations are for the subject. Because there appears to be a tendency in these research studies to use "seasoned" subjects (i.e., those who are in the scientific field or have participated in these studies before), a detailed explanation of the procedures might seem to be unnecessary to the investigator.

Nonetheless, the written details of the experimental procedure must be sufficient to inform a potential subject who has never anticipated in this type of study and to remind one who has done so. A video, PowerPoint presentation, or photographs might help the subject to visualize what will occur to him/her during the study. If it is a laboratory study, it may help to have the subject place their arm into the cage. If the subject is expected to use an aspirator, training on its use should occur prior to the beginning of the study. A demonstration of what a landing and probing feels like might be appropriate because the dermal sensitivity of individuals will vary.

The length of time that the study would take should be clear in the informed consent document, including whether the test would be repeated. The process for randomizing subjects to the test or experimental group should be included. In field studies that take the entire day, it might be explained whether food would be provided to the subjects.

A clear discussion of the stopping rules should be included, especially for the field studies. For example, to discontinue participation, does the subject raise their hand, return to the van, or find a study monitor to express a desire to stop.

Another basic element of informed consent is a description of any reasonably foreseeable risks or discomforts to the subjects. Inclusion of a Material Data Safety Sheet is not sufficient to adequately inform the subject as far as all the inherent risks and benefits of study participation. For insect repellent studies, three types of risks are reasonably foreseen.

The first is the risk of being bitten. The informed consent document should give an estimate of the potential number of bites that a subject could receive in the control and experimental groups. A statement that the researcher strives for each subject to receive few to no bites is not sufficient. Additionally, should a subject have an allergic reaction to the insect bites, medical procedures and remedies that would be present should be clearly described in addition to any available follow-up treatment (e.g. will subjects be given an antibiotic or steroid cream for their bites?

The second risk is that of a sensitivity or allergic reaction to the experimental product. A synopsis of the animal studies and any human data should be given to the subject along with a discussion of the theoretical risk of a reaction occurring. Emergency care procedures should be presented for a subject who has a reaction to either the bite itself or the repellent.

The third and most serious risk is the potential for acquisition of vector-borne illness as a result of insect bites. The severity of these illnesses should be clearly explained, even if the researcher believes the risk is minimal to non-existent due to either through the use of disease-free insects in a laboratory study or the selection a disease-free zone for the field study. The risk of insect-borne diseases might not occur to a subject who normally does not work in the field. The investigator should make sure that the subject clearly understands both the risks of disease transmission and what symptoms to look for with any potential insect-borne diseases. One suggestion might be to test the subject, either verbally or in writing, about their understanding of the procedures and the risks.

Another basic element of the informed consent process is a clear and complete description of any benefits to the subjects or others that are reasonably expected to result from the research. It should be very clear in the informed consent document that there are likely to be no direct benefits to the subject as a result of study participation. The only potential benefit is to society at large to have an arsenal of insect repellents available. Additionally payment for participation in a research study can not be considered a benefit of the study.

Each research participant should be told the extent, if any, to which confidentiality of the records identifying the subject should be maintained. The researcher should be careful to only include those organizations that have jurisdiction over the study and might therefore have the right to inspect the records. It is equally important that access to the records is limited to as few individuals as possible and that strict confidentiality procedures be developed and are strictly adhered to.

Since insect repellent studies should be classified as research involving more than minimal risk, the subject should be clearly told if the researcher will cover medical treatment if an injury occurs (this issue was reviewed by the Board in more detail in response to question g.) , including not only treatment during the research study but long-term care, if needed (e.g. in those circumstances in which a study subject contracts an insect-borne disease). A phone number should be provided to volunteers so that they can obtain additional information about the risks and benefits of study participation, and their rights as study subjects.

The subject should clearly be told that the study is voluntary and refusal will not result in any loss of benefits or privileges. For studies that use only one control group, it is especially important for a subject assigned to the control group to understand that they can withdraw even if this withdrawal might invalidate the study. It should also be clear that the subject does not need to give a reason for withdrawal from the study. The consequences of a subject's decision to withdraw from the study should be addressed, including how it will affect any payment for participation in the study.

Students or employees used as research subjects in this study are considered "vulnerable subjects" because they might feel coerced into participating either by their supervisor, thesis advisor, or even fellow students/employees. It should be clear that participation in these types of studies is neither a condition of employment nor an academic requirement for students. An explanation of whom to contact if the subject feels coerced should be provided. This contact should not be associated with the investigator and the subject should be guaranteed anonymity. Employees who report directly to the investigators or study sponsors, and students of the investigator should be excluded from the study.

The subjects should also be told that they will be informed if any new information is found during the course of the study that might affect the subject's willingness to participate. Additionally the subject should be told that they will be informed if it is found that either a test site, or a laboratory strain of insects used is discovered to have a higher level of disease than previously thought.

The informed consent document should be written in a language understandable to the subjects and the subjects should be informed about any potential conflicts of interests that the researchers have.

HSRB Consensus and Rationale

The consensus of the HSRB was that informed consent should comply with all of the requirements of 40 CFR 26.1116 of the Agency's final human studies rule. To comply with the human studies rule, consent information for pesticides studies must include: (a) detailed information on the procedure (e.g., number of insect bites or landings anticipated, nature apparatus or field context, length of time of exposure); (b) a clear statement of the risks involved (e.g., discomfort from bites, risk of vector-borne disease, medical consequences of the disease, treatments available for the disease); (c) the voluntary nature of participation (e.g., statements that eliminate the perception of coercion for students or employees; specific instructions on how to signal desire to withdraw from the study); (d) the fact that there was no immediate direct benefit to the subject in participating as well as a description of alternative available repellents; and (e) other steps outlined above. In addition, informed consent information should be as detailed for experienced subjects as for naïve subjects.

**Charge to the Board**

i. Does the HSRB recommend that the draft guideline be revised? If so, please explain what aspects or sections might improve with revision.

**Board Response to the Charge**

The Board recommended that the Agency refer to Board responses to previous questions to address revisions to the draft guideline.

**Review of HSRB Protocol Criteria**

Before the Board reviewed the presented proposed human studies research, the Board developed science and ethics criteria as a guide for its evaluation of such studies. Reference to such criteria would be helpful for the Agency, study investigators, and other members of the public to understand the Board's approach for the review of proposed human studies. The relative emphasis placed by the Board on each criterion may be applied case-by-case and may vary with the nature of the chemical product, study design, and participants. Specific studies may also call for additional criteria. A list of the science and ethics criteria identified by the Board are provided below:

Science Criteria

The criteria for the evaluation of the scientific quality of studies involving human subjects was based on a series of questions which the Board agreed needed to be addressed by the details provided in the study protocol.

1) Is a valid scientific question addressed by the study?
2) Are existing data adequate to answer the scientific question?
3) Are new studies involving human subjects necessary to answer the question?
4) What are the potential benefits of the study?
5) What is the likelihood that the benefits would be realized?
6) What are the risks? Are they serious or irreversible?
7) Is the purpose of the study clearly defined?
8) Are there specific objectives/ hypotheses?
9) Can the study as described achieve these objectives or test these hypotheses?
10) What is the sample size and how is it derived?
11) What is the basis for the proposed dose levels and formulations in the study?
12) Is there a plan allocating individuals to treatment?
13) Can the findings from this study be generalized beyond the study sample?
14) Is there a justification for the selection of the target population?
15) Are participants representative of the population of concern? If not, why not?
16) Are the inclusion/exclusion criteria appropriate?
17) Is the sample a vulnerable group?
18) Will the measurements be accurate and reliable?
19) Are measurements appropriate to the question being asked?
20) Are adequate quality assurance procedures described?
21) Can the data be statistically analyzed?
22) Is the statistical method appropriate to answer the question?
23) Are point estimates accompanied by measures of uncertainty?
24) Do laboratory conditions simulate real-world conditions?
25) Are field conditions representative of intended use?
26) Does the protocol include a stop rule plan, medical management plan, and a safety monitor?

Ethics Criteria

The criteria for the ethical acceptability of environmental research protocols involving human dosing and intentional exposure proposed by the Board are grounded in the general criteria for IRB approval found in Subpart K of the Agency's human studies rule (40 CFR 26.1111 and 1116). This approach is similar to that taken by the National Academy of Sciences (2004) in formulating the criteria for scientific and ethical acceptability (recommendation 5-1) and participant selection (recommendation 5-2).

Scientific Validity and Social Value

One of the most important criteria for the ethical review of protocols in fact is scientific. The research design must be sound (i.e., scientifically valid) and the risks of the research must be reasonable (or balanced) in relation to the importance of the knowledge that may reasonably be expected to result. Absent a sound research design, the prospect of the research generating usable knowledge is severely diminished. Although the risks to research participants may be balanced against anticipated benefits to these same subjects, much environmental research (such as intentional exposure studies) will not offer any direct benefit to the research subjects themselves (See NAS recommendation 3-1.).

The justifiable risks to which research subjects justifiably are exposed should be directly proportional (i.e. reasonable or balanced) to the importance of the knowledge expected to be gained. In other words, the information to be gained from the research study must be "worth knowing". The NAS took this approach in recognizing that scientific accuracy alone is insufficient justification for exposing research subjects to anything more than "no identifiable risk." For example, there must be a "reasonable certainty of no harm" to research subjects if the only benefit of the research is to improve the scientific accuracy of extrapolating animal to human data (NAS recommendation 4-1). As such, a research protocol needs to describe the benefits of the knowledge that may be obtained so that the reasonableness of the risks can be judged against the importance of that knowledge. In addition to improved scientific accuracy of risk assessment, such benefits may include a more stringent regulatory standard, new public health measures that could be adopted, or new products that may protect public health.

Federal regulations state that an IRB should not consider the possible effects of the research on public policy when evaluating those research risks that fall within the responsibility of the IRB (40 CFR 26.1111 (a) (2)). Nevertheless, the public policy implications of the knowledge that may result from the research does affect the importance of that information. Further, the Human Studies Review Board is not limited by Subpart K in evaluating the ethical acceptability of a proposed research study. As such, scientific accuracy alone may be an insufficient justification for the importance of a research project. The protocol should address the potential benefit of improved scientific accuracy, and to whom this benefit would accrue. As recognized by the NAS in recommendation 4-2, studies that may have a potential public health or environmental benefit could involve a somewhat higher level of risk while not causing any lasting harm to research subjects.

Minimizing Research Risk

The research should not expose any human subjects to unnecessary risk (40 CFR 26.1111 (a) (1)). This ethical principle has a number of important corollaries. First, the use of human subjects must be absolutely necessary in order to answer an important scientific question that could not otherwise be answered by using animal models. In addition, any intentional dosing studies can only be justified if observational studies would neither answer the question nor be feasible. Admittedly, the judgment of feasibility may be ethically difficult especially if the only consideration is time and expense. Second, the elimination of unnecessary risk means that there is no way to answer the scientific question that involves less risk if human subjects are to be used. Third, the scientific protocol should involve no additional exposure of study participants to risk unless absolutely necessary. The ethical responsibility for "using procedures already being performed on the subjects" translates, in the environmental context, to studying those situations in which human subjects are exposed to environmental toxins as part of their usual activities without increasing their exposure to those same toxins. In addition to the ethical priority of animal over human studies, there is an ethical priority for observational research over intentional dosing research involving environmental toxins if scientifically appropriate. Whether a study meets the scientific and ethical criteria necessary to justify the exposure of human subjects to potential risk can only be evaluated in the context of a given research protocol if the investigator and/or sponsor specifically addresses alternative means of obtaining the desired data.

Equitable Selection of Subjects

The selection of subjects should be equitable (40 CFR 26.1111 (a) (3)). In practical terms, this means that the selection of subjects should reflect the scientific purposes of the research and not the availability of a particular population. This ethical criterion may be especially problematic in the context of environmental hazards research. Often the exposure to environmental hazards in the workplace or at home is greater for those who are either socioeconomically or educationally disadvantaged. As such, subject selection based purely on scientific design may be insufficient protection for the research subjects, with additional safeguards. The need for such safeguards must be assessed within the specific context of a particular protocols based on an in-depth knowledge of the community within which the research will take place. The ability to "minimize the possibility of coercion or undue influence" (40 CFR 26.1116) may require the involvement of representatives from the community from whom the research subjects will be drawn and within which the research will take place. A research protocol also must include specific measures for assuring the equitable selection of subjects, including recruitment practices, incentives (financial or otherwise), impact on employment, and the possibility of retaliation. In addition, any incentive for participation (whether financial or otherwise, such as time off from work) should not be included in the analysis of risks and potential benefits of the research.

Informed Consent

The information that is included in the informed consent process and documentation should include all of the information found under the general requirements for informed consent in 40 CFR 26.1116. There are a few specific features of the informed consent information that are worth highlighting in the context of environmental research. First, EPA regulations do not allow for a waiver of either informed consent or the written documentation of informed consent.

Second, the informed consent information must include the identity of the pesticide and its mode of action if the research involves intentional exposure of subjects to a pesticide (40 CFR 26.1116 (e)). Given the vulnerability of the research subjects that are likely to be enrolled in environmental research (as discussed above), the default position for any research on environmental toxins (whether observational or intentional) should be that the risks of any potential pesticide exposure be included in the informed consent information. However, if the risks of the toxins are not part of the research, but instead are part of daily work life, this should be made clear. Third, the alternatives to research participation (40 CFR 26.1116(a)(4)) should include all steps that might minimize the risk of exposure to environmental hazards, up to and including removing oneself from that environment. Fourth, as noted previously, the HSRB supports the view that research subjects should receive needed medical care for research related injuries at no cost to themselves (consistent with NAS recommendation 5-5). As such, the oft-used informed consent template statement that "no program of compensation is available" would be unacceptable in human dosing or pesticide exposure research. The HSRB acknowledges that the determination that any given injury may be research-related might be difficult when the protocol combines observational or interventional procedures with non-research related exposure to environmental toxins. Nevertheless, the principle of providing medical care for research related injuries at no cost to research subjects must be affirmed. Fifth, the voluntary nature of participation must be carefully and explicitly described during the consent process. Investigators, study sponsors and pesticide registrants are obligated to ensure that neither employment status nor economic need creates a coercive context for study participation. Finally, the process and documentation of informed consent needs take into account special circumstances that may arise in the context of any given research setting, including language barriers, literacy, comprehension, employment status, and the confidentiality of screening tests such as for pregnancy.

Subject Safety

The research protocol must also discuss provisions for assuring the safety of subjects enrolled in the research, both during and after the research has been completed. This obligation goes beyond simply "monitoring the data collected" to include procedures for collecting real-time exposure data to the environmental toxins during the research, and procedures for intervening should the health of research subjects be at risk from the environmental toxins (regardless of whether the exposure is intentional or not). The protocol should describe in detail any procedures for reversing experimentally-induced harms.

IRB Approval

The HSRB believed that the ethical analysis of a research protocol requires information concerning the potential risks to human subjects, measures proposed to minimize risks, the nature and magnitude of all the expected benefits, and to whom they may accrue, alternative means of obtaining information comparable to what would be collected for the proposed research, and the balance of risks and benefits of the proposed research (40 CFR 26.1125(a)). Further, the HSRB believes that an IRB is unable to make the determinations required under 40 CFR 26.1111 absent this information. As such, the HSRB expects this information to be found in the protocol submitted to the responsible IRB. Although an IRB may be able to gather this information from other sources, the lack of this information in the protocol and the lack of a

substantive discussion of these issues in the IRB minutes would raise doubt about the adequacy of the IRB review.

**Insect Repellent Product Performance Efficacy Studies**

**Study EMD-003 from Carroll-Loye Biological Research**

**Charge to the Board**

a. Does the proposed research described in study EMD-003 appear likely to generate scientifically reliable data, useful for assessing the efficacy of the repellent?

**Board Response to the Charge**

The protocol submitted for review by the HSRB outlined studies to evaluate the efficacy of IR3535 as a tick repellent in human subjects. The protocol described a laboratory study in which the movement of the Western black-legged tick (*Ixodes pacificus*) up the forearm was to be determined. Studies in humans are required to assess the efficacy of such repellents because laboratory animals differ in their attractiveness to the pest, and therefore do not provide an accurate assessment of efficacy in humans. A more general protocol (CL-001), which provided additional information relevant to study conduct, was also submitted for review in combination with protocol EMD-003.

Overall, the protocol for EMD-003 was poorly prepared, with numerous errors in referring to the nature of the material to be applied (as a lotion, aerosol or spray) and the evaluation of repellency against mosquitoes was indicated in the rationale provided for the study. Furthermore, the protocol indicated that the dose to be applied was 1 mg formulation/600 $cm^2$, when in fact, the authors of the protocol intended the applied dose to be 1 gram/600 $cm^2$. These mistakes were not considered to be fatal errors in the protocol, but suggested a lack of attention to the details of protocol preparation and review by the investigators. Staffers from the USEPA provided comments on the numerous shortcomings of the proposed study, and the HSRB fully concurred with these weaknesses.

IR3535 is commercially available, and there is a large amount of toxicology data suggesting that it is a compound of low toxic potential. Therefore, human subjects are unlikely to be at risk of experiencing adverse effects relative to exposure to the proposed formulations.

The HSRB recognized three major limitations to the protocol as submitted to the HSRB for review. These limitations include: (1) the lack of a clear rationale underlying the conduct of the study; (2) the lack of identification and characterization of the formulations to be tested and (3) the scientific design of the study. Of these issues, which are discussed in more detail below, the design of the study was seen as the most significant shortcoming of the proposed work.

With respect to the clear rationale for the conduct of the study, the HSRB understood that all new formulations must be evaluated for efficacy, and that such studies must be conducted in human subjects to be valid. However, the investigators failed to identify what was new about the

formulations being studied and failed to identify the potential benefit of the formulations. This shortcoming was considered to be minor and could readily be addressed by providing such additional information in the study protocol.

With respect to the formulations to be evaluated, the investigators provided tables listing the percent of active ingredient along with incipients used to formulate the spray, aerosol and lotion to be used in the study. However, there was no additional information regarding when the formulations would be prepared relative to study execution, whether the formulations would be characterized analytically to confirm active ingredient composition, and whether the stability of the formulations was to be determined. This information is critical to the overall valid execution of the study and could be remedied by providing such detail in the protocol.

The major limitation with the scientific conduct of the study concerned the study design and data collection. In particular, the protocol outlined a study using six test subjects for each product formulation, with two additional subjects serving as a negative and a positive control. No information was provided to justify the group sizes used in the research. Given the nature of these studies to evaluate tick repellency, the HSRB considered that a test in which each subject served as his own control (using one arm for the untreated, negative control and one arm for the test) was a more appropriate design that would also be more powerful statistically and more likely to generate reliable results.

Additional questions were raised by the HSRB concerning how subjects would be trained to accurately and consistently collect information regarding the number of ticks crossing or repelled from the arm skin. The protocol defined that a crossing is scored by the movement of a tick by at least two centimeters toward the elbow starting from a line at the wrist, and that subjects select a new tick from a pool of unused, prescreened ticks every 15 minutes. There was no information made available to the Board as to how subjects were trained and qualified to establish that they could collect accurate data on tick movement. The Board agreed that such information was important for establishing good quality control of the data collection concerning repellency.

HSRB Consensus and Rationale

Overall, the HSRB concluded that there were numerous technical deficiencies in protocol EMD-003, and the information provided in the general protocol (CL-001) did not make up for the deficiencies in the specific protocol. Therefore, the Board concluded that the available protocol did not warrant moving forward with the study.

**Charge to the Board**

b. Does the proposed research described in Study EMD-003 from Carroll-Loye Biological Research appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

**Board Response to the Charge**

Background

The study proposed was to evaluate the efficacy of a compound known as IR3535 as a tick repellent in human subjects. The study is to be conducted by Carroll-Loye Biological Research, a private research laboratory in Davis, California by using healthy volunteers and a controlled laboratory environment. Two protocols were submitted for review, a general protocol (CL-001) that provided considerable background information about tests of insect repellency in general, and the protocol for analysis of the efficacy of IR353 as a tick repellent evaluated here.

For this protocol, the efficacy of IR3535 as a tick repellent would be determined by placing Western black-legged ticks (*Ixodes pacificus)* on IR3535-treated and –untreated forearms and measuring the speed and distance that moving insects would penetrate into the treated area.

Strengths and Limitations

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in the EPA's Initial Ethics Review (USEPA 2006b). This study, it was argued, would provide critical data on the efficacy of IR3535 as a tick repellent. IR3535 is commercially available and has been used as a repellent in Europe for years with no evidence of toxicity, so the subjects enrolled in this study were unlikely to be at increased risk of experiencing adverse side effects upon exposure. The ticks used for the study also were bred and raised in a laboratory environment and are considered to be pathogen-free, minimizing the risk of vector-borne diseases.

The Board concluded, however, that given the deficiencies noted by the Agency, the proposed research described in Protocol EMD-003 did not comport with the applicable requirements of §40CFR26, particularly subpart K. Carroll-Loye Biological Research and the IRB of record also failed to obtain or to provide all of the documents necessary to be in compliance with the requirements of §40CFR26, subpart M. The IRB, for example, refused to release copies of the minutes documenting the discussion of EMD-003 protocol, preventing the Board from evaluating whether or not they considered fully the issues listed under the EPA's Final Human Studies Rule as part of their review. Furthermore, the protocol and supplementary documents submitted to the Board were poorly written which, while not a fatal flaw in and of itself, should have precluded IRB and HSRB review and approval.

The HSRB recognized several significant limitations to the protocol, as submitted to the Board for review. There was, for example, lack of a clear rationale justifying the conduct of the study as designed. The Board's concerns about this are discussed in detail with respect to the companion protocol submitted by Carroll-Loye, EMD-004, but it was felt that absent any clear scientific rationale for conducting such a study, exposure of human subjects to the risks inherent in this protocol would be unnecessary and unjustifiable.

Additional limitations of the study protocol provided to the Board can be grouped into two broad categories: (1) concerns about equitable study subject selection and recruitment; and (2) questions about whether or not the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent.

Subject Recruitment

The Board expressed concern about the potentially coercive nature of study subject recruitment. Although the study is to be conducted by Carroll-Loye Biological Research, a private research laboratory in Davis, California, the Principal Investigator of the study and Co-Owner of the research laboratory, Dr. Scott P. Carroll, also is an adjunct faculty member of the Department of Entomology at the University of California, Davis. As the majority of research participants will be recruited from the University's student population, including from Dr. Carroll's own department, the protocol and consent documents need to be altered to define clearly the mechanisms in place to prevent any coercive enrollment, as well as the additional concerns listed below.

Voluntary Informed Consent

The Board believed that the protocol and consent documents, as provided, lacked sufficient information to ensure that all study participants were adequately informed about the risks, benefits and alternatives to participation in the study. It was unclear, for instance, that participation in the study would have no direct benefit for volunteers or that the study was being conducted solely for marketing research. The major risks of participation in the study also needed to be more clearly identified in the informed consent form and in supplementary documents provided to study subjects. For example, one additional risk that the study investigators may have failed to consider arises from the plan to pre-screen female volunteers in order to exclude any subjects who may be pregnant. In accordance with the newly promulgated provisions in the EPA's final human studies rule (§40CFR26.1701 - 26.1704), minors and pregnant women are explicitly excluded from participation, the latter being confirmed by requiring all female volunteers to undergo a self-administered over-the-counter pregnancy test on the day of the study. Because many of the volunteers are undergraduate or graduate students at a nearby college, the unexpected revelation that a subject may be pregnant could have a profound psychological or social impact; Dr. Carroll also may have a professional relationship with these students through his affiliation with the University. These risks should be specifically addressed, and the Board recommended that a separate consent document for female volunteers be prepared that addresses these risks as well as the safeguards established by study investigators to ensure that the results of over-the-counter pregnancy tests would be kept private.

Study investigators also may wish to provide either a more detailed explanation of the study protocol – including a detailed method for manipulating the ticks used in the experiment and a clear description of the study's duration.

Finally, it was felt that the informed consent documents should be re-written to: (a) comport with the reading and comprehension level of the likely subject population; and (b) clarify the section on compensation for research related injury.

HSRB Consensus and Rationale

The Board concurred with the initial assessment of the Agency that the study submitted for review by the Board failed to meet the ethical requirements established in the Agency's final human studies rule (§40CFR26).

The Board determined the proposed research described in this study did not comport with the applicable requirements of §40CFR26, subparts K and L. The study documents submitted for review also failed to comply with the requirements of §40CFR26, subpart M. However, the deficiencies noted, while significant, were not irreparable.

**Study EMD-004 from Carroll-Loye Biological Research**

**Charge to the Board**

a. Did the proposed research described in Study EMD-004 from Carroll-Loye Biological Research appear likely to generate scientifically reliable data, useful for assessing the efficacy of a test substance for repellent ticks?

**Board Response to the Charge**

Introduction

The Board began its review noting that this protocol addresses repelling insects, not ticks. The Board concluded that the proposed research should generate scientifically useful data for assessing efficacy. Protocol EMD-004 describes a test of the efficacy of 3-[N-butyl-N-acetyl]-aminopropionic acid, ethyl ester (IR3535) to repel mosquitoes in field experiments. It describes the formulation and dose of the repellent and the number of replications (6-10 for each formulation). The components of the three formulations were provided by the Agency. There would be one to two untreated controls and one to two positive (DEET-treated) controls. Two locations would be used, in or adjacent to the Central Valley in California and the Florida Keys. The experiment would be double-blinded. The compound has a very low toxicity profile in animal tests and has been used in Europe for over 20 years as a repellent for many years without reports of adverse effects in humans.

General Scientific Criteria

- The scientific question was stated (i.e., to test the efficacy of IR3535 in repelling mosquitoes).
- It was not clear whether existing data were adequate to answer the question.
- It was not clear whether new studies involving human subjects were necessary; however, if the repellency had never been tested with North American mosquitoes, the tests may be necessary.
- The potential benefits of the study were clear, i.e., that an effective repellent would be available that would have either greater efficacy and/or fewer drawbacks than what was currently approved.
- It was likely that the benefits would be realized (i.e., efficacy as a repellent) because there was a long positive history on this compound from its European use.

- The risks were not specifically noted.
- The most likely relevant risk would be disease transmitted by the mosquitoes, if the mosquitoes carried pathogens, and some mosquito-borne diseases (e.g., West Nile virus-mediated disease) were serious. The protocol did not indicate the likelihood of the mosquitoes in the two test areas to be carriers of disease organisms that could be transmitted to humans. However, using the fewest number of untreated controls would provide the least risk of disease to the participants. The protocol did not indicate whether all the inert ingredients in the formulations are GRAS compounds or have documented lack of toxicity at the exposure levels anticipated.

Study Design Criteria

- The purpose of the study was clearly defined (i.e., efficacy testing).
- There were specific objectives/hypotheses (i.e., that IR3535 is an effective repellent).
- The study as described can test this hypothesis.
- The sample size and how it was derived was not clear, but seems to have been taken from the guidelines. The number of subjects listed in section 9.1.3 of the protocol listed potentially more subjects than in the table in section 8.3.2. It was not clear if the stated number of subjects would be repeated in both locations. The basis for the dose levels and formulations were not provided. There were no controls with just the formulation matrix without the repellent.
- There was a plan allocating individuals to treatments.
- The findings from this study can probably be generalized beyond the study sample.

Participation Criteria

- There was partial justification for the selection of the target population.
- The participants were representative of the population of concern.
- The inclusion/exclusion criteria were appropriate.
- The sample was not a vulnerable group.

Measurement Criteria

- The measurements were expected to be accurate and reliable.
- The measurements were appropriate to the question being asked.
- Quality assurances issues did not appear to be addressed.

Statistical Analysis Criteria

- The data should be able to be analyzed statistically if the efficacy with time was the subject of the analysis and the comparisons are made across time. However, if there is only one untreated control (which would be more protective against possible disease transmission), then there would be difficulties with statistical analysis with comparisons to the untreated control.
- The statistical method seems to be appropriate.
- Measures of uncertainty were not addressed.

Laboratory and Field Conditions

- No laboratory experiments were proposed in this protocol, probably because of the data already available due to the compound's long previous use.
- The field conditions were representative of the intended use.
- The protocol did not include a stop rule plan, medical management plan, and a safety monitor.

HSRB Consensus and Rationale

It was not clear whether new studies involving human subjects were necessary. If the repellency had never been tested with North American mosquitoes, however, the tests were probably necessary. The potential benefits of the study were clear, i.e., that an effective repellent would be available that would have either greater efficacy and/or fewer drawbacks than what was currently approved. However, empirical evidence or procedures to determine risks to subjects (e.g., risks of contracting a vector-borne disease) were not adequate. It was not clear if the stated number of subjects would be repeated in both testing locations. The basis for the dose levels and formulations were not provided. There were no controls with just the formulation matrix without the repellent. Therefore, the Board concluded that some of the more critical deficiencies in information identified above would have to be adequately addressed before this protocol could receive a positive recommendation.

**Charge to the Board**

b. Did the proposed research described in Study EMD-004 from Carroll-Loye Biological Research appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

Brief Overview of the Study

The proposed study would evaluate the efficacy of three different skin applied formulations of an already registered and marketed (in Europe) insect repellent IR3535. There would be two study sites, one located in central California and the other located in the Florida Keys. The test compounds would be administered to a standardized skin surface area, with a comparison to one positive control and one negative control. The subjects allocated to the intervention groups would be blinded to the treatment. The chosen outcome measures are "percent reduction in the rate of alightments" and "complete protection time." The protocol stated that there would be 6 to 10 subjects per treatment group, with one subject per control group. However there was no discussion of sample size justification. As discussed below, the protocol lacked any discussion of risks.

Ethics and Regulatory Compliance

Subpart K of the Agency's final human studies rule requires that the investigator submit to the EPA all information that pertains to the IRB review of proposed research (40 CFR 26.1115a) as well as additional information specified in 40 CFR 26.1125, if not already included in the IRB documentation. The information requested under 40 CFR 26.1125 includes a discussion of the potential risks to human subjects, the measures proposed to minimize these

risks, expected benefits if any and to whom, alternative means to obtain comparable information, and the balance of risk and benefits of the research. In addition, subject information sheets and approved written informed consent agreements should be provided, along with any information about recruitment and the presentation of this subject information. Finally, the investigator should provide copies of all correspondence with the IRB, including official notification of IRB review and approval.

In the case of this protocol, the principal investigator made a request to the reviewing IRB (Independent Investigational Review Board Inc. located in Plantation, Florida) for the documents required under 40 CFR 26.1125. The response from the IRB, dated May 12, 2006, did not include the minutes of IRB meetings at which the protocol was discussed. As a result, the Board was unable to assess whether the IRB discussed or was even aware of the controversial issues raised by this protocol. The IRB did provide templates of two different forms, the EPA Protocol Checklist and Research Evaluation Form. Although these forms were fairly comprehensive, the Board was not provided with copies of the forms used for the specific protocol review and thus cannot assess whether or not the forms were used or the content of the IRB analysis and discussion. The membership roster of the IRB was included. Although the membership was diverse and meets the regulatory requirements, there was no scientific member that appears to have sufficient expertise in the scientific issues involved in field testing of insect repellents to assure that the IRB was qualified to make an adequate assessment of this protocol. The scientific and ethical assessment may have been adequate, but the lack of IRB minutes made this determination impossible. In effect, the IRB response was to provide procedural documentation of the IRB's compliance with 40 CFR 26.1115 but to withhold any substantive documentation that this procedural compliance resulted in an adequate ethical and scientific review of the submitted protocol. As such, the proposed research failed to meet the requirements of 40 CFR part 26, subpart K.

The investigator, to his credit, remedied some of these deficiencies in a supplementary document submitted to the EPA as part of the Board's review. This undated document was presumably written after the IRB review. In this document, the investigator addressed the potential risks to human subjects, the measures proposed to minimize these risks, the nature and magnitude of all expected benefits of the proposed research and to whom they would accrue, the balance of risks and benefits of the proposed research, and alternative means of obtaining information comparable to what would be collected through the proposed research.

Several observations are in order. First, none of this material, including the discussion of risks and benefits can be found in the protocol submitted to the IRB. The absence of this information in the protocol further compounds the uncertainty created by the absence of minutes showing how the IRB made the determinations required under 40 CFR 26.1111. The information about the potential risks to human subjects, the measures proposed to minimize these risks, the nature and magnitude of all expected benefits of the proposed research and to whom they would accrue, and the balance of risks and benefits of the proposed research should be part of the research protocol submitted for initial IRB review. Otherwise, the IRB lacked sufficient information to make an appropriate assessment of the proposed research. This was not to say that the protocol would fail to meet the criteria for IRB approval, only that there was no evidence that the IRB had sufficient information or expertise to make these determinations. Second, the

protocol did minimize the risk of vector-borne diseases by limiting the untreated control group to a single subject who was experienced in field biology or entomology. The risk was minimized further by using an outcome measure that does not require biting, but rather preparatory activities on the part of the mosquito after lighting on the subject followed by aspiration and removal of the mosquito. However, this approach raised concerns about the scientific adequacy of the protocol design. Third, the investigator addressed the question of alternative means of obtaining information by arguing that the protocol was designed in compliance with previous EPA guidelines for registration of these products. The Board did not take a position on whether the protocol was indeed in compliance with these previous guidelines. However, there was sufficient discussion by the Board of the new draft EPA guidance on "Insect Repellent Product Performance Testing" to cast doubt on the scientific adequacy and necessity of the approach taken in this protocol.

Finally, the Board agreed with the ethical deficiencies noted by the EPA (USEPA 2006c) With the exception of amending the protocol to include the applicability of additional standards of ethical conduct and the process of informing appropriate regulatory authorities of any amendments or deviations from the approved protocol, all of these deficiencies related to the informed consent discussion and document. These included a more accurate discussion of subject assignment, a more extensive discussion of the risks (with specific information about the risk of vector borne diseases), the correction of an important typographical error in the pregnancy section, a clarification of the section on compensation for research related injury, a clarification of the lack of direct benefit to research subjects and additional information under the heading of confidentiality. The Board also discussed the topic of pregnancy testing and whether there should be a separate consent for such testing. As many of the research subjects may be in a professional relationship to the principal investigator (such as graduate students or colleagues), a protocol and consent document needs to discuss how the confidentiality of such pregnancy testing would be protected.

HSRB Consensus and Rationale

The Board thus concluded that the proposed research described in Study EMD-004 from Carroll-Loye Biological Research did not comport with the applicable requirements of 40 CFR part 26, subpart K. The proposed research does comport with subpart L, as pregnant women and children were excluded. Although the ethical concerns identified by the Board could be remedied, there were sufficient questions raised about the adequacy of the research design to cast doubt on whether the proposed research would meet the criteria for IRB approval found under 40 CFR 26.1111(a) (1). In other words, absent a sound research design, any exposure of human subjects to risk would be unnecessary and unjustifiable.

**Board Response to the Charge**

**Occupational Handler Exposure Monitoring Studies**

**Charge to the Board**

The Agricultural Handlers Exposure Task Force (AHETF) had submitted protocols for five pesticide exposure studies that are part of a larger research program the AHETF is conducting. The premise of the AHETF research program is that data can be used generically by various stakeholders (e.g., applicants, registrants, EPA, and others) for calculating exposures for the occupational handlers of pesticides. The scope of the AHETF research program was very broad in that it intends to address exposures related to many job functions in agriculture and also to assess generally the impacts of various parameters on exposure (e.g., How do changes in the pounds of pesticide handled or acres treated affect exposure levels?). The protocols submitted for HSRB review described studies to measure exposures for five specific scenarios.

The Agency believed these studies had the potential to improve EPA's ability to assess the risks of using pesticides because the data would reflect current agricultural practices, equipment and techniques and would allow for more refined exposure estimates. Further, the monitoring techniques to be used for these studies have been standardized for use across the AHETF research program. These more refined and reliable data would allow the Agency to estimate better how worker exposure levels are affected by changes in various factors such as the amount of active ingredient handled, type of application equipment used, application rate used, volumes handled, and personal protective equipment used.

It should be noted, however, that the use of the data generated in this study by the EPA and other stakeholders would depend upon the nature of the results. For example, the adequacy of the field or laboratory quality control data may dictate that correction factors are applied to adjust monitored exposure levels to account for losses from field samplers or low performing analytical methods.

1. AHETF Closed System Mixing/ Loading of Liquids Protocol (AHE34)

a. Does the proposed research described in Study No. AHE34 from the Agricultural Handlers Exposure Task Force appear likely to generate scientifically reliable data, which would be useful, together with other data, for assessing the potential levels of pesticide exposure received by people when mixing, loading or applying a liquid pesticide with closed systems? [Note: In a few cases, corresponding application events are also to be monitored; the same question applies to those elements of the study.]

b. Does the proposed research described in Study No. AHE34 from the Agricultural Handlers Exposure Task Force appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

2. AHETF Airblast Application to Trellis Crops in the West Protocol (AHE36)

a. Does the proposed research described in Study No. AHE36 from the Agricultural Handlers Exposure Task Force appear likely to generate scientifically reliable data, which would be useful, together with other data, for assessing the potential levels of pesticide exposure received by people when making an airblast application of a pesticide to a trellis crop under conditions found in the western United States? [Note: In a few cases, corresponding mixing/loading events are also to be monitored; the same question applies to those elements of the study.]

b. Does the proposed research described in Study No. AHE36 from the Agricultural Handlers Exposure Task Force appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

3. AHETF Airblast Application to Trellis Crops in the East Protocol (AHE37)

a. Does the proposed research described in Study No. AHE37 from the Agricultural Handlers Exposure Task Force appear likely to generate scientifically reliable data, which would be useful, together with other data, for assessing the potential levels of pesticide exposure received by people when making an airblast application of a pesticide to a trellis crop under conditions found in the eastern United States? [Note: In a few cases, corresponding mixing/loading events are also to be monitored; the same question applies to those elements of the study.]

b. Does the proposed research described in Study No. AHE37 from the Agricultural Handlers Exposure Task Force appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

4. AHETF Closed Cab Airblast Application to Orchards Protocol (AHE38)

a. Does the proposed research described in Study No. AHE38 from the Agricultural Handlers Exposure Task Force appear likely to generate scientifically reliable data, which would be useful, together with other data, for assessing the potential levels of pesticide exposure received by people when making an airblast application of a pesticide to orchard crops? [Note: In a few cases, corresponding mixing/loading events are also to be monitored; the same question applies to those elements of the study.]

b. Does the proposed research described in Study No. AHE38 from the Agricultural Handlers Exposure Task Force appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

5. AHETF Fixed-Wing Aerial Application Protocol (AHE42)

a. Does the proposed research described in Study No. AHE42 from the Agricultural Handlers Exposure Task Force appear likely to generate scientifically reliable data, which would be useful, together with other data, for assessing the potential levels of pesticide exposure received by people making an aerial application of a pesticide from fixed-wing aircraft? [Note: In a few cases, corresponding mixing/loading events are also to be monitored; the same question applies to those elements of the study.]

b. Does the proposed research described in Study No. AHE42 from the Agricultural Handlers Exposure Task Force appear to comport with the applicable requirements of 40 CFR part 26, subparts K and L?

**Board Response to the Charge**

For the Board's review of the agricultural handler protocols, the Board decided to focus its analysis addressing the common strengths, limitations and overall conclusion of the five protocols.

**Scientific Considerations**

Study Overview

The pesticide handler exposure study protocols submitted to the HSRB were part of a larger project that was initiated in December 2001 by the Agricultural Handler Exposure Task Force (AHETF). The project will produce a generic agricultural handler exposure database (AHED™). EPA and other regulatory agencies would use this database to calculate pesticide handler exposures across a wide range of work conditions. All of the protocols follow a similar pattern. They involve personal measurements of inhalation and dermal exposure among a group of workers who conduct what is referred to as a "scenario"; that is, the study participant would conduct a specified job task with specified equipment, handling a particular product formulation that contains one of six pesticides.

These studies can be referred to as scripted, so as to distinguish them from purely observational studies. Workers are asked to conduct their work activities under a set of scripted conditions similar, but maybe not identical, to those they experience in their normal work activities. The overall plan for the exposure database had been discussed with regulators from EPA, California EPA, and Health Canada on a regular basis. The presentation of these protocols to the HSRB is the first independent scientific review of the task force project.

The task force had proposed 33 handler exposure scenarios, distinguished by equipment type, work task, and pesticide formulation. The task force has already conducted or initiated 14 exposure studies, and has sponsored four studies. In addition to the five protocols presented to the HSRB, the task force planned to conduct approximately 40 additional studies over the next several years.

Each of the protocols focuses on one primary exposure scenario, but all of the protocols include more than one scenario. The five protocols reviewed by the HSRB are summarized in Table 1.

Table 1. Exposure Scenarios Included In The Five AHETF Protocols (N = anticipated number of independent observations for a particular scenario)

| | AHE34 | AHE 36 | AHE37 | AHE38 | AHE42 |
|---|---|---|---|---|---|
| **Scenario 1** | Closed system mix-load of a liquid | Open cab trellis crop airblast application | Open cab trellis crop airblast application | Closed cab orchard crop airblast application | Closed cockpit fixed-wing aircraft application |
| **N** | 10 | 5 | 5 | 8 | 7 |
| **Scenario 2** | Open or closed cab airblast application | Closed cab trellis crop airblast application | Closed cab trellis crop airblast application | Open pour mix-load of a liquid | Open pour mix-load of a liquid |
| **N** | 0-3 | 4 | 4 | not specified | 0-3 |
| **Scenario 3** | Closed cockpit fixed-wing aircraft application | Open pour mix-load of a liquid | Open pour mix-load of a wettable powder | | Closed system mix-load of a liquid |
| **N** | 0-3 | 0-4 | 4 | | 0-3 |
| **Scenario 4** | Closed cockpit rotary-wing aircraft application | Closed system mix-load of a liquid | | | |
| **N** | 0-3 | 0-4 | | | |
| **Total N** | 13 | 13 | 13 | not specified | 10 |
| **Chemical** | Malathion 8 (80% a.i.) | Malathion 8 (80% a.i.) | Diazinon 50% WP | Carbaryl 4lb a.i./gal | Chlorothalonil 6lb a.i./gal |
| **Location** | CA | West (CA) | East (NY) | FL and GA | Pacific NW |

The task force studies are using six different pesticides: three organophosphorus insecticides (acephate, diazinon, malathion), one carbamate insecticide (carbaryl), one organochlorine fungicide (chlorothalonil), and one triazine herbicide (simazine). A description of the selection criteria for these compounds was provided as a part of the task force documentation package. These selection criteria did not include the toxicity of the compounds, and toxicity was not discussed in the Agency reviews of the protocols. However, oral comments from task force representatives at the June HSRB meeting indicated that the formulations of these pesticides have been selected such that all fall into EPA toxicity categories III or IV; i.e., relatively low toxicity formulations. It was also stated that all workers in these studies wear long-sleeve shirts, long-legged pants, as well as socks and shoes. Protective equipment, such as chemical-resistant gloves and eye protection, are provided to the workers if required by the pesticide label.

The database to be developed from the task force studies is intended to supersede an existing database – the Pesticide Handler Exposure Database (PHED). This database was

developed in the late 1980's and early 1990's through the compilation of existing data. These data were drawn from both registrant-sponsored studies and studies published in the scientific literature. The studies included in PHED used a different method for measuring dermal exposure. This method, known as the "patch technique" (deposition coupons distributed over body regions attached to the outer layer of clothing), has served as the standard method for such studies since the 1960's. When coupled with a hand rinse technique, it provides an estimate of exposure to all body surfaces.

The documents submitted by the AHETF in support of the proposed exposure studies consisted of the following:

- Cover letter dated May 24, 2006
- Analytical method validation reports for 5 of the 6 pesticides (missing simazine)
- List of 33 exposure scenarios
- Description of selection requirements for surrogate compounds
- 32 standard operating procedure (SOP) documents
- A generic field exposure monitoring protocol
- 5 exposure study protocols: AHE34, 36, 37, 38, and 42
- IRB documents related to each protocol

In addition, the HSRB received an EPA review for each protocol, EPA, Office of Pesticide Program guideline documents, and several general documents on pesticide handler exposure. Finally, the AHETF provided public comments (AHETF 2006) containing comments on the EPA review of the five protocols.

Critique of Study

General Scientific Criteria

The primary aim of these studies is to generate personal measurement data on pesticide handlers suitable for use in an agricultural handler exposure database. The notion that such a generic database for pesticide handlers can be developed is supported by substantial scientific evidence. This evidence indicated that occupational pesticide exposure in agriculture is largely process rather than chemical-dependent (excluding chemicals with high volatility). Thus, if sufficient data can be collected on the key variables that influence exposure, then a database can be developed to estimate exposure for a wide range of exposure scenarios. A major concern of the HSRB was that these protocols included too many variables, and that, even when combined with the full complement of studies proposed, the database would be inadequate for meaningful exposure estimates.

The process that has guided the exposure database project had some significant limitations. The database project has been developed over the past 5 years by a pesticide industry task force with the input of regulatory staff from EPA, California, and Canada. Such an ambitious undertaking would have benefited from an initial independent scientific peer review, particularly of the study design and statistical plan, as the HSRB finds itself raising some fundamental questions mid-stream in the project. Input from the labor community would also

have enhanced the project regarding procedures such as subject recruitment, selection of pesticides to be used in individual studies, and informed consent. The purpose of the project, after all, is to develop data to estimate worker risks. It seems reasonable to give those who would be taking the risks an opportunity to contribute to the design of the project.

In regard to justification for new human studies, the Agency currently uses an existing generic pesticide handler exposure database, known as PHED. It is recognized that new data have not been added to PHED in a number of years, and that the existing data have a number of scientific limitations. However, the inadequacy of PHED was not documented in the protocols. The Agency had not provided a compelling justification for these new human studies in the materials provided.

Benefits of the study were not described in the protocols. However, the AHETF comments (AHETF 2006) and the EPA review documents provided some general information regarding the role of a handler exposure database in EPA's regulatory process. It was not possible to determine the likelihood that the benefits would be realized, since the protocols did not include a description of the full database and how it would be used.

Study Design Criteria

The purpose of these studies was clearly defined. The objective was to collect high quality personal measurement data for use in a generic exposure database. The protocols reviewed by the Board should be able to produce such data.

Approximate sample sizes were presented in the protocols and discussed in more detail in the Agency reviews of the protocols. Within each protocol, the sample sizes for particular scenarios were quite small. All of the protocols contained multiple scenarios, with sample size per scenario ranging from 1-10 (see Table 1). The inability to define exactly how many samples would be collected in each proposed study was understandable, since the task force was attempting to take advantage of 'real-world' conditions. Weather, logistical challenges, and grower decisions regarding pest management can all affect the number of workers available for a given study. The effort to study exposures under realistic conditions required expenditure of significant resources, and was viewed by the HSRB as highly commendable. The HSRB understood that these protocols should not be viewed as "stand-alone" studies, since data from these studies would be combined with other similar studies. Presumably all of the data collected in these five studies would be allocated to one of the 33 exposure scenarios outlined by the task force. Under these circumstances, there was insufficient information for the HSRB to evaluate the adequacy of the sample size.

In regard to dose levels, participants would be handling varying amounts of pesticides under variable exposure conditions. The HSRB presumed that the conditions outlined in the protocols and reviewed by the Agency all fall within parameters on the label. Actual dose during these studies would likely be lower than normal, due to the wearing of a whole-body cotton garment, and strict observance of label instructions.

Participation Criteria

Participants are referred to as 'replicates' both in the AHETF protocols and in the Agency reviews. This term is problematic from a scientific perspective, since it is used to refer to both a series of independent observations (e.g., three persons doing the same thing one time) and a series of repeated measures (e.g., one person doing the same thing three times). This language needs to be altered such that different terms are used for repeated measures on one person as compared to observations on unique individuals. The AHE34 protocol, for example, indicated that "ten different mixer/loader workers (or replicates) will be monitored . . . each mixer/loader replicate should be performed by a separate worker." This type of awkward description could be eliminated through use of unambiguous terminology.

The protocols indicated that the participants would be "experienced workers" recruited through their employers, but there was no detailed description of the recruitment procedures, nor were there clear inclusion/exclusion criteria other than age and pregnancy status. It was hard to tell whether the workers who volunteer for a protocol exposure study would be representative of the typical worker. Random sampling from a group of eligible workers would improve this aspect of the protocols.

Measurement Criteria

AHETF investigators are using three different methods to measure skin exposure: cotton garments (whole body dosimeters), hand rinse, and face/neck wipes. The cotton garments should be able to capture pesticide that would normally be deposited on skin. However, no method for preventing or monitoring garment breakthrough was presented. If breakthrough occurs, the dermal exposure measurements would underestimate true exposure. The hand rinse method and face/neck wipe both measure the amount of material that can be removed from the skin at the particular time of the sampling. This amount is some fraction of the total material deposited on the skin, since some of the material would have been absorbed into the skin. This method is likely to underestimate the true exposure. Published laboratory and field studies have indicated that the fraction of the amount deposited on skin that can be removed by rinsing or wiping can be quite variable, depending on the nature of the chemical, its formulation, skin characteristics, and the length of time the chemical has been in contact with the skin. In particular, the face/neck wipe method may seriously underestimate exposure to these surfaces. This method was not among the methods presented by the Agency in its 875 guidelines (Occupational and Residential Exposure Test Guidelines: OPPTS 875.1100 Dermal Exposure – Outdoor), and has not been validated. The accuracy of these measurements could be improved through the conduct of laboratory removal efficiency studies. The 875 guidelines do not require removal efficiency studies, but they do indicate that investigators should address this concern. If such method validation studies are contemplated, the HSRB recommended that they be conducted as independent scientific studies published in the peer-reviewed literature.

In summary, all of the methods for dermal exposure measurements have the potential to underestimate exposure. The study investigators should acknowledge this problem in the protocols, and explain what steps, if any, they have taken to improve or verify the accuracy of the measurements.

The protocols stated that hand rinse and wipe samples may be collected multiple times during the work period (e.g., prior to eating, whenever a worker would normally wash hands), and that this would vary from worker to worker. The protocols did not explain how multiple measurements from a single worker would be combined. They also did not discuss whether or not samples across workers with different rinse/wipe regimens can be considered comparable. For example, can the amount of pesticide recovered in a single hand rinse from a worker at the end of the study period be put in the same database as that from a worker who had four hand rinses across the study period? The answer would seem to be "no" from a sampling perspective, given the nature of dermal absorption processes.

The quality assurance components of the protocols are of high quality. There was substantial documentation regarding the reliability of analytical methods available for each of the sampling media to be used. There were detailed standard operating procedure documents for field and laboratory quality assurance activities.

Statistical Analysis Critera

An inadequate statistical analysis plan was provided in the protocols. The HSRB identified this deficiency as the most critical scientific limitation for these protocols. There was a need for a more professional and comprehensive treatment of statistical issues in the analysis of data, and in the design of individual protocols. Chief among these issues was the question of statistical power. It is critical to address the HSRB's concern that the present design calls in most cases for a single observation per experimental condition. In other words, it appears that the present studies are intended to be parsed in terms of formulation, container size, frequency of worker activity, equipment, air temperature, wind speed, relative humidity, amount of cloud cover, rainfall, crop, amount of material handled, rate of application, acreage treated, and geographic location (along with other possible qualifiers). As a result, the number of variables to be evaluated appears to approach or even exceed the total number of subjects for a given scenario. One may hope that some useful information might yet emerge from a properly performed analysis of the full data set coming from studies involving different chemicals, sites, and conditions. What is needed now, however, is a cogent and thoughtful discussion, in the protocol, of just what can be accomplished along these lines, and an explanation of how it can be accomplished. Further thought may lead to the conclusion that the current data-gathering plan is in fact overly optimistic in regard to the issue of statistical power. It would then be essential to restructure the plan and change the study design to ensure that the enormous effort in this large and important project would not be wasted.

If the goal of these studies was to estimate the distribution of exposures across a variety of application scenarios, it would be important to include true repeated measures for at least some of these scenarios to assess the extent of within-worker variability.

Laboratory and Field Conditions

The protocol states that the field conditions were selected to be representative of real-world use of pesticides. In this regard, participants are experienced workers, are allowed to wear

their own clothing, and conduct normal work activities in an actual agricultural setting. Studies are distributed across the U.S. and across the year in an attempt to develop a range of exposure conditions. While laudable in scope, it is important to restate the concern that the large number of variables included in these studies may prove extremely problematic for analysis.

The issue of potential heat stress was discussed at length at the HSRB public meeting. The HSRB concluded that the protocols should include explicit criteria for halting a study due to heat stress risk.

The protocols stated that workers would be monitored "during a period of time representative of a full day's work". The protocols also indicated that monitoring times would conform to a "typical" workday. However, none of the protocols defined the typical work period for the specific tasks to be studied. Instead, the protocols stated that monitoring time "will involve work periods with a target of 4 hours." This language suggested that even four hours of monitoring might not be achieved in some cases, and there was no indication that workdays as long as 8 or 9 hours would ever be monitored. It was not clear to the HSRB that these studies would necessarily reflect a full workshift or a "typical" workday. Many factors can influence the length of the workday, including weather conditions, and the need to "get the job done" due to pest pressures or the stage of crop development. Fatigue is an important factor that can affect exposure, and usually inattention occurs at the end of the day. The HSRB recommended that the protocols document the time of a typical workday (or the range of these times) for each scenario, and that the monitoring time be based on this information.

Special Concerns Regarding Use of Diazinon in Protocol AHE37

Protocol AHE37 involves handling a wettable powder (50WP) formulation of diazinon. Workers would be monitored during open cab trellis crop airblast applications and open pour mixing-loading operations. The HSRB was concerned that these practices are not consistent with current Agency policy. The Agency's May 2004 interim registration eligibility document (IRED) for diazinon stated that engineering controls are required during handling. The "IRED Facts for Diazinon" states, "All application equipment must use lock and load engineering controls. All wettable powder formulations must be packaged in water-soluble bags. Closed cabs are required for all ground equipment, except for applications to apples." The IRED Executive Summary further stated, "Occupational exposure to diazinon is of concern to the Agency. For agricultural uses of diazinon, most mixer/loader/applicator risk scenarios currently exceed the Agency's level of concern (i.e., MOEs are less than 100 for dermal exposure and MOEs are less than 300 for inhalation exposure). Taking into consideration both the risks and benefits of these uses, EPA has determined that most agricultural uses may continue with the adoption of the following mitigation measures: . . . engineering controls for mixers and loaders and closed cabs for applicators for all application scenarios . . ." The above statements indicate that open pouring of diazinon is not permitted, and that open cab airblast applications are not permitted in trellis crops.

HSRB Consensus and Rationale

The five studies presented for HSRB review were components of a large-scale exercise to create a contemporary database on occupational exposure to agricultural pesticides. The undertaking is in itself likely to be worthwhile in quantifying and improving our understanding of the exposures of and risks to pesticide handlers. The potential benefits are large and the risks appear to be relatively modest. However, the materials supplied for HSRB review failed to deal adequately with risks and benefits. None of these protocols can be properly evaluated in regard to scientific validity because they lacked: (1) a developed rationale documenting the need for new data; (2) a clear and appropriate plan for the handling of the data (including its statistical analysis), and (3) an explanation of the uses to which the data would be put and adequate sample sizes and protocols for repeated measures to appropriately estimate exposures within individuals and between scenarios. These points need to be addressed, at least briefly, in each specific protocol and, more fully, in a separate and new "governing document" that is not simply a generic description of the planned activities.

Additional validation studies are recommended to determine the extent to which dermal exposure measurements may underestimate true exposure. Laboratory-based removal efficiency studies or field-based biomonitoring studies could be conducted to achieve this goal. Such studies should be published in the peer-reviewed literature. Broader participation of the scientific community and of parties with a direct interest in the database project, such as the labor community, would likely improve the quality of the database and enhance the credibility of its use in risk assessments.

The HSRB recommended that specific criteria for cessation due to heat stress be included in these worker exposure protocols, and that the protocols included a heat stress management plan. In addition, the HSRB recommended that the length of each study should be truly representative of a full workday, and that each protocol should document the basis for the proposed duration of the study.

The HSRB was gratified to receive the Agency's response to its query regarding the use of diazinon in the AHE37. It is the understanding of the HSRB that the Agency would inform the AHETF that it needs to identify a pesticide other than diazinon in this protocol to evaluate exposures associated with open pour activities and applications using open cabs, and that the Agency would ensure that future protocols comply with the most current risk mitigation measures specified in IREDs and REDs.

**Board Response to the Charge**

**Ethical Considerations**

Background

These five studies are part of a series of studies that are to be conducted by the AHETF, a coalition of 19 pesticide registrants that was formed in December 2001 to share resources in the

design, evaluation, and development of a proprietary agricultural mixer/loader and applicator exposure database for use in regulatory risk assessments.

The study protocols were designed by AHETF investigators after joint discussions with the US EPA, Health Canada, and the California Department of Pesticide Regulation, in accordance with the recommendations of such guidance documents as: 1) US EPA, Occupational and Residential Exposure Test Guidelines, Series 875.1000 through 875.1600 (1996); and 2) US EPA, Working Draft - Occupational and Residential Exposure Test Guidelines, Series 875 Group-B, Postapplication Exposure Monitoring Test Guidelines Version 5.4 (1998). The supporting and supplementary study documents also assert compliance with the Good Laboratory Practice (GLP) Standards established by the 1972 amendment to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (§40CFR160). Finally, these protocols were reviewed and approved by the Western Institutional Review Board (WIRB) of Olympia, Washington, prior to submission to the Agency.

The aims of these studies are to provide critical exposure information for individuals who mix, load, and apply agricultural pesticides. Agricultural producers ("growers") would be recruited by the study coordinators several months prior to initiation of the study; in exchange for their participation in the study, each grower would receive, free of charge, an amount of liquid pesticide equivalent to the normal quantity mixed and loaded into closed-mixing systems and spray rigs for the duration of the study (expected to be a single day). Participating growers also may be asked to recruit other growers and pesticide applicators into the research study.

Study investigators would recruit agricultural handlers on-site; volunteers would receive $100/day for their participation in addition to their regular pay. Voluntary informed consent would be solicited by study investigators, and will be documented using a standardized informed consent form. Because the study participants would be recruited from a pool of experienced agricultural workers who routinely mix and load liquid pesticides as part of their normal duties, the AHETF had argued that participation in this study presents a negligible increase in pesticide exposure risk to volunteers. In accordance with the newly promulgated provisions in the EPA's Final Human Studies Rule (§40CFR26.1701 - 26.1704), minors and pregnant women are explicitly excluded from participation, the latter being confirmed by requiring all female volunteers to undergo a self-administered over-the-counter pregnancy test on the day of the study.

Dermal exposure to pesticides would be ascertained through hand rinses and face/neck wipes, as well as the use of long cotton underwear – as a surrogate for skin – to be worn under the study participant's clothing. In addition to the long underwear, all participants would be required to wear long sleeved shirts and long pants, shoes plus socks, in accordance with accepted worker protection standards. Volunteers may wear their own clothing provided they are freshly laundered; alternatively, the AHETF would provide freshly laundered clothing. Any personal protective equipment (PPE) that may also be required, such as chemical resistant gloves and protective eyewear, will be provided. At the conclusion of the four-hour study observation, the long underwear would be removed and subjected to laboratory analyses to estimate whole-body dermal pesticide exposure. Study participants would also be asked to wear OSHA Versatile Samplers (OVS) outfitted with glass filters, XAD-2 sorbent, and tygon tubes to measure

inhalation exposure. The tubes would be attached to the volunteer's collars with the openings positioned in their breathing zones. By using such state-of-the-art monitoring techniques, the AHETF argues, this study would provide critical exposure information for individuals who mix/load liquid agricultural pesticides.

Strengths and Limitations

These studies would provide critical exposure information for individuals who mix/load and apply agricultural pesticides. It is also believed that the monitoring techniques proposed for these studies represent the current state-of-the-art. However, the Agency also recognized that use of the data resulting from this studies would take careful scrutiny and may require a number of adjustments depending upon the results. Finally, the overall design of these studies should be considered in the context of the goals of the AHETF which are to develop a broad-based database that can be generically used as a predictive tool for estimating exposures to pesticide handlers and that the interpretation of the results of these studies may or may not necessitate the need for additional monitoring data.

The Board concurred with the factual observations of the strengths and weaknesses of the studies, as detailed in the EPA's Initial Ethics Review of the AHETF Template Protocol and each individual study protocol. The Board concluded that, given the deficiencies noted by the EPA, the proposed research described in the AHETF Template Protocol and each individual study protocol do not comport with the applicable requirements of §40CFR26, subparts K and L. Furthermore, the AHETF and WIRB failed to provide all of the documents necessary to be in compliance with the requirements of §40CFR26, subpart M.

Although public comments from several members of the AHETF helped assuage some of the Board's concerns, the members of the HSRB believed that further comments about this protocol were warranted. The comments below are grouped into four broad categories: (1) whether the study was designed to adequately minimize risk to study participants; (2) whether the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent; (3) whether study participants would be adequately compensated in the event of a study-related injury; and (4) whether appropriate alternatives to participation are provided.

Minimization of Risks to Study Participants

This study proposes to measure dermal and inhalation exposure to liquid pesticides by agricultural handlers who usually perform pesticide mixing, loading, and application as part of their daily routine. However, it was unclear to Board members, given the semi-scripted nature of the protocol provided, as to whether or not study participants would be exposed to greater quantities of these compounds than would normally occur. Are the studies proposed purely observational in nature, or are study investigators intervening by requesting that study participants use different types and quantities of pesticide, or different mixing, loading, and application methods, than they normally would? If the latter is true, the assumption that this study represents a negligible increase in pesticide exposure risk to volunteers may be unfounded. Several Board members also expressed concern that the additional requirements for donning and

removing the equipment used to measure pesticide exposure may inadvertently lengthen the participant's normal work day. If so, this should be clearly described during the consent process, as should the question of whether the $100 paid for study participation is expected, in whole or in part, to compensate for the extension of the work day.

The protocol failed to detail the approach taken to ensure that agricultural handlers are adequately trained in the proper mixing, loading, and application of these compounds. Although pesticide mixing instructions and Material Safety Data Sheets are made available to study participants, given that many agricultural workers may not be fluent in English (or may even be illiterate), a clear plan for ensuring that volunteers are properly educated in minimizing their exposure to these compounds should be included. Furthermore, study investigators may want to make arrangements to provide volunteers with the results of the study following completion.

One of the greatest risks to study participants is heat-related illness, given that dermal exposure to pesticides will be determined by asking volunteers to wear long underwear in addition to their normal protective equipment (e.g., long sleeved shirts and long pants, and other applicable protective gear). Although study coordinators are expected to be vigilant for signs of heat-related illness among volunteers, in order to minimize the risks posed to the study participants the protocol also should include: a) explicit starting and stopping criteria based on a quantifiable measure like ambient temperature or heat index; and b) a clear description of the symptoms of heat-related illness in the informed consent documents. There should also be a clear plan for reporting any heat-related illness (or, for that matter, any other adverse event) to the study investigators, Western IRB, and the EPA.

Because some of the study participants may be undocumented immigrants, measures to ensure strict confidentiality should be developed. Many undocumented workers, for example, may be loathe to report any adverse study-related event requiring medical attention or hospitalization if they believe that their illegal status will be reported to immigration authorities. Alternatively, study investigators may wish to require documentation of citizenship or immigration status as part of the inclusion criteria for recruiting study participants. In addition, because many pregnant day-laborers may fear job loss in the event that their employer learns of their condition, extra care should be taken to keep the results of over-the-counter pregnancy tests private.

Voluntary Informed Consent

Several Board members felt that the AHETF protocol, as provided, lacked sufficient safeguards to ensure that all study participants were adequately informed about the risks, benefits and alternatives to participation in the study. For example, it was felt that the informed consent documents provided were written at too high of a reading and comprehension level. Given the sociodemographic characteristics of the farm worker population, many of the study participants may have limited education, may speak English as a second or even a third language, or may even be illiterate. Study investigators should develop a clear consent document which – in addition to including a more detailed description of risks (including the risks of the pesticides being handled) as described previously, as well as a clear distinction between what comprises research versus normal activities – is written at a lower grade-level and translated into the

various languages likely to be spoken by study participants. A brief oral test of comprehension should also be developed, with volunteers required to demonstrate a clear understanding of the purposes and the risks of the study prior to enrollment.

The Board also expressed concern about the potentially coercive nature of the study, given the potential for study participants to believe that there is a direct relationship between study investigators and growers. Absent additional safeguards, the "gift" of study pesticide to the growers may contribute to undue influence on employees to participate in the research. Western IRB, in its initial review of several of the AHETF protocols, recommended that "extra care" be taken during the recruitment and consent process to minimize coercion or undue influence on study participants. However, no documentation was provided to the HSRB as to how the AHETF addressed WIRB's concern. For example, there was no evidence to suggest that AHETF researchers solicited the help of the farm-workers themselves or other community leaders to ensure that study participants would not be covertly or overtly coerced into participating in the study. The rights of participants to withdraw from the study at any time also should be emphasized. It is unclear from the informed consent or other study documents, for instance, as to whether volunteers are entitled to receive monetary payment even if they chose to withdraw during the course of the study. Although the Board was reassured during the discussion that sufficient alternate work was available, the protocol also failed to specify that workers would still be paid for a day's labor even if they refused to participate in the research.

Compensation for Injury to Study Participants

The study protocol and informed consent documents state that: "If [a study participant is] injured as a result of being in this study, treatment will be available from a health professional at a nearby medical facility. The costs of such treatment will be covered by the AHETF. This does not cover any injuries resulting from [the volunteer's] normal activities." Given the nature of the study design, however, it is unclear whether a distinction between injuries resulting from normal work activities versus participation in this study can be made. Two of the symptoms of heat exhaustion, for example, are dizziness and loss of coordination – will study coordinators be able to distinguish between an accidental injury caused by clumsiness versus an injury resulting from potentially-unrecognized symptoms of heat-related illness? In light of these concerns, the Board recommends that the AEHTF cover medical treatment for all participant illness and injury occurring during the study period (i.e., the day of the test).

Alternatives to Participation

As noted above, the design of this study involves collaboration between the researchers and growers in which the growers receive, free of charge, a particular pesticide that they are required to apply to their fields on the day of the study. That arrangement will lead in many circumstances (except of the few coincidental instances when the grower had already planned to use that chemical on that day) to a change in the pesticide being applied by the grower.

The following question thus arises: What alternatives are offered to agricultural handlers working for that grower who choose not to participate in the study? One option is that they could be offered the choice of applying that pesticide that day, but not needing to participate in any

other study procedures (such as wearing the long underwear). Some members of the Board believed that if that is the only alternative to participation, then this aspect of the study would not comply with 40 C.F.R. Part 26, Subpart K. A primary purpose of the EPA rule is to prevent a person from being intentionally exposed to a pesticide without their voluntary informed consent. The EPA emphasized this point when it promulgated the final version of its rule, commenting that the term "research involving intentional exposure" covers "any research on a substance, unless the subjects of the research retain complete control over whether, when, and how they are exposed to the substance." 71 Fed. Reg. 6138, 6146 (2006).

Some members of the Board accordingly concluded that for agricultural workers who had pre-existing expectations of earning money working for the grower on the day of the study (either as employees or as independent contractors with contractual expectations of working that day), the protocol must provide them alternatives for earning that same amount of money that do not require them to apply the pesticide used in the study. Acceptable alternatives could include applying some other pesticide they have in the past applied, performing some other task they regularly perform, or being paid their expected earnings without needing to work. Absent such alternatives, the protocol would appear to be inappropriately coercing such persons into applying the study compound or else losing the money they expected to earn that day.

HSRB Consensus and Rationale

The Board concurred with the initial assessment of the Agency that the studies submitted for review failed to meet the ethical requirements established in the 40CFR26.

The Board determined the proposed occupational handlers exposure studies do not comport with the applicable requirements of 40CFR26, subparts K and L. However, the deficiencies noted, while significant, were not irreparable.

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