December 18, 2007

EPA-HSRB-07-03

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

Subject: June 27-29, 2007 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: (1) Carroll-Loye Picaridin Mosquito Repellency Protocol LNX-001; (2) ICR Picaridin Mosquito Repellency Protocol ICR 1 A 044; (3) Acrolein; (4) 4-Amino Pyridine and; (5) Antimicrobial Exposure Assessment Task Force (AEATF) and Agricultural Handler Exposure Task Force (AHETF) Research Programs. The enclosed HSRB report addresses the Board's response to EPA charge questions at its June 27-29, 2007 meeting. A summary of the Board's conclusions is provided below.

Carroll-Loye Picaridin Mosquito Repellency Protocol LNX-001

- The protocol LNX-001 to study the efficacy of a cream formulation and a pump spray formulation of picaridin for repelling mosquitoes is sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy of these formulations against mosquitoes.

- The Board concurred with the assessment of the Agency that the protocol LNX-001 submitted for review by the Board, if revised as suggested in EPA's review, would meet the applicable requirements of 40 CFR 26, subparts K and L. In addition, with the submission of the amended protocol, the Board believed that the protocol meets the applicable requirements of 40 CFR 26, subparts K and L.

ICR Picaridin Mosquito Repellency Protocol ICR 1A 044

- If amended and consistent with the Board's concerns and recommendations, the protocol ICR 1A 044 studying the efficacy of two aerosols formulations of picaridin for repelling mosquitoes would be sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy of these formulations against mosquitoes.

- The Board concurred with the assessment of the Agency that the protocol ICR 1A 044 submitted for review by the Board, if revised as suggested in both EPA's review and by the Board, would meet the applicable requirements of 40 CFR 26, subparts K and L.

Acrolein

- The Board concluded that the Weber-Tschopp et al. study contains information sufficient for assessing human risk resulting from potential acute inhalation exposure. In addition, the study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of acute inhalation exposure to acrolein for the population tested but may not be generalizable to younger or older groups.

- There was not clear and convincing evidence that the conduct of the Weber-Tschopp et al. study was fundamentally unethical. In addition, despite the lack of adequate information to assess the affirmative, most of the HSRB agreed that there was not 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.

4-Amino Pyridine

- The Board concluded that the three clinical studies, Grijalva et al. 2003, Segal et al. 1999, and Van Diemen et al. 1993, were sufficiently sound, from a scientific perspective, to be used to derive a point of departure for estimating risk to humans from exposure to 4-AP. Thus considering the three studies, an estimate of the LOAEL of 0.07 mg/kg/day was determined. The Board was reluctant to endorse the use of a 5 mg/day (0.07 mg/kg-day) LOAEL, given the multiplicity of side effects seen among patients receiving this dose, and the steep dose-response curve of 4-AP. Thus, the Board cautioned that this conclusion comes with a degree of uncertainty and advised the Agency to take such uncertainty into account when using the published information to arrive at a point of departure for 4-AP.

- The Board concurred with the initial assessment of the Agency that for each of these three clinical studies (Segal et al., 1999; Grijalva et al., 2003; and Van Diemen et al., 1993), there was no clear and convincing evidence that the conduct of the study was fundamentally unethical, or that the conduct of the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

AEATF and AHETF Research Programs

- Risks and Benefits of Handler Research

The AHETF and AEATF governing documents have provided the HSRB with a detailed and thoughtful analysis of expected benefits and risks associated with the conduct of human exposure monitoring. The Board recommended that the particular arrangements for providing the test substance be outlined in the individual protocols. Finally, the Board concurred with the AHETF and AEATF that the database developed from these studies will improve the quality of risk assessments, and that they should be considered a valuable societal benefit, provided that the data collected are accurate, or at the least do not underestimate real-world exposures.

The Board recommended that AHETF pay more careful attention to the issue of safety when asking participants to operate equipment with which they do not normally work. In particular, AHETF should be more explicit about the level of competency expected of workers when operating such equipment. The Board commended the AHETF for developing clear guidelines for stopping work based on a heat index. However, the Board concluded that the approach described in the governing documents was not fully protective of workers, and recommended that additional attention be given to this matter, including consideration of a lower heat index threshold for stopping work.

- Addressing Potential Sources of Underestimation Bias

The Board recommended that the validity of dose estimates based on passive dosimetry be reassessed given the variability of previous laboratory data. Given some uncertainty as to exposure to hands, face and neck for various scenarios, the Board concurred with the Agency's intention to evaluate data provided by the task forces for each scenario to determine the relative contribution from skin residues versus whole body dosimeters. The Board recommended that the Task Forces either generate data supporting the efficiency of removing their surrogate pesticides from skin by washing and by wiping, or accept the automatic adjustments being proposed by the Agency; in fact, the adjustment for wipes could even be increased beyond that being proposed for washing. This recommendation is consistent with the advice provided in the 1997 OECD guidance document: "The best that can be achieved for a hand wash or hand rinse method is a laboratory validation of the efficiency of recovery of material from the hands of human volunteers." In addition, the Board suggested a validation study for recovery efficiency of wash or wipe samples could be tested *in vitro* and not require human exposure testing.

The HSRB was comfortable with not including concurrent biomonitoring in the protocols. In fact, the Board recommended that the use of additional monitoring units was more appropriate than the inclusion of biomonitoring in these programs.

- QA/QC Controls

Overall, the Standard Operating Procedures (SOPs) outlining the overall administration, report generation and quality assurance (QA) oversight seems reasonably complete. The Board noted two major areas that should be expanded and/or revised for additional clarity, namely the SOPs that focused on data quality and sample integrity and compliance.

- Design of Scenario-Level Sampling

The Board acknowledged the great complexity of study design development, given the many variables associated with exposure and the practical constraints that arise in the conduct of human exposure studies. The Board commended the Agency and the task forces for their efforts in developing a detailed discussion of sampling strategies. The Board remained concerned that the number of variables is large, and that the relative importance of these variables has not yet been defined adequately. For both the AHETF and AEATF studies, the Board recommended that prior to data collection, the Agency ensure that the critical variables associated with

exposure are ranked, accompanied by an appropriate rationale and justification for this ranking. This ranking would then inform the study design in terms of how sampling sites and individual participants are selected.

The Board recommended that the following information was necessary for it to provide its scientific advice on scenario-specific information:

- Scenario specific information detailing variables that might influence exposure and its effect;
- A feasible sampling strategy including specifics of population to be tested (including its size), a list of relevant variables and how they would be collected and analyzed;
- Information on relationship between scenario-specific exposure assessment and the representative exposure in such scenarios in the target population;
- Essential environmental variables including site description, temperature, humidity, wind levels as well as subjects' external clothing, work history and type of pesticide application;
- Relevant data on inter-subject variability;
- Data analysis plan.

The major limitation of non-random sampling is that it provides no means for estimating the error associated with any estimate based on the sample. The exposure distributions based on this type of sample might or might not be anywhere close to the true exposure distributions and there is no way to tell if the results are representative or not. If the estimated exposure distributions will be used for regulatory purposes it is particularly important to base those estimates on samples that at least approximate a random sample and that permit obtaining data-driven estimates of uncertainty around quantities of interest. Error estimates and other estimates relevant to determination of quantities in the AEATF and AHETF reports are based on strong and un-testable assumptions.

- Statistical Justification of Number of Cluster

Purposive sampling method is used as a surrogate for probability sampling, no additional information seems to be needed for the HSRB to assess the adequacy of the justification for the number of clusters and the number of monitoring units in specific AHETF and AEATF II study proposals (however, as noted previously, the Board raised serious concerns about the purposive sampling strategy).

As the sample size justification for the AEATF II program is based on the ICC estimate from the AHETF program, it is recommended that the AEAFF II program update the proposed sample size based on an analysis of whether the ICC estimates agree with that from the AHETF program.

- Within-Worker Variability

The lack of choosing a within-person design is limiting if the Agency wishes to obtain data on usual exposures. However, if the Agency wishes to collect data on one-day exposures, their approach is not limiting, but the Board recommends that as many handler as possible be observed as costs would allow.

- Subject Recruitment and Enrollment Issues

The Board agreed that the Governing Documents and associated Standard Operating Procedures do include comprehensive and appropriate protections for human subjects.

The Board agreed that the handling of language differences is an area requiring further refinement and is appropriate to protections for human subjects. Related issues include mechanisms to ensure understanding and voluntariness in the consent process.

Sincerely,

Celia B. 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 the mention of trade names or commercial products constitute a recommendation for use. Further information about the EPA Human Studies Review Board can be obtained from its website at http://www.epa.gov/osa/hsrb/. Interested persons are invited to contact Paul Lewis, Designated Federal Officer, via e-mail at lewis.paul@epa.gov.

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

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* Not in attendance at June 27-29, 2007 Public Meeting

** Become HSRB member August 31, 2007

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INTRODUCTION

From June 27-29, 2007, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB) met to address scientific and ethical issues concerning:

A. Proposed Carroll-Loye Picaridin Insect Repellent Efficacy Study LNX-001

EPA requires data from efficacy studies using appropriate insect species to support claims of greater efficacy than have previously been approved.

EPA's regulation, 40 CFR §26.1125, requires the sponsor or investigator to submit to EPA, before conducting a study involving intentional exposure of human subjects, materials describing the proposed human research in order to allow EPA to conduct scientific and ethics reviews. In addition, EPA's regulation, 40 CFR §26.1601, requires EPA to seek HSRB review of the research proposal.

In previous meetings the HSRB has reviewed and commented favorably on several proposed insect repellent efficacy protocols to be conducted by Carroll-Loye Biological Research, submitted by Dr. Scott Carroll. Dr. Carroll has submitted a proposal for new research to evaluate the efficacy of two conditionally registered repellent products containing the active ingredient picaridin. The research protocol, identified as LNX-001, describes a field study of the efficacy of the test formulations against mosquitoes. The proposal bears many similarities to the protocols EMD-004, SCI-001, and WPC-001 that the HSRB has previously reviewed.

EPA has reviewed Dr. Carroll's protocol and has concluded that, with some required refinements, it appears likely to generate scientifically sound, useful information and to meet the applicable provisions of the EPA regulations in 40 CFR part 26, subparts K and L. The sponsor wishes to submit the data to EPA later this year to satisfy the requirement to provide efficacy data imposed when it received a conditional registration for picaridin. In the interest of providing a thorough and timely response to the proposal, and because EPA finds the protocol generally meets applicable scientific and ethical standards, EPA is presented this protocol for review at the Board's June 2007 meeting.

B. Proposed ICR Picaridin Insect Repellent Efficacy Study

EPA requires data from efficacy studies with human subjects to support claims of efficacy of a new pesticide product intended to repel insects that transmit human diseases.

EPA's regulation, 40 CFR §26.1125, requires the sponsor or investigator to submit to EPA, before conducting a study involving intentional exposure of human subjects, materials describing the proposed human research in order to allow EPA to conduct scientific and ethics reviews. In addition, EPA's regulation, 40 CFR §26.1601, requires EPA to seek HSRB review of the research proposal.

Dr. Niketas Spero has submitted a proposal for new research to evaluate the efficacy of two new formulations of a skin-applied repellent product containing picaridin, to be conducted by Insect Control & Research, Inc. (ICR). The research protocol, identified by Protocol ID G0590307001A044, describes a field study of the efficacy of the test formulations against mosquitoes.

EPA has reviewed ICR's protocol and has concluded that, with a number of required revisions, it appears likely to generate scientifically sound, useful information and to meet the applicable provisions of the EPA regulations in 40 CFR part 26, subparts K and L. The sponsor wishes to submit the data to EPA later this year in support of an application to register one or more new picaridin products. In the interest of providing a thorough and timely response to the proposal, and because EPA finds the protocol can meet applicable scientific and ethical standards, EPA is presented this protocol for review at the Board's June 2007 meeting.

C. Completed Inhalation Study with Acrolein

In its reregistration program EPA reexamines the safety of previously registered pesticides. The Agency is currently reviewing pesticides containing the active ingredient acrolein. Acrolein is registered for use as a biocide in agricultural and industrial water supply systems. It is also formed as a byproduct in various industrial processes and is a component of cigarette smoke.

In a review of the published scientific literature, EPA identified a study published in German in 1977 in which human subjects were exposed to acrolein for various durations and at varying concentrations in an inhalation chamber. Researchers collected data on subjective irritation sensations and on eye-blink and respiratory rates. The Agency intends to use the results of this study in its hazard assessment to derive a "point of departure" (POD) for assessing acute toxicity resulting from acute exposure to this chemical.

The Agency's regulation, 40 CFR §26.1602, requires EPA to seek HSRB review of an EPA decision to rely on the results of any study if the research was "initiated before April 7, 2006, and the research was conducted for the purpose of identifying or measuring a toxic effect." EPA has reviewed the study, applying the standards in 40 CFR §§26.1703 and 26.1704. Those provisions state:

> **§26.1703 Prohibition of reliance on research involving intentional exposure of human subjects who are pregnant women (and therefore their fetuses), nursing women, or children.**
>
> Except as provided in §26.1706, in actions within the scope of §26.1701 EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.
>
> **§26.1704 Prohibition on reliance on unethical research with non-pregnant, non-nursing adults conducted before April 7, 2006**

Except as provided in §26.1706, in actions within the scope of §26.1701, EPA shall not rely on data from any research initiated before April 7, 2006, if there is 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), or was significantly deficient relative to the ethical standards prevailing at the time the research was conducted. This prohibition is in addition to the prohibition in §26.1703.

The Agency's reviews concluded that the data were scientifically sound and that there was no clear and convincing evidence that the conduct of the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time the research was conducted. Nor was there evidence to show that the subjects included nursing or pregnant women or children.

D. Completed Studies on the Therapeutic and non-Therapeutic Effects of Administration of 4-aminopyridine

In its reregistration program EPA reexamines the safety of previously registered pesticides. The Agency is currently reviewing pesticides containing the active ingredient 4-aminopyridine (4-AP). 4-AP is registered by EPA as a bird repellent under the name Avitrol. It has also been investigated as a drug to treat various neurological diseases, and was recently approved for the treatment of chronic functional motor and sensory deficits resulting from Guillain-Barré syndrome.

In a review of the published scientific literature EPA identified three studies in which human subjects were exposed to 4-AP to evaluate whether it alleviated neurological symptom in patients with either spinal cord injury or multiple sclerosis. These clinical trials also report on the non-therapeutic effects of 4-AP. The Agency intends to use the results of these studies to derive a point of departure for assessing the risks to humans resulting from all potential durations of exposure–acute, short term, intermediate or subchronic, and chronic exposure.

The Agency's regulation, 40 CFR §26.1602, requires EPA to seek HSRB review of an EPA decision to rely on the results of any study if the research was "initiated before April 7, 2006, and the research was conducted for the purpose of identifying or measuring a toxic effect." EPA has concluded that the three studies with 4-AP are subject to HSRB review under 40 CFR §26.1602. The Agency reviewed the studies, applying the standards in 40 CFR §§26.1703 and 26.1704. Those provisions state:

§26.1703 Prohibition of reliance on research involving intentional exposure of human subjects who are pregnant women (and therefore their fetuses), nursing women, or children.

Except as provided in §26.1706, in actions within the scope of §26.1701 EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

§26.1704 Prohibition on reliance on unethical research with non-pregnant, non-nursing adults conducted before April 7, 2006

Except as provided in §26.1706, in actions within the scope of §26.1701, EPA shall not rely on data from any research initiated before April 7, 2006, if there is 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), or was significantly deficient relative to the ethical standards prevailing at the time the research was conducted. This prohibition is in addition to the prohibition in §26.1703.

The Agency's reviews concluded that the data were scientifically sound and that there was no clear and convincing evidence that the conduct of any of the research was fundamentally unethical or was significantly deficient relative to the ethical standards prevailing at the time the research was conducted. None of the studies included as subjects nursing or pregnant women or children.

E. Background Materials Relating to the Design of Research on the Levels of Exposure Received by Pesticide Handlers

Under FIFRA, EPA requires that all pesticide products must be "registered" before they may be sold or distributed in commerce. The applicant for registration has the burden of demonstrating that its pesticide will not cause "unreasonable adverse effects on the environment." Among other potential risks, EPA requires applicants to provide information that allows EPA to assess the potential for adverse effects on people who mix, load, or apply a pesticide (referred to as pesticide "handlers.") Accurately characterizing handlers' potential exposure is essential to EPA's risk assessment and regulatory decision-making.

EPA currently relies on a collection of exposure studies mostly contained in the Pesticide Handlers Exposure Database (PHED) to develop estimates of handlers' potential exposure. When dealing with pesticide that have low volatility, EPA assumes that, if field data are corrected for chemical-specific losses under field conditions, the amount of exposure a handler receives is independent of the chemical composition of the pesticide he is using, and that his exposure depends on the amount of active ingredient handled, as well as the particular activity, the particular type of pesticide formulation and the particular type of equipment used. The Agency uses the PHED data to develop estimates of "unit exposures" – expressed as an amount of exposure per amount of active ingredient handled – for specific scenarios. (A scenario is defined by the activity, formulation, and equipment, e.g. applying a liquid formulation by using airblast equipment in an open cab.) Using this information, EPA estimates handlers' potential exposures for each use of a pesticide and compares those levels with toxicity data. If the comparisons show potential exposure is acceptably low, EPA concludes there is no risk to handlers. If, however, the comparisons show that in some scenarios a handler may receive unacceptably high exposure, EPA takes actions to mitigate the risk. The range of possible actions to reduce handlers' exposure includes requiring the use of personal protective equipment, reduced application rates, changes in formulation, use of specific types of application equipment or engineering controls, or prohibition of the use pattern.

The data currently used to estimate handlers' potential exposure has a number of limitations. The Agency believes that data from new handler exposure studies would provide a much sounder basis for estimating potential exposure. In particular, new data should provide a basis for characterizing the distribution of unit exposures across the population of handlers performing activities in each scenario. Two industry groups have arisen to undertake the research necessary to develop new databases – the Agricultural Handlers Exposure Task Force (AHETF) and the Antimicrobials Exposure Assessment Task Force II (AEATF). The AHETF is focusing on studies that relate to the use of pesticides in agriculture, and the AEATF will characterize exposures received by people while handling antimicrobial pesticides, e.g., disinfectants, materials preservatives, etc.

Both Task Forces would like to initiate research soon – the AEATF during the winter of 2007–2008, and the AHETF during the pesticide use season in 2008. At least some Task Force studies would involve intentional exposure of a human subject. EPA's regulation, 40 CFR §26.1125, requires the sponsor or investigator to submit to EPA, before conducting research involving intentional exposure of a human subject, materials describing the proposed human study in order to allow EPA to conduct scientific and ethics reviews. In addition, EPA's regulation, 40 CFR §26.1601, requires EPA to seek HSRB review of the research proposal.

The HSRB has considered the prospect of new handler research at two previous meetings. In June 2006 the Board reviewed five proposed protocols developed by the AHETF. The Board raised questions and made numerous comments on both scientific and ethical aspects of the proposals.

Over the past year EPA and the Task Forces have worked hard to address the issues identified by the HSRB. In response to scientific concerns raised by the HSRB, EPA analyzed the existing handler exposure database and relevant scientific literature, and presented its analysis to the FIFRA Scientific Advisory Panel (SAP) in January 2007. The Agency asked the SAP to comment on, among other topics, the "limitations [of existing data] and on EPA's conclusion that additional data could improve significantly EPA's ability to estimate worker exposure." The SAP report was released April 2, 2007, and is available at: http://www.epa.gov/scipoly/sap/meetings/2007/january/january2007finalmeetingminutes.pdf . At its April meeting the HSRB received a copy of the SAP report and a presentation by two members of the Panel that prepared the report.

In addition, for the April 2007 HSRB meeting EPA prepared a draft document identifying the major elements of the recruitment and enrollment processes that should be considered by investigators as they prepare protocols for handler exposure research. The document discussed broad principles which should be considered in the course of research design. In the future, through a participatory process involving investigators, workers, and other stakeholders EPA intends to add to the document specific best practices, and to identify publicly available resources that contain additional discussion, information, and guidance relevant to the implementation of general ethical principles in occupational exposure research. The draft document is available at: http://www.epa.gov/osa/hsrb/files/meeting-materials/apr-18-20-2007-public-meeting/DraftFrameworkForDevelopingBest-Practices0315007.pdf

Both the AHETF and AEATF have prepared extensive materials explaining and justifying their proposed research, and have revised these materials in response to EPA comments. These materials provided to the HSRB for discussion at its June 2007 meeting, generally explain the scope of the proposed research programs and describe the general framework for conducting the research. In addition, each Task Force has provided Standard Operating Procedures which will guide the conduct of the studies. These materials provide essential background information to support the Board's evaluations of Task Force protocols and related materials at subsequent meetings. Because EPA regards the proposed studies as "research involving intentional exposure of human subjects," EPA regulations require the Agency and the Board to review these proposals before the investigators initiate the studies.

This report transmits the HSRB's comments and recommendations from its June 27-29, 2007 meeting.

REVIEW PROCESS

From June 27-29, 2007, 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 (72 Federal Register 108, 31323). At the public meeting, following welcoming remarks from Agency officials the Board then heard presentations from the Agency on the following topics:

- A research proposal from Carroll-Loye Biological Research to evaluate the efficacy of two conditionally registered products containing picaridin in repelling mosquitoes in the field.

- A research proposal from Insect Control & Research, Inc. to evaluate the efficacy of two unregistered products containing picaridin in repelling mosquitoes in the field.

- A completed study measuring the effects on human subjects of acute inhalation exposure to acrolein.

- Three completed studies of the efficacy and side effects of 4-aminopyridine used as a therapeutic agent.

- Extensive background materials concerning research to quantify the level of exposure received by people who mix, load, and apply pesticides. These materials were prepared by the Agricultural Handlers Exposure Task Force and by the Antimicrobial Exposure Assessment Task Force.

The following oral comments were presented at the meeting:

(1) Scott Carroll, Ph.D. representing Carroll-Loye Biological Research and Ghona Sangha, Ph.D. representing LANXESS Corporation addressing the proposed Carroll-Loye picaridin insect repellent efficacy study LNX-001;

(2) Mr. Niketas Spero and Robin Todd, Ph.D. representing ICR, Inc. addressing the ICR picaridin mosquito repellency protocol;
(3) Richard Collier, Ph.D. of Landis International representing the Agricultural Handlers Task Force, John Ross, Ph.D. of infoscientific.com, representing the Agricultural Handlers Task Force, Ray McAllister of CropLife America, Inc., and Larry Holden, Ph.D. of Sielken and Associates Consulting, Inc. addressing the design of research on the levels of exposure received by pesticide handlers.

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 protocols and Agency evaluation of the protocol).

CHARGE TO THE BOARD AND BOARD RESPONSE

Proposed Carroll-Loye Picaridin Insect Repellent Efficacy Study LNX-001

Charge to the Board

If the proposed research described in Protocol LNX-001 from Carroll-Loye Biological Research is revised as suggested in EPA's review, does the research appear likely to generate scientifically reliable data, useful for assessing the efficacy of the test substances for repelling mosquitoes?

Board Response

The active ingredient picaridin in two formulations will be tested in the field by the Carroll-Loye company for picaridin's ability to repel mosquitoes. Picaridin is also known as Icaridin and KBR 3023. Picaridin has a history of use as an insect repellent principally outside the US. The active ingredient will be formulated into a 20% pump spray and into a 20% cream. All experiments will be conducted using Good Laboratory Practices. A dosimetry experiment with 10 individuals will be performed to determine the amount of product that would be utilized by people using the product as directed.

The experiment will be a field study. Two locations in California could be used, either in the Central Valley or in southern California. A mixture of *Culex* and *Aedes* species will be present at these sites.

Legs and/or arms will be tested. There will be two experienced persons serving as negative controls (i.e., without any repellent product) to confirm mosquito biting pressure. Experimental subjects, in pairs, will monitor landings with intent to bite (LIBe's) during a one minute interval each 15 minutes, until the First Confirmed LIBe (FCLIBe) can be determined. Stopping rules will be employed. The Complete Protection Time (CPT) will be determined, expressed as mean and standard deviation plus 95% confidence interval, if data are normally distributed, and methods are described to assess normality.

With respect to the science criteria established earlier by the HSRB, the following assessments are made:

General HSRB Scientific Criteria

- The scientific question was stated (i.e., to test the efficacy of picaridin formulated as either a pump spray or a cream in repelling mosquitoes).
- Existing data were not adequate to answer the question of efficacy of these new formulations.
- Because existing data were not adequate to answer the question of efficacy, new studies involving human subjects are necessary.
- The potential benefits of the study are 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 is likely that the benefits would be realized because repellent efficacy will be determined in carefully designed field experiments.
- The risks are minimal because the active ingredient is of very low toxicity, the other formulation ingredients are of very low toxicity, the mosquitoes will be aspirated before they have an opportunity to bite, and the regions selected will not have evidence of West Nile Virus.
- The most likely relevant risk would be irritation from mosquito bites, but participants will be instructed to remove mosquitoes before they are bitten, or the possibility of infection with West Nile Virus, but the regions selected will have no evidence of the virus. Serology tests will be performed on captured mosquitoes.

Study Design Criteria

- The purpose of the study is clearly defined (i.e., efficacy testing).
- There are specific objectives (i.e., to determine the Complete Protection Time that picaridin in two formulations displays as a mosquito repellent).
- There was a formally stated hypothesis; however, it is broad and untestable. This does not detract from the value of the study because a hypothesis is not really necessary for an efficacy study such as this.
- The sample size will be 10 individuals per product along with 2 experienced individuals to confirm mosquito biting pressure. A dosimetry experiment prior to the field experiment will quantify the amount of repellent being used.
- There is a plan allocating individuals to treatments.
- It is anticipated that the findings from this study can be generalized beyond the study sample.

Participation Criteria

- There is justification for the selection of the target population (i.e., selection primarily or completely from the existing Volunteer Data Base, comprised of individuals previously participating in similar studies or interested in doing so, who routinely are active outdoors, and are routinely exposed to mosquitoes).
- The participants will be representative of some of the population of concern. However, there are others in the population unlike these participants who are likely to use these products, but it would either be unethical to test them or would be less appropriate to test

them. The participating population, while not completely representative, is considered appropriate and reasonable.
- The inclusion/exclusion criteria are appropriate.
- The sample will not be a vulnerable group.

Measurement Criteria
- The measurements will be accurate and reliable as defined. The endpoint will be the First Confirmed Landing with Intent to Bite (FCLIBe). While this was viewed as an appropriate endpoint by many of the Board members, there was some concern that the confirming LIBe criterion was not a sufficiently protective/conservative, and that the first unconfirmed LIBE should be the endpoint.
- The measurements will be appropriate to the question being asked.
- Quality assurance will be a part of the experimental plan.

Statistical Analysis Criteria
- The data were designed to be analyzed to calculate Complete Protection Time with a range of variability. There was concern that specific criteria from the standpoint of statistics for the selection of 10 subjects were not provided or available. There was also concern about the handling of censored data.
- Measures of uncertainty were addressed.

Laboratory and Field Conditions
- Laboratory experiments are not proposed, except for the dosimetry
- Field experiments will be appropriate.
- The study will include a stop rule plan, medical management plan, and a safety monitor.

EPA's science analysis identified a deficiency in the lack of a stated hypothesis, and one was subsequently added. However, the objective of this study, i.e, length of time of efficacy in repelling mosquitoes in the field, is clear and the lack of a formally stated hypothesis, or a vague and broad hypothesis, does not detract from the scientific value of the study. EPA also identified a deficiency in a lack of an explanation for the negative control in the dosimetry experiment; however, it is a necessary control to determine whether any factors besides the formulated product (e.g., sweat) might alter the weight of the dosimeters. EPA also noted that the method of measuring the treatment area was not described. The Board concurred with this deficiency and urged that it be addressed. There were also two statistical deficiencies identified, and the Board urged greater consideration of statistical issues with respect to determination of sample size and analysis of the data.

HSRB Consensus and Rationale

The protocol LNX-001 to study the efficacy of a cream formulation and a pump spray formulation of picaridin for repelling mosquitoes is sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy of these formulations against mosquitoes.

Charge to the Board

b. If the proposed research described in Protocol LNX-001 from Carroll-Loye Biological Research is revised as suggested in EPA's review, does the research appear to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board Response

Background on Study

The proposed study would evaluate the efficacy of two different skin-applied formulations of an already registered and marketed insect repellent, Icaridin (registered by the Agency as Picaridin). Icaridin is also known under the registered trade name Bayrepel™ and marketed under the brand name Autan.

The research is to be conducted by Carroll-Loye Biological Research, a private laboratory in Davis, CA. The sponsor of this study is LANXESS, Inc. of Pittsburg, PA. The submitted documents assert that the study will be conducted in accordance with the ethical and regulatory standards of 40 CFR 26, Subparts K and L, as well as the requirements of FIFRA §12(a)(2)(P), the U.S. EPA's Good Laboratory Practice (GLP) Standards described at 40 CFR 160, and the California State EPA Department of Pesticide Regulation study monitoring (California Code of Regulations Title 3, Section 6710). Finally, the protocol was reviewed and approved by an independent human subjects review committee, Independent Investigational Review Board (IIRB), Inc., of Plantation, FL prior to submission to the Agency.

The revised research protocol submitted consists of two interdependent studies: 1) a dosimetry study, performed under controlled laboratory conditions, designed to determine the amount of an insect-repelling compound, known as KBR 3023 (picaridin; Icaridin), that normal subjects would typically apply when provided with one of two compound formulations (lotion or pump-spray); and 2) an efficacy study, performed at field sites in Central California and/or Southern California, designed to measure the effectiveness of 20% KBR 3023 (Picaridin; Icaridin), as a mosquito repellent. Dosimetry will be determined either by passive dosimetry using self-adhesive roll-gauze (spray and aerosol formulations) or by direct measurement of compound application (lotion formulation). The efficacy of 20% KBR 3023 (picaridin; Icaridin) as a mosquito repellent will be determined by measuring the ability of the two formulations to prevent mosquito landings (defined as "Lite with Intent to Bite"; LIBe) under field conditions. Mosquitoes will be aspirated mechanically after landing but prior to biting; prior to initiation of the efficacy study, all volunteers will be trained, using laboratory-raised, pathogen-free mosquitoes in a controlled laboratory setting, both to recognize a mosquito landing with the intent to bite (LIBe) and to remove such mosquitoes with an aspirator. The strengths and weaknesses of each study design are described above.

The dosimetry study will enroll 10 healthy volunteers, each of whom will apply both formulations. These same subjects may or may not participate in the efficacy study. The efficacy study will be conducted at two field sites located in Central and/or Southern California, depending on the season. A total of twenty study participants will take place in the two field trials; ten volunteers will test the lotion formation and ten will test the pump spray formulation. For each field trial, two additional untreated control subjects (experienced field-workers or

frequent participants of Carroll-Loye-conducted repellency studies) will be enrolled to determine ambient LIBe pressure under field conditions; such measurements are necessary to determine 20% KBR 3023 (picaridin; Icaridin) efficacy as a mosquito repellent. Each control subject may or may not participate in both field trials, thus a total of two to four control subjects may be enrolled. The test compounds would be administered to a standardized skin surface area, with a comparison to the two control participants. Each untreated subject will be attended by two assistants who will aspirate mosquitoes prior to biting, thus minimizing risk of exposure to vector-borne illnesses. In addition, three alternate subjects will be enrolled to: 1) replace any subject who withdraws from participating; and 2) protect the confidentiality of any subject excluded from the study as a result of pregnancy or other potentially stigmatizing condition, as described below. The number of participants enrolled in this study thus will total a minimum of 25 volunteers and a maximum of 37 volunteers, a number that appears to be adequately justified (Carroll 2007a; Carroll 2007b).

Ethics and Regulatory Compliance Review

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in the EPA's initial Science and Ethics Review, dated May 24, 2007 (Carley and Sweeney 2007a). With the provision of an amended protocol on June 14, 2007 (Carroll 2007b), the proposed research described in Protocol LNX-001 comports with the applicable ethical and regulatory requirements of 40 CFR 26, subparts K and L.

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. As submitted to the Agency, the amended protocol (Carroll 2007a; Carroll 2007b) meets the regulatory requirements of 40 CFR § 26.11159 . For example, the original and amended protocols were reviewed and approved by IIRB. Documentation previously provided to the EPA by IIRB indicates 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.

With respect to study design, the risks to participants are minimal and justified by the likely societal benefits, including data on the efficacy of 20% KBR 3023 (picaridin; Icaridin) as a mosquito repellent. The nature and likelihood of any side effects or adverse events are described clearly in the informed consent documents (with separate documents outlining the risks for treated volunteers and experienced, untreated controls). The risks to study participants are three-fold: 1) allergic reaction to test materials themselves; 2) exposure to biting arthropods; and 3) possible exposure to arthropod-borne diseases.

Reasonable attempts have been taken to minimize any potential harm, and plans for the medical management of any side effects or adverse events have been developed. Although 20% KBR 3023 (picaridin; Icaridin) is not currently used as an insect repellent in the United States, for example, repellent formulations containing 20% KBR 3023 are commercially available in Europe and Australia, and have been used for years with little evidence of toxic effects. Laboratory analyses, as summarized by Dr. Ghoma Sangha of LANXESS at the public meeting of the HSRB, also suggest that participants enrolled in this study are unlikely to be at increased risk of experiencing adverse side effects upon exposure to the test materials.

Reactions to mosquito bites are usually mild and easily treated with over-the-counter steroidal creams. Excluding subjects who have a history of such severe skin reactions will minimize the risk of a subject experiencing a severe physical reaction to a mosquito bite. In addition, the study protocol is designed specifically to minimize the likelihood that a mosquito will bite, through the use of clear stopping rules, limited exposure periods, pre-bite aspiration and joint observation.

To minimize the risk that study subjects will be exposed to illnesses such as West Nile Virus, field tests of repellent efficacy will be conducted only in areas where known vector-borne diseases have not been detected by county and state health or vector/mosquito control agencies for at least one month. Finally, mosquitoes collected while attempting to bite control and treated subjects during the field tests will be subjected to multiplex RT-PCR assays for several known arthropod-borne diseases—including West Nile Virus, Western Equine Encephalitis Virus, and St. Louis Encephalitis Virus—with clear plans to contact study participants and alert them if a transmissible pathogen is detected.

In accordance with the provisions in the EPA's final human studies rule (40 CFR §§ 26.1701-1704), 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. The use of so-called "alternate" subjects ensures that the results of over-the-counter pregnancy tests would be kept private; that study participants may be designated as alternate subjects and automatically excluded from participation allows for potentially pregnant volunteers to withdraw without compromising their confidentiality.

Finally, the study protocol also included several mechanisms designed to minimize coercive subject recruitment and enrollment. For instance, 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. The majority of research participants will be recruited from the University's student population, including from Dr. Carroll's own department, but the protocol specifically excludes any student or employee of the Study Director and includes a substantial waiting period between recruitment and study enrollment and an interview by Dr. Carroll designed to minimize coercive subject recruitment and enrollment. In addition, compensation for study participation is not so high as to unduly influence enrollment. It is important to note, however, that the planned use of a convenience sample of study participants may limit the broad applicability of the study results to the general population; this fact is noted by the study investigators in the protocol.

HSRB Consensus and Rationale

The Board concurred with the assessment of the Agency that the protocol LNX-001 submitted for review by the Board, if revised as suggested in EPA's review, meets the applicable requirements of 40 CFR 26, subparts K and L. In addition, with the submission of the amended protocol, the Board believed that the protocol meets the applicable requirements of 40 CFR 26, subparts K and L.

Proposed ICR Picaridin Insect Repellent Efficacy Study

Charge to the Board

If the proposed research described in ICR's proposed picaridin protocol is revised as suggested in EPA's review, does the research appear likely to generate scientifically reliable data, useful for assessing the efficacy of the test substances for repelling mosquitoes?

Board Response

This protocol was submitted with Confidential Business Information (CBI) redacted. The name of the sponsor was withheld as was the concentration of the active ingredient, picaridin. A workgroup of the HSRB had decided prior to the HSRB review that the information being withheld was not critical to the scientific review of the protocol.

The protocol was clearly written and described a field study to be conducted on two picaridin aerosol formulations at two locations. The locations, selected to achieve different species composition of mosquitoes, would be: Site 1: Savannah-Ogeechee Canal Museum and Nature Center, Savannah, GA, and Site 2: Pine Island, Lee County Mosquito Abatement District, FL. The testing would last up to 14 hours on any particular test day.

There is an adequate pool of test subjects from individuals previously tested for repellent efficacy from which the specific subject pool will be selected. Fourteen individuals will be selected for the conduct of the protocol at each site, 10 test subjects, 2 alternates, and 2 untreated controls. The treated subjects will have one test formulation on one arm and the other on the other arm. A standard amount, as indicated in EPA test guidelines, will be applied to the subjects' arms; no dosimetry study will be conducted to determine likely consumer use rate. The treated skin will be exposed to potential mosquito bites for 5 minutes out of each 30 minutes. During the remaining 25 minutes of each 30 minute interval, the subjects will be in a screened area that is impervious to mosquitoes. The treated area of skin will not be covered during these 25 minute intervals to avoid any rubbing off of the product. The endpoint will be time to First Confirmed Bite (FCB), monitoring bites from only those mosquitoes which have all 6 legs on the skin. Two negative controls will be selected randomly; these individuals are not anticipated to receive bites. No positive control will be conducted. The ICR staff will record the data.

There was concern raised by the Board because of the lack of a dosimetry study. The Board understands that a standardized amount of the repellent is the guideline. However, the Board considered a dosimetry experiment as a valuable addition to the protocol and recommended that it be included so that the most likely amount of product applied by the consumer will be tested.

The Board recommended that a sample of mosquitoes be taken from those biting the participants so that subsequent serology for pathogenic viruses can be performed; this information can be provided to the participants to assist them in medical management if there is any evidence of diseases present in the mosquitoes.

The Board also had concerns about the use of the First Confirmed Bite as the endpoint. The Board understands that the FCB is at present the guideline endpoint, and appreciates that it may be the more rigorous criterion for efficacy. However, the Board has been impressed with the added safety to participants of the Landing with Intent to Bite (LIBe) endpoint on some of the previously reviewed protocols, even though this endpoint is not that stated in the current guidelines. There is insufficient information to judge how reflective the LIBe is of the bite. The Board is also cognizant of the possibility of a lack of consistency in the labels if new criteria are introduced, and the resultant confusion and/or unfairness to consumers who wish to compare products. However, balancing these issues (consistency of endpoints, safety to test subjects, and accuracy of the results) needs to be a priority item for EPA; thus the Board strongly urged EPA to confirm or update its guidelines with the best possible balance of safety, consistency with past studies, and accuracy of the results (including statistical validity).

With respect to the science criteria established earlier by the HSRB, the following assessments are made:

General HSRB Scientific Criteria

- The scientific question was stated (i.e., to test the efficacy of picaridin formulated as one of two aerosols in repelling mosquitoes).
- Existing data were not adequate to answer the question of efficacy of these new formulations.
- Because existing data were not adequate to answer the question of efficacy, new studies involving human subjects are necessary.
- The potential benefits of the study are 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 is likely that the benefits would be realized because repellent efficacy will be determined in carefully designed field experiments.
- The risks are low because the active ingredient is of very low toxicity, and the regions selected will not have evidence of West Nile Virus during the previous week, although this one week interval may not be sufficient protection. Because the protocol specifies that the test subjects must be bitten, there is the possibility of infection from virus-bearing mosquitoes in the repellent-treated subjects.
- The most likely relevant risk would be irritation from mosquito bites, or the possibility of infection with West Nile Virus or other mosquito-borne diseases, but the regions selected

will have no evidence of the West Nile Virus during the week prior to the test. A more intensive medical management plan should be described.

Study Design Criteria

- The purpose of the study is clearly defined (i.e., efficacy testing).
- There are specific objectives (i.e., to determine the protection time that picaridin in two formulations displays as a mosquito repellent).
- There was no formally stated hypothesis; however, this does not detract from the value of the study.
- The sample size will be 10 individuals (plus selection of 2 alternates) along with 2 experienced individuals to confirm mosquito biting pressure. A dosimetry experiment prior to the field experiment will not be performed, but the standardized amount in the guidelines will be used. The Board recommended that a dosimetry test be performed to quantify the amount of repellent a consumer would apply, and that this be used in the field tests.
- There is a plan allocating individuals to treatments.
- It is anticipated that the findings from this study can be generalized beyond the study sample.

Participation Criteria:

- There is justification for the selection of the target population (i.e., selection primarily or completely from the existing Volunteer Data Base, comprised of individuals previously participating in similar studies).
- The participants will be representative of some of the population of concern; however, there are others in the population unlike these participants who are likely to use these products, but it would either be unethical to test them or would be less appropriate to test them. Even though the participant group is not totally representative, the participating population is considered appropriate and reasonable.
- The inclusion/exclusion criteria are appropriate, except that individuals over the age of 55 should not be participants.
- The sample will not be a vulnerable group.

Measurement Criteria

- The measurements will be accurate and reliable as defined. The endpoint will be the First Confirmed Bite (FCB), which is the endpoint in the testing guidelines. While this would be consistent with the tests that have been conducted in the past, there was some concern that the bite would place the participants at greater risk than necessary because of the possibility of contracting a mosquito-borne disease.
- The measurements will be appropriate to the question being asked, with the possible exception of the exclusion of bites from mosquitoes which do not have all 6 legs on the skin.

Statistical Analysis Criteria

- The data were designed to be analyzed to calculate mean time to FCB, plus standard deviation and 95% confidence interval. There was concern that specific criteria from the standpoint of statistics for the selection of 10 subjects were not provided or available.

- Information on determining the normality of data was not included.
- The originally-suggested exclusion of "outliers" should be abandoned and the data from all participants should be included.
- Measures of uncertainty were addressed, but need to be considered in relationship to whether the data are normally distributed or not.

Laboratory and Field Conditions

- Laboratory experiments are not being proposed.
- Field experiments will be appropriate.

EPA's science analysis identified a deficiency in the lack of a stated hypothesis, and there was a promise to add a hypothesis about a test of a specified length of repellent efficacy; however, the objective of this study, i.e., determination of the length of efficacy in repelling mosquitoes in the field, is clear and the lack of a formally stated hypothesis does not detract from the scientific value of the study. In reality, for this type of study, it does not matter whether there is a hypothesis of a specific time or not because the study's data will indicate what the length of efficacy is for that particular product, which is what the EPA needs for its regulatory purposes. EPA also identified a deficiency in a lack of information about determination of the normality of the acquired data, and the handling on non-normally distributed data (should this be the case), and the Board concurred that such information is needed. The Board concurred with EPA regarding the necessity for addressing the identified deficiencies in statistical approaches and Good Laboratory Practices.

HSRB Consensus and Rationale

If amended and consistent with the Board's concerns and recommendations, the protocol ICR 1A 044 studying the efficacy of two aerosols formulations of picaridin for repelling mosquitoes would be sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy of these formulations against mosquitoes.

Charge to the Board

If the proposed research described in ICR's proposed picaridin protocol is revised as suggested in EPA's review, does the research appear to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board Response

Background on the Study

The proposed study (Spero 2007) would evaluate the efficacy of two different skin-applied formulations of picaridin-based insect repellents. Picaridin is already registered and marketed as an insect repellent in the United States, under the registered trade name Bayrepel™ and marketed under the brand name Autan. The goal of this study is to evaluate the efficacy of two formulations under field conditions.

The research is to be conducted by ICR, Inc., a commercial organization based in Catonsville, Maryland; ICR provides testing and regulatory consulting services for companies developing and marketing pesticides and insecticides in the United States and Canada. The study is managed by toXcel, LLC of Gainesville, Virginia. The sponsor of this study is unknown; because of claims of CBI, the documents for this study were provided to the HSRB in redacted-form, with neither the sponsor's identity nor the exact composition of the two test formulations provided. The submitted documents assert that the study will be conducted in accordance with the ethical and regulatory standards of 40 CFR 26, Subparts K and L, as well as the requirements of FIFRA §12(a)(2)(P), and the U.S. EPA's Good Laboratory Practice (GLP) Standards described at 40 CFR 160. Finally, the protocol was reviewed and approved by an independent human subjects review committee, Essex Investigational Review Board (EIRB), Inc., of Lebanon, NJ prior to submission to the Agency.

Efficacy of the two picaridin-based formulations will be evaluated under field conditions by using healthy volunteers. The study will be performed at two field sites in Georgia (Savannah-Ogeechee Canal Museum and Nature Center) and Florida (Pine Island, Lee County). The effectiveness of the two compounds as mosquito repellents will be determined by measuring the ability of each formulation to prevent mosquito bites under field conditions. The strengths and weaknesses of the study design are described above.

The efficacy study will enroll a total of up to 28 subjects. 14 subjects will participate in field tests in Georgia and 14 in Florida. Of the 14 participants at each site, twelve will be treated and test the effectiveness of the two Picaridin-based repellent formulations. The study protocol justifies the enrollment of twelve treated participants at each field site, with ten volunteers needed to obtain statistical validity and an additional two participants enrolled as alternates (Spero 2007). The compounds will be applied to 250 cm^2 patches of skin on the forearms of each study participant; one compound will be applied to the right forearm and one to the left forearm, with the effectiveness of each formulation simultaneously evaluated. Treated skin will be exposed for five minutes at half-hour intervals, with repellency of each formulation ascertained by measuring the time from application to "breakdown" of repellency. "Breakdown" is defined in the protocol as either two bites in a single five-minute exposure period, or one bite in each of two consecutive exposure periods. Treated study participants will work in pairs, observing mosquito landings and alerting attendant ICR staff of potential bites; ICR staff will determine whether to count an event as a bite. Probes (i.e., "bites" where the mosquito punctures the skin but does not collect blood) and bites from mosquitoes that do not fully alight (i.e., all six legs on the surface of the exposed skin) will not be counted as bites. Once breakdown has occurred for a particular repellent formulation, no further exposure of the subject's treated skin will occur.

Two participants at each site, chosen by lottery, will remain untreated and will be monitored to determine ambient mosquito biting pressure under field conditions. A 250 cm^2 patch of untreated skin will be exposed for five minutes at half-hour intervals, with the ambient biting pressure determined by counting the mosquitoes landing on the skin. A minimum rate of 1 and a maximum rate of 10 landings per minute is necessary for the field trial to be conducted. Landing mosquitoes will be brushed away by attendant ICR staff, and a small number will be collected for later laboratory identification.

Ethics and Regulatory Compliance Review

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in the EPA's Science and Ethics Review, dated May 24, 2007 (Carley and Sweeney 2007b). If the recommended changes included therein are incorporated, the proposed research described in Protocol ICR 1A 044 is likely to meet the ethical and regulatory requirements of 40 CFR 26, subparts K and L. As submitted to the Agency, however, the current protocol (Spero 2007) fails to meet the applicable requirements of 40 CFR 26, subparts K and L.

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 documents 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.

The supporting documentation provided by EIRB and submitted to the Agency appears to meet the regulatory requirements of 40 CFR 26.1115a and 40 CFR 26.1125. A description of EIRB procedures was provided to the EPA with a claim of confidentiality, so was not available for review by the HSRB. Agency staff, however, reviewed the documentation provided by EIRB and determined these procedures and policies to be in compliance with the applicable standards of the Common Rule (45 CFR 46, Subpart A). The minutes from the meetings at which Protocol ICR 1A 044 was discussed, however, provide minimal information and are inadequate in that there is no substantial discussion of the ethical issues such a study design would be expected to raise. The minutes are little more than a list of editorial changes to the protocol and consent form while noting that there were "no controverted issues" raised by any EIRB member present (Spero 2007). Nevertheless, the protocol as submitted to the Agency is substantially compliant with the regulatory requirements of review and documentation in 40 CFR § 26.1125, minor deficiencies notwithstanding.

With respect to study design, the risks to participants (if properly minimized) are justified by the likely societal benefits, including data on the efficacy of these new picaridin-based formulations of mosquito repellents. The risks to study participants are three-fold: 1) allergic reaction to test materials themselves; 2) exposure to biting arthropods; and 3) possible exposure to arthropod-borne diseases. Although plans for the medical management of any side effects or adverse events have been developed, it is not unreasonable to expect greater efforts to be taken to minimize any potential harm. Allergic reactions to the test materials themselves are unlikely. Picaridin is commercially available and has been used at higher doses as a repellent with little evidence of toxic effects, so the subjects enrolled in this study are unlikely to be at increased risk of experiencing adverse side effects upon exposure to the test materials. The inert ingredients are

also widely used in cosmetic and personal care products, and have previously been reviewed and approved for use in other pesticide products registered by the Agency under FIFRA. It is disturbing to note, however, that picaridin is listed in the informed consent documents as a seemingly innocuous Toxicity Category IV compound. This may mislead participants into believing that picaridin is less toxic than it actually is, given that the EPA lists picaridin as a Toxicity Category III compound for acute oral, dermal and eye exposure. In toXcel's response to the Agency's science and ethics review of the protocol, however, Dr. Micah Reynolds indicates that this misleading information has been corrected in the informed consent documents (Reynolds 2007).

The endpoints of the study protocol require two mosquito bites—the second confirming the first within 30 minutes—to document breakdown of repellent efficacy. Reactions to mosquito bites are usually mild and easily treated with over-the-counter steroidal creams; such a cream, in addition to calamine lotion and rubbing alcohol, will be provided to study participants to alleviate minor symptoms associated with mosquito bites. Excluding subjects who have a history of such severe skin reactions will minimize the risk of a subject experiencing a severe physical reaction to a mosquito bite. Given the risk of contracting arthropod-borne disease in field conditions, it is unclear, however, if confirmed mosquito bites are necessary to measure repellent efficacy; previous repellent protocols reviewed by the HSRB have used an alternative endpoint, "Landing with Intent to Bite" (LIBe). Use of confirmed bites rather than alternative endpoints should be justified, as well as the exclusion of probes and bites from mosquitoes that do not fully alight as evidence of repellency breakdown. This justification was given in subsequent documents submitted to the Agency as well as in public testimony provided to the Board, and these justifications seem adequate. In written comments provided to the Agency (Reynolds 2007), for example, ICR and toXcel justify reliance on time to first confirmed bite (TFCB) rather than LIBe as the primary study endpoint by citing current and proposed Agency guidelines for efficacy testing; OPPTS 810.3300 and 810.3700 specify use of first bite or first confirmed bite for determining protection time. The Board is aware that landings with intent to bite as an endpoint is not part of the Agency's guidelines. However, many Board members argued—from both a scientific and an ethical perspective—that the Agency should give considerable weight to revising these guidelines to specify use of LIBe for determining protection time in future studies submitted to the EPA for registration.

The protocol states that to minimize the risk that study subjects will be exposed to arthropod-borne illnesses such as West Nile Virus, field tests of repellent efficacy will be conducted only when "cases" of known vector-borne diseases have not been detected by local vector/mosquito control agencies for at least one week prior to initiation of the field trials. However, disease prevalence and incidence can change rapidly. Conducting field trials in areas that have been certified as "disease-free" only for a single week may be inadequate to minimize the potential risk of vector-borne disease for study participants, and the Board recommended that the protocol be amended such that trials only be conducted in areas in which known arthropod-borne viruses have not been detected in weekly testing for at least one month prior to initiation of the field studies. As researchers also will be collecting mosquitoes for species identification, to further minimize the risk of arthropod-borne disease these mosquitoes should be subjected to serologic or DNA-based assays for known arboviruses, with clear and workable plans to contact study participants and alert them if a transmissible pathogen is detected. Given that many of

these diseases can have long incubation periods in infected individuals (e.g., 2-14 days for West Nile Virus), and that many of the symptoms of arthropod-borne illnesses can be diffuse or subacute, simply following up with study participants via phone two weeks after the completion of the field studies is insufficient.

Study investigators also should clarify in both the protocol and the informed consent document whether or not "cases" refers to actual reports of human disease, or the detection of pathogens in the local mosquito population by using sentinel flocks or other laboratory based methods. In the site-specific informed consent documents, the term "case" is used to describe both actual cases of human disease and detection of known arboviruses in mosquito pools in adjoining paragraphs detailing the risk to study participants of vector-borne disease. This may be confusing to study participants, and interfere with their accurate assessment of the risks. The Board recommended that reference to rates of West Nile Virus and other arthropod-borne diseases (human "cases") be deleted from the informed consent documents, as should the suggestion that the species known to transmit these viruses most frequently are uncommon at the sites where the field trials will be conducted. These statements may lead study participants to underestimate the risk of exposure to these agents. Although these illnesses were rare in Georgia and Florida in 2006, for example, rates of vector-borne diseases like West Nile Virus were substantially higher in previous years and it is difficult for even the most seasoned arbovirologist to predict what the likely incidence will be in the field test areas in 2007 and 2008.

Finally, as effects of arthropod-borne diseases are particularly severe in the elderly or those with compromised immune systems, it may be prudent to: 1) exclude participants greater than 55 years of age (rather than the current protocol's current 65 years and older), and 2) explicitly describe the risk for those with immune disorders—many with asymptomatic HIV-disease, for example, may consider themselves healthy enough for study participation yet may be at increased risk of illness. In written comments provided to the Agency (Reynolds 2007), ICR and toXcel apparently agree with the recommended change to the protocol's age-based inclusion and exclusion criteria.

In accordance with the newly promulgated provisions in the EPA's final human studies rule (40 CFR §§ 26.1701-1704), 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 field study. In order to protect the confidentiality of these results, however, it may be prudent to have study participants conduct the pregnancy tests just prior to travel to the sites in Georgia and Florida, with an investigator-confirmed test performed before exposure at the field site, so that pregnant participants can self-exclude themselves from study participation prior to travel; exclusion of female participants once they have arrived at the field sites may otherwise be difficult to explain while ensuring that the results of over-the-counter pregnancy tests are kept private. Additional procedures to ensure confidentiality are also recommended. As noted in the Agency's science and ethics review (Carley and Sweeney 2007b), identification of study participants by first name and last initial was inappropriate and it is rather surprising that the EIRB did not request the use of unique identifiers to further protect participant identity. In written comments provided to the Agency (Reynolds 2007), however, ICR and toXcel apparently agree with this assessment and have modified to protocol accordingly to require the use of unique numerical identifiers.

A number of additional concerns should also be raised. First, a more detailed explanation of study recruitment is needed, particularly a description of plans to minimize coercive subject recruitment and enrollment. Although compensation for study participation is not so high as to unduly influence enrollment, it may be appropriate to exclude all employees and contractors of ICR, toXcel and the sponsor (as well as family members), not just full-time employees of ICR, in order to minimize coercion; in written comments provided to the Agency (Reynolds 2007), ICR and toXcel apparently agree with this assessment and have modified the protocol. Enrollment and informed consent procedures should also be described in greater detail; informed consent is a process, not just a discrete moment in time. Although the informed consent document will be described and discussed with potential subjects—in person or via telephone—prior to initiation of the field studies, discussion of the risks and benefits of study participation should be ongoing. It is insufficient to simply state that, on the evening prior to the field trials, "[investigators] will review with [study participants] the specifics of the study as described in the ICD" (e.g., Spero 2007, 53). A detailed explanation of study procedures for risk and benefit is required. Furthermore, the additional risks to untreated control subjects (chosen by lottery rather than via separate enrollment of more experienced study participants) should be clearly listed in both the protocol and the informed consent document. However, it should be noted that mosquitoes are supposed to be aspirated from untreated controls prior to biting

Finally, there was considerable debate about whether or not the recruitment of research subjects from distant sites, with transport to field sites in Georgia and Florida, might be coercive with respect to enabling study withdrawal; volunteers may be less willing to withdraw from study participation if withdrawal involves considerable inconvenience and delay in returning home or if they believe that they have some reciprocal obligation to the researchers for the travel and lodging. In addition, given the amount of time involved and the additional risks associated with study-related travel and out-of-state housing (though not directly related to the study intervention), researchers and study sponsors should more clearly justify the recruitment and transport of experienced study participants from across the United States rather than recruiting and enrolling volunteers from the local populations in Savannah, Georgia and Lee County, Florida. At least one member of the Board, for example, believed that a more ethically-appropriate study design would involve recruitment of local research participants and specialized training sessions for those volunteers.

HSRB Consensus and Rationale

The Board concurred with the assessment of the Agency that the protocol ICR 1A 044 submitted for review by the Board, if revised as suggested in both EPA's review and by the Board, would meet the applicable requirements of 40 CFR 26, subparts K and L.

Completed Inhalation Study with Acrolein

Charge to the Board

The Agency has concluded that the Weber-Tschopp et. al (1977) study contains information sufficient for assessing human risk resulting from potential acute inhalation exposure. Please

comment on whether the study is sufficiently sound, from a scientific perspective, to be used to estimate a safe level of acute inhalation exposure to acrolein.

Board Response

The Board began by highlighting the toxicological evaluation of acrolein as prepared by the Agency for Toxic Substances and Disease Registry (ATSDR, 2005) and the EPA Integrated Risk Information System (IRIS 2003). The information below is reproduced from these reviews. Acrolein is toxic by inhalation, oral, and dermal exposures (toxicity category I for all routes). It is a potent irritant to the mucous membranes. As such, its toxicity is exerted at the point of contact with tissues. Signs and symptoms resulting from inhalation exposure to airborne acrolein may include irritation of the nose, throat and lungs, pulmonary edema, lung hemorrhage, and death. The nasal tissues appear to be the most sensitive target of inhalation exposure, with onset of noticeable irritation occurring in seconds (0.3 ppm). Higher airborne concentrations of acrolein (2– 5 ppm) result in increasingly severe manifestations of irritation over the entire respiratory tract. Oral acrolein exposure may result in gastrointestinal discomfort, vomiting, and stomach ulceration and/or hemorrhage. The stomach epithelium appears to be the most sensitive target for oral exposure (0.75 mg/kg). Higher concentrations of ingested acrolein have primarily resulted in increasingly severe irritation effects in the stomach (2 mg/kg and higher). Exposure to acrolein vapors or liquids may cause stinging of the eyes, lacrimation, and reddening, ulceration, or necrosis of the skin (10% acrolein solution). The eye appears to be the most sensitive target for exposure (0.3 ppm). Histological changes in respiratory and gastrointestinal epithelium have been observed from both inhalation and oral exposures, respectively. Changes in body and organ weights, hematology, and serum biochemistry, as well as developmental effects have been observed. Some of these effects are believed to be secondary effects of gastrointestinal and/or respiratory tract irritation (i.e., loss of appetite and weight loss due to gastrointestinal irritation). Inhaled acrolein is retained primarily in the upper respiratory tract (Egle, 1972) because of its high solubility and reactivity. Draminski et al. (1983) identified a low level of acrolein derived conjugates in the urine of rats following oral dosing. Orally administered acrolein is excreted (as metabolites) in the urine, feces and as carbon dioxide. The main pathway of metabolism for acrolein is the addition of GSH to the activated double bond followed by conversion to mercapturic acid. A second pathway is that of epoxidation of the double bond followed by attack on the epoxide by glutathione. A third pathway is addition of water to acrolein to form 3-hydroxypropionaldehyde, which can be further metabolized and ultimately incorporated into normal metabolic pathways (Parent et al., 1998). Exposure of the general population occurs primarily through atmospheric contact (HSDB, 2003).

EPA reported mean ambient acrolein concentrations of 14.3 $\mu g/m^3$ (6.2 ppb), ranging from 8.2 to 24.6 $\mu g/m^3$ (3.6 to 10.7 ppb), for two urban locations based upon data from 1961 to 1980 (U.S. EPA, 1993). Acrolein has been detected in exhaust gases from both gasoline engines (0.05-27.7 mg/m^3) and diesel engines (0.12-0.21 mg/m^3) (IARC, 1995). Concentrations in indoor air may exceed outdoor levels 2- to 20-fold times (Environment Canada, 2000). Levels between 2.3 and 275 $\mu g/m^3$ have been reported in smoky indoor environments such as bars and restaurants (IARC, 1995). In residences where wood stoves were used, concentrations from 0.7-6.0 $\mu g /m^3$ have been reported (IARC, 1995). IARC (1995) noted that the acrolein concentrations

in the smoke from various cigarettes ranged from 3-220 μg/cigarette. Levels as high as 463-684 μg/cigarette were reported (Kuwata et al., 1979). Jones et al. (1999) reported concentrations of acrolein in mainstream smoke ranging from 10 – 140 μg per cigarette, and estimated concentrations in side stream smoke in the range of 100 – 1700 μg per cigarette (IRIS 2003)" It is fairly well known that the annoyance effect of environmental tobacco smoke (ETS) is likely due to acrolein, as indicated in the EPA document on ETS (1992, reprinted by NIH in 1992-93); Weber (ibid.) indicated it was more pronounced for nasal irritation than eye irritation, though probably not the primary cause of irritation from ETS.

While the Weber-Tschopp et al. study is the focus of the review, the EPA IRIS review (2003) reports another human exposure study by Sim & Pattle (1957) in which 12 volunteers were exposed in a chamber to 0.8 and 0.12 ppm acrolein for 10 and 5 minutes, respectively; the volunteers reported it was extremely irritating to all exposed mucosal surfaces. No chronic studies of human exposure to acrolein have been reported. NIOSH has recommended that the concentration in workroom air be limited to 0.1 ppm averaged over an 8-hour shift. The ATSDR toxicology profile stated that "acrolein exposure levels were very comparable for the appearance of cellular changes in nasal epithelium of animals and onset of nasal irritation in humans" (Weber-Tschopp et al., infra vide), implying that acute nasal effects are similar.

The Weber-Tschopp et al. study provides the most comprehensive description of acute effects in humans. For this research, healthy male and female college student volunteers were exposed to acrolein in a 30 m^3 chamber at an 0.1 hourly air exchange rate in 3 trials:
(1) A continuous exposure at constantly increasing acrolein concentrations,
(2) Discontinuous short exposures to successively increasing concentrations, and
(3) Constant concentration for one hour.

Acrolein was injected with a micro liter syringe, vaporized and blown into the test chamber via a carrier gas stream. Acrolein concentration in the test chamber was quantitatively determined and results were reproducible [sd = 0.023 ppm = 3.8%].

In the first experiment, 31 male and 22 female students in groups of three participated. One trial with acrolein and one control trial under identical conditions but without acrolein were performed with each subject. Students were exposed to increasing acrolein concentration from 0 to 0.6 ppm in the first 35 minutes and to a constant 0.6 ppm concentration in the last 5 minutes. The subjects had to fill out a questionnaire every 5 minutes. The questions were: Is air quality good? Acceptable or bad? And do you have a desire to leave the chamber? After that, two subjects in each group were immediately compared for eye blinking frequency. With the third subject the breathing frequency during the entire exposure was measured. Eye irritation was significantly higher ($p<0.01$) than controls at 0.09 ppm and above. Nasal irritation was significantly higher ($p<0.01$) than controls beginning at 0.26 ppm. Throat irritation was experienced at 0.43 ppm and above. Eye blinking rate was experienced at 0.26 ppm and above ($p<0.01$). Respiration rate decreased by 25% ($p<0.01$) at 0.6 ppm concentration.

In the discontinuous short exposure experiment there were 42 students (17 males and 25 females). The subjects in groups of 4 were each exposed 5 times for 1 ½ minutes to variously high acrolein concentrations (0, 0.15, 0.3, 0.45, and 0.6 ppm). After a minute of exposure, they

were given the questionnaire form to fill. Between each exposure they were allowed to recuperate in a clean room for 8 minutes. The same controls from the first experiment were used. Eye and nasal irritation was significantly higher ($p<0.05$) than controls beginning at 0.3 ppm and 0.06 ppm, respectively. Throat irritation was not evident.

In the constant one hour exposure duration, 46 students in groups of threes (21 males and 25 females) were exposed to 0.3 ppm acrolein concentration for 60 minutes. Measurements of eye blinking frequency, breathing frequency and subjective symptoms of irritation were taken at the beginning of exposure and during exposure. Measurement of control values were obtained in the subjects at the beginning of exposure. Eye, nose and throat irritation increased significantly ($p < 0.01$), reached a plateau after 20-30 minutes of exposure, while eye blinking frequency plateaued after 10 minutes. Respiratory rate decreased 20% after 40 minutes exposure ($p<0.01$) in 16 subjects. The severity of the annoyance significantly increased almost immediately after acrolein was introduced. Eye, nose and throat irritation and eye blink frequency increased with increasing exposure duration. After 40 minutes, the subjective irritation reached a constant intensity while eye blink frequency after 10 minutes reached a definite rate. Throat irritation, which was insignificant in the other exposures, reached significance after only 10 minutes at this long exposure. There was a significant individual correlation (p between <0.05 and <0.01) between eye blink frequency and the subjective eye irritation. Every person with a sharp increase in eye blink frequency also had a sharp increase of eye irritation.

The volunteers were asked about the air quality during the exposure if it was good, bad or for the desire to leave the chamber and the degree of irritation to the eyes, nose and throat. The effects to continuous exposure as well as discontinuous exposure increased with acrolein concentration. Some indication of adaptation to the annoyance, not irritating effects of acrolein was suggested by the study investigators as intermittent exposures were more annoying. The eyes were more sensitive than the nose to the irritating effects of acrolein.

In the continuous exposure the irritation was significantly greater both in the eyes and nose than in the discontinuous short exposures which the investigators attribute to an increase in the sensitivity of both organs as a function of increasing exposure time. Throat irritation in both experiments was not as sensitive a criterion: in continuous exposure it increased significantly through 0.43 ppm, in discontinuous exposure it showed no change. The eye blink frequency of 34 subjects in the continuous trial was a function of the acrolein concentration. It increased from 0.17 ppm to 0.26 ppm ($p<0.01$) and it doubled at about 0.3 ppm. The breathing frequency of 19 subjects in the continuous exposure trial decreased slightly with increasing acrolein concentration. This decrease was statistically significant at 0.6 ppm ($p<0.05$). At this concentration the decrease in breathing frequency reached 4 breaths per minute - a decrease corresponding to about 25%. An increase in irregular breathing frequency in 11/19 subjects compared to controls was observed, very soon after the addition of acrolein but mostly in the second half or last third of the exposure time. Nearly half of the subjects displayed more or less pronounced tendency to lengthen the expiration cycle or more rarely the inspiration cycle holding the breath toward the end of the acrolein exposure.

Based on the results of this investigation, it was concluded that the threshold for the effects measured are: Eye irritation 0.09 ppm; Nasal irritation 0.15 ppm; Eye blink frequency

0.26 ppm; Breathing frequency 0.30 ppm; Throat irritation 0.30 ppm. The investigators concluded also that the threshold value for irritation is at least at the lower end of the spectrum, i.e., around 0.1 ppm.

Based on the nose and throat irritation and a decrease in respiratory rate in humans exposed to acrolein, ATSDR derived an acute-duration inhalation MRL of 0.003 ppm calculated from the LOAEL of 0.3 ppm from the Weber-Tschopp et al. 1977 study. They also derived an intermediate duration inhalation MRL of 0.04 ppb from their extrapolation based on animal nasal epithelial metaplasia. EPA also has an RfC for acrolein. EPA concluded in its WOE that "the study demonstrated that subjective eye irritation was the most sensitive indicator for the acute acrolein exposure in humans with a threshold effect of 0.09 ppm (0.2 mg/m^3). Protection of the eyes from the irritating effects of acrolein will protect against other respiratory effects of nasal and throat irritation and breathing effects which occurred at slightly higher thresholds."

Critique of Weber-Tschopp et al. Study

Strengths

This was a very well designed and conducted study in a lab with known scientific ability, QA capabilities, and institutional ethical review. Acrolein monitoring, chemical analysis, and low variability in chamber concentrations are indicative of the study's strength. A large number of healthy subjects were used (though the total number of subjects involved is unknown), including controls (though the controls may not have been very "blind"). The measurements of the subjects were performed well with appropriate methods, the most important being the objective measurements. The three studies looked at intermittent and continuous exposures over a sufficient range of concentrations and of times for the acute effects to be manifested. LOAELs could be determined from these data. The study appears to meet the HSRB scientific criteria.

Weaknesses

No positive controls were used, and negative controls may have been biased by not being completely "blind". There is no justification for the sample size, so there may have been a false negative rate in some comparisons where statistically significant differences were not found. The periods between studies are unknown. The results may not be generalizable to younger or older groups.

HSRB Consensus and Rationale

The Board concluded that the Weber-Tschopp et al. study contains information sufficient for assessing human risk resulting from potential acute inhalation exposure to acrolein. In addition, the study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of acute inhalation exposure to acrolein for the population tested but may not be generalizable to younger or older groups.

Charge to the Board

Please comment on the following:

1) Is there clear and convincing evidence that the conduct of the study was fundamentally unethical?

2) Is 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

Introduction

This is a report of third party research involving human subjects that was conducted prior to April 6, 2006 and was not conducted with the intention of submission to EPA under the pesticide laws. Rather, this study was conducted in order determine the relative contribution of acrolein to the irritating effects of cigarette smoke. The study was conducted at the Institute for Hygiene and Occupational Physiology, Swiss Federal Engineering College, in Zurich Switzerland. Financial support for the study was provided by the Association Suisse des Fabricants de Cigarettes, located in Fribourg, Switzerland.

Because the document was not submitted to EPA, 40 CFR §26.1303, which requires documentation of ethical conduct of studies submitted after April 6, 2006, does not apply. In addition, because the study was conducted prior to that date, it is not subject to those documentation requirements. 40 CFR §26.1602(b)(2) requires HSRB review, 40 CFR §26.1703 forbids EPA reliance on research involving intentional exposure of pregnant or nursing women or children, and 40 CFR §26.1704 forbids EPA reliance on pre-rule research if there is "clear and convincing evidence" that its conduct was fundamentally unethical or significantly deficient relative to standards prevailing when it was conducted. When evidence concerning subject age and reproductive status is both absent and unobtainable, EPA's policy is that §26.1703 does not prohibit reliance on a study.

This report describes three sub-studies:

A) Continuous exposure to acrolein concentrations increasing over 40 minutes from zero to 0.60 parts per million (ppm)

B) 90-second exposures separated by 8-minute recovery periods to concentrations increasing from 0.15 to 0.60 ppm

C) Continuous exposure over 60 minutes to constant concentration of 0.30 ppm.

All sub-studies were conducted in a 30-cubic meter (m^3) chamber using healthy research subjects. Subjective measures of annoyance were obtained from the responses of the research volunteers to questions about air quality ("good", "acceptable", or "poor"), a wish to leave the room ("no", "don't know", or "yes"), and perceived eye, nose, and throat irritation (1=not at all; 2=a little; 3=medium; 4=strong). Objective measures of response were recorded only for tests A and C and included measurement of eye blink rate for two out of three subjects and respiratory rate and depth for the remaining one out of three subjects.

There were 53, 42, 46 subjects in sub-studies A, B and C respectively. It is not known whether this represents a maximum of 141 individual subjects, a minimum of 56 individual subjects ([based on 25 women, which is the largest number of women in any of the three sub-studies, and 31 men, which is the largest number of men in any of the three sub-studies], or some number in between. This range of somewhere between 56 and 141 individual research subjects in the three sub-studies assumes that no subject participated more than once in a given substudy. The only information provided about the subjects is the gender distribution, and that they were "healthy college students."

There is no indication in the results or discussion sections of the publication that any subjects withdrew from participation in any of the three sub-studies.

The presentation by EPA described the compound acrolein as "highly toxic," although clarification from HSRB members familiar with acrolein indicated that at the exposure levels of this study, acrolein was not considered "highly toxic." Airborne concentrations higher than those used in this study (between 2 and 5 ppm) result in increasing irritation over the entire respiratory tract. Relative to this, the doses used in this study were not considered highly toxic. Acrolein is highly irritating, has an odor threshold, and manifests toxicity at the point of contact, rather than leading to systemic toxicity.

Critique of Study

The prevailing standard is assumed to be the 1975 version of Declaration of Helsinki (DoH), because the study was published in 1977. However, the previous version of the DoH (1964) may have been in effect if the study was conducted prior to 1975. In addition, because this study was not performed by medical doctors, the DoH may not have applied, regardless of when the study was conducted.

Because this study was published in 1977, the report is missing much information on which to base a thorough assessment of the ethical conduct of the study. The number of individual subjects used could range from 56 to 141 and how many may have participated in more than one test is unknown. There also is little information about the subjects themselves: whether any were students or employees of the investigators; how they were recruited; and what they were told about risks, their freedom to withdraw, or the informed consent process. The description of expressing a "wish" to leave the chamber is problematic because it does not necessarily indicate that a subject would leave if given the opportunity; a subject may have "wished" to leave but might not actually leave. The Agency provided a more direct translation of the original German language publication. This offered the Board a more exact understanding of the actual question being asked of the research volunteers, which was whether they would have preferred to leave the room, rather than did they want/need to leave the room. There is no information concerning compensation, possible undue influence, or the applicable version of the DoH, or whether, in fact, the DoH would have been applicable to research. There was no justification for the sample size, which raised questions as to whether more subjects than necessary were placed at risk. Another issue concerns whether the study should have been stopped when indications of a wish to leave (e.g., 72 % of subjects expressed a wish to leave the chamber at 20 minutes but did not withdraw from the study) became evident. The subjects were

tested in groups of three and it is unclear whether the investigators analyzed the data as it was obtained to determine irritation levels or if the analysis was not performed until all subjects had been tested. Thus, it is unknown whether the study should have been stopped sooner, as accumulating evidence suggested irritation was occurring.

40 CFR §26.1703 forbids EPA from using research involving intentional exposure of pregnant or nursing women or children; the research subjects were described as college students, and thus were likely to have been at least 18 years old. Approximately half the subjects were female, but the report does not indicate their reproductive or nursing status.

The Board was in agreement that there was a great deal of information lacking in the published report of this study. In addition, several members of the HSRB expressed discomfort in having to assess the ethics of the research in this situation. Many members agreed with the recommendation to EPA that as they decide whether or not to use this information, they should carefully consider whether use of this information would lead to more protective standards and consider whether the information from the animal studies would suffice for its risk assessment work.

Although a majority of members found no clear and convincing evidence that the research was significantly ethically deficient or was so deficient as to place subjects at risk or seriously impair the informed consent process, members felt that they were limited by the standard of "clear and convincing evidence", and were uncomfortable with assessing the conduct of this study in the absence of information necessary to make that assessment.

One Board member disagreed with the majority opinion, with the Board member stating that the study was significantly deficient relative to ethical standards prevailing at the time it was conducted. This position was based on an analysis of the potential benefits of the study (to the extent that these benefits could be predicted at the time the study was initiated) and the potential risks to individual research volunteers. Prevailing ethical standards at the time, as described for example in the Declaration of Helsinki (Tokyo revision, 1975), included a commitment to the idea that, to be ethically acceptable, research with human subjects must have a favorable risk-to-benefit ratio and "cannot be legitimately carried out unless the importance of the objective is in proportion to the inherent risk to the subject" (Declaration of Helsinki, 1975, section I.4). In the judgment of this HSRB member, the potential benefits of the study did not justify the risks to research subjects. In EPA's presentation, acrolein was characterized as "highly toxic" and there was no intent of the research to provide therapeutic benefit or diagnostic results, and no benefit at all to the subjects. Intentional exposure to this highly toxic substance thus constituted an inappropriate risk-to-benefit ratio. However, subsequent to the publication, information from this study was applicable and considered during development of the Clean Air Act. Data from this and similar research was instrumental in developing tobacco smoke exposure regulations and laws that banned tobacco smoke in many places. The societal benefits of these activities, in the forms of occupational and societal regulations and worker protection standards, have been substantial by reducing tobacco smoke exposure to many people. This and other studies were considered scientifically sufficient given the standards of the time to justify placing regulations on tobacco smoke. However post hoc benefit is not relevant to the prospective risk-benefit

balance and the potential societal benefit at the time of study conduct was not felt by one Board member to be reasonable in relation to the risks to subjects.

HSRB Consensus and Rationale

There was not clear and convincing evidence that the conduct of the Weber-Tschopp et al. study was fundamentally unethical. In addition, despite the lack of adequate information to assess the affirmative, most of the HSRB agreed that there was not 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.

Completed Studies on the Therapeutic and non-Therapeutic Effects of Administration of 4-aminopyridine

Charge to the Board

The Agency's weight-of-evidence (WOE) document for 4-aminopyridine describes the study design and results of three clinical trials (Grijalva et al. 2003, Segal et al. 1999, and Van Diemen et al. 1993). The WOE document also discusses the Agency's conclusion that these studies provide sufficient information to establish a point of departure for the assessment of the risk to humans resulting from all potential durations of exposure to 4-AP. Please comment on whether the studies are sufficiently sound, from a scientific perspective, to be used to derive a point of departure for estimating risk to humans from exposure to 4-AP.

Board Response

To inform this question, EPA provided HSRB with a number of background documents, including an extensive review of the health and environmental effects of 4-AP conducted by the Agency in January 1989, as well as the WOE document and electronic copies of the 3 published studies on which the proposed estimates of risk are to be based.

Salient background information includes the following. 4-AP exerts its major biological actions by blocking fast acting potassium channels. This effect enhances or prolongs action potentials in muscle and nerve, and also increases transmitter release at neuronal synapses in the periphery and brain. 4-AP is acutely toxic in animals and humans. Toxicity at low doses is primarily mild, including tremors, sweating, and salivation. The dose-effect curve is very steep. Higher doses cause muscular incoordination, seizures, and death. There is little evidence of metabolism, most of the drug appears unchanged in urine. There is little selectivity between humans and other mammals, or birds. The compound is tightly bound to soil particles and persists in the environment for a year or longer under many circumstances.

4-AP has been used or considered for therapy of botulism, overdose of non-depolarizing muscle relaxant drugs in surgical anesthesia, for spinal cord injury, and for certain demyelinating disorders such as multiple sclerosis and Guillain-Barré syndrome (FDA approval for the latter use was granted by FDA under the orphan drug program in December 2006).

Present EPA concern is focused on the use of 4-AP as a "bird repellant" (eg., Avitrol). This terminology appears somewhat misleading as the chemical does not drive birds away by adverse sensory stimuli such as odor but by poisoning, which elicits distress calls that warn the flock to avoid a baited area. The database on animal studies is quite extensive. In fact the 1989 EPA document presents tabular information on LD50 values for a wide variety of vertebrates and invertebrates and numerous species of birds. The WOE document, however, states that "there are no reliable animal toxicity studies to derive an appropriate point for departure for assessing human health risk". The limitation appears to be that the animal studies have chiefly focused on LD50 values—ideal for comparing potency across species and genera but insufficient to establish a point of departure such as NOAEL, LOAEL, or BMD10. Hence the perceived need to rely on human toxicity studies. It should be noted, however, that the 1972 study by Mistov and Uzunov (summarized by EPA, 1989) did reveal dose-related histopathology in white rats treated for 1 or 6 months with 1 to 5 mg/kg 4-AP. Thus, the LOAEL in rats appears to lie at 5 mg/kg or lower. It should also be noted that tabular data in EPA 1989 show rats to be one of the less sensitive species with regard to 4-AP toxicity.

EPA's WOE document concluded that 4-AP has short residency time in the body, and therefore "one can conclude that a single PoD value is sufficient for risk assessments of 4-AP for different potential exposure scenarios (short-, intermediate- or long-term exposures)." The EPA analysis also noted that the minimal daily oral dose of 4-AP producing side effects ranged from 5 to 30 mg/day. EPA reviewers also concluded that 4-AP has a very steep dose-response relationship. In conclusion, the Agency proposed a 5 mg/day (0.08 mg/kg-day) as a LOAEL, and a point of departure for risk assessments.

The WOE document considers three published human clinical studies, 1. MRID 47093602 (Segal et al., 1999); 2) MRID 47093601 (Grijalva et al., 2003); and 3) MRID 47093603 (Van Diemen et al., 1993

While the studies were conducted primarily to evaluate efficacy, they all included a discussion of safety. The investigators frequently indicated that subjects were able to "tolerate" doses, which is quite understandable, given the severity of the disease/injury in these patients. In many cases it was not clear which subjects (at which doses) suffered side effects. The lowest oral dose tested in these studies was 5 mg/day. The Agency translated this dose to 0.08 mg/kg-day.

A critique of the three studies is provided below. As clinical studies, the three reference studies have major weaknesses of design or outcome.

Segal et al.

Segal et al. (1999) created an active control group of 5 spinal cord injury patients, each of whom received doses of 6 mg/day, as well as 16 patients in a high dose group. It was not clear from the article whether any of the active control patients experienced side effects. The article simply states, "Nervousness, giddiness or dizziness, and gastrointestinal upset manifesting as mild abdominal cramping or nausea were the most frequent side effects." The frequency of these side effects was not provided. The article also states, "All side effects were transient, self-

limited, or disappeared with changes in dosage or the timing of drug ingestion to coincide with meals or snacks." In summary, it was not clear from this study the extent to which patients who received a 6 mg/day, the lowest dose in the study, experienced side effects.

Grijalva et. al.

Grijalva et al. (2003) completed a study with 21 spinal cord injury patients. The lowest dose in the study was 5 mg/day. The authors reported that 56 probable adverse reactions were registered over the 26-week study. Adverse effects (dry mouth, dizziness, and gastritis) began with 4-AP at 5 or 10 mg/day. Fourteen patients receiving 4-AP treatment had 26 probable adverse reactions. The authors report the frequency of these side effects, but did not specify which side effects occurred at which dose level.

Van Diemen et al.

Van Diemen et al. (1993) conducted a randomized, double-blind, placebo-controlled cross-over trial with 70 multiple sclerosis patients. In the intravenous phase of the study, parathesias occurred at a minimal dose of 1 mg. In the oral phase of the study (69 patients), 54 of 69 (78%) patients experienced at least one side effect. Table 2 in the article indicated that 75 side effects were observed in those patients who received the minimal daily dose of 5 mg, so presumably some of the patients reported multiple side effects. At 5 mg/day, these side effects included paresthesias/dysesthesias (15), dizziness/light-headedness (36), gait instability (11), nausea/vomiting (9), and restlessness/anxiety (4).

Conclusion of Studies

Overall the therapeutic effects as noted from each of the three studies were of minor degree or of marginal statistical significance, particularly as concerns improved function after spinal cord injury. Powerful placebo effects were noted, further weakening confidence in treatment-related improvements. These weaknesses do not in themselves impair the potential usefulness of these studies in defining toxic endpoints. Unfortunately, clinical studies are typically not designed in a manner that allows one to estimate toxic endpoints with confidence, but rather to establish efficacy at doses that are not "overly toxic" in relation to a therapeutic benefit.

As for toxic signs and symptoms, the results of the Grijalva study are difficult to interpret because adverse reactions were reported by 56% of the treated patients and, apparently, by a still higher proportion of the placebo controls. The Segal study (1999) is weakened, by the absence of a placebo control. For this reason it is uncertain which if any adverse effect is truly treatment-related. Actually, Segal et al did not specifically report adverse effects but merely stated that "nervousness, giddiness or dizziness, and GI upset like mild abdominal cramping or nausea were the most frequent side effects. It therefore seems reasonable to state that neither the Grijalva study nor the Segal study by itself is sufficiently sound for the purpose of deriving a point of departure for estimating risk to humans from exposure to 4-AP. On the other hand, the Van Diemen study (1993) was comparatively rigorous and provided a wealth of detail on the occurrence and intensity of treatment-related side effects. Unfortunately, this study did not employ doses low enough to establish a NOAEL but it does seems to indicate that a total daily

oral dosage of 5 mg was associated with definite but mild discomfort unaccompanied by changes in blood chemistry or EEG.

HSRB Conclusion and Rationale

The Board concluded that the studies that the three clinical studies, Grijalva et al. 2003, Segal et al. 1999, and Van Diemen et al. 1993, were sufficiently sound, from a scientific perspective, to be used to derive a point of departure for estimating risk to humans from exposure to 4-AP. Thus considering the three studies, an estimate of the LOAEL of 0.07 mg/kg/day was determined. The Board was reluctant to endorse the use of a 5 mg/day (0.07 mg/kg/day) LOAEL, given the multiplicity of side effects seen among patients receiving this dose, and the steep dose-response curve of 4-AP. Thus, the Board cautioned that this conclusion comes with a degree of uncertainty and advised the Agency to take such uncertainty into account when using the published information to arrive at a point of departure for 4-AP.

Charge to the Board

Please comment on the following:

1) Is there clear and convincing evidence that the conduct of any of the three clinical studies (Segal et al., 1999; Grijalva et al., 2003; Van Diemen et al., 1993) any of the clinical studies was fundamentally unethical?

2) Is there clear and convincing evidence that the conduct of any of the clinical studies was significantly deficient relative to the ethical standards prevailing at the time the research was conducted?

Board Response

Three studies are being evaluated. The EPA is seeking to use these studies to derive a point of departure for estimating risks to humans from exposure to 4-aminopyridine (4-AP). Each of them is a completed and published study, and the information about the studies is derived solely from the published articles.

The earliest of the three studies, by Van Diemen and colleagues, was conducted at the Free University Hospital in Amsterdam in the early 1990s, for the primary purpose of determining the efficacy and safety of 4-AP in treating persons with multiple sclerosis. This was a cross-over study, in which subjects were randomized between 4-AP and placebo. The initial portion of the study involved the use of intravenous 4-AP, while a second phase used oral 4-AP. It is stated in the article that the study was approved by the "ethical committee" of the hospital where it was conducted, and that the informed consent of all subjects was obtained. No specific ethical standard is mentioned in the article, although presumably this study would have been governed by the 1989 version of the Declaration of Helsinki.

The second of the three studies, by Segal and colleagues, was conducted at the VA Medical Center in Long Beach California in the late 1990s, for the primary purpose of determining the efficacy and safety of 4-AP in treating persons with chronic traumatic spinal cord injury. One group of subjects was randomized between low and high doses of oral 4-AP, while another group of subjects (which had previously been exposed to this compound) received only the high dose. It is stated in the article that the study was "institution-approved", and that the written informed consent of all subjects was obtained. No specific ethical standard is mentioned in the article, although because this study was conducted in a VA hospital which also holds a Federal-Wide Assurance from the federal Office for Human Research Protections, presumably this study was governed by the Common Rule (45 CFR 46 Subpart A).

The third of the studies, by Grijalva and colleagues, was conducted at the Specialties Hospital, Centro Médico Nacional Siglo XXI, in Mexico City, in 1999 and 2000, for the primary purpose of determining the efficacy and safety of 4-AP in subjects with long-term spinal cord injury. This was a cross-over study in which subjects were randomized between oral 4-AP and placebo. It is stated in the article that the study was approved by the local research committee of the hospital where it was conducted, and by the National Research Council of the Instituto Mexicano del Seguro Social, and that all subjects were fully informed about the study and signed an "informed consent letter." No specific ethical standard is mentioned in the article, but the Institute Mexicano del Seguro Social has an IRB listed with OHRP, and holds a Federal-Wide Assurance, and thus presumably this study was governed by the Common Rule (45 CFR 46 Subpart A).

Critique of Studies

Because each of these three studies was completed prior to the effective date of the EPA's final rule, "Protections for Subjects in Human Research" (April 7, 2006), the Board is required to evaluate these studies under a rule that allows their results to be used by the EPA unless there is clear and convincing evidence that either a study was fundamentally unethical, or that it was significantly deficient relative to the ethical standards at the time the research was conducted. The consequence of that review standard is that in the absence of information about particular aspects of a study, all uncertainties must be resolved in favor of the study having been properly conducted. Given that circumstance, the Board believed it would be appropriate, in the future where such studies that pre-date the effective date of the final rule are being reviewed, for the EPA (as it has reported to the Board with other similar studies previously) to attempt to collect additional information about the studies, such as the records of IRB review including any consent forms.

Given that such additional information was not available to the Board with regard to these three studies, the Board can only rely on the information presented in the published reports regarding these studies. Those reports indicate that in each case the study was reviewed by an IRB or an equivalent type of body. In addition, in each instance, it was stated that informed consent of the subjects was obtained. With regard to that aspect of the studies, there is no evidence before the Board suggesting that the consent obtained was not appropriate under the then-applicable standards.

With regard to the relationship between benefits and risks, each of the studies involved an examination of 4-AP as a treatment for a very serious medical condition, either multiple sclerosis or spinal cord injury. Given the information that was known about the possible risks of using 4-AP in each instance, there was no evidence before the Board suggesting that there was not an appropriate relationship between risks and benefits under the then-applicable ethical standards.

In none of the published reports for these three studies is there any evidence suggesting that any subjects under age 18 were enrolled, or that any pregnant or nursing women were enrolled. Given that circumstance, it appears that the provisions of 40 CFR § 26.1703 have been complied with.

HSRB Consensus and Rationale

The Board concurred with the initial assessment of the Agency that for each of these three clinical studies (Segal et al., 1999; Grijalva et al., 2003; and Van Diemen et al., 1993)., there is was no clear and convincing evidence that the conduct of the study was fundamentally unethical, or that the conduct of the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

Design of Research on the Levels of Exposure Received by Pesticide Handlers

Risks and Benefits of Handler Research

Charge to the Board

Will the Task Forces' Governing Documents considered in conjunction with the additional study- and scenario-specific information specified above provide an adequate basis for assessing whether the risks of conducting a particular study are justified by the expected benefits of the proposed research? If not, what additional information should be provided for an IRB, EPA, and the HSRB?

Board Response

The Agency has provided the HSRB with a document entitled, "AHETF Human Research Monitoring Program". This report serves as the "governing document" for the pesticide handler studies sponsored by the Agricultural Handlers Exposure Task Force. Section 4 of the report discusses study benefits, section 5 discusses risks to subjects, and section 6 provides a benefit-risk comparison.

The Board also received a document entitled, "Governing Document for a Multi-Year Antimicrobial Chemical Exposure Monitoring Program". Section 9 of this report discusses study benefits, sections 10 through 12 discuss risks to subjects, and section 13 provides a benefit-risk comparison.

Both documents indicated that no direct benefits will accrue to study participants, and that the risks to participants must be justified by societal benefits. The primary societal benefit

cited by the authors was the ability for EPA and other regulatory agencies to use new handler exposure to data to improve the quality of worker risk assessments. The AHETF authors pointed out that growers or landowners who participate in these studies would benefit through use of the test substance at no cost. However, the Board questioned whether the free pesticide would indeed be considered a benefit, as opposed to a means to encourage growers to participate in the research. In addition the Board questioned whether such a benefit would also be appropriate to study participants (e.g. commercial applicators).

The documents also indicated that the database would be a benefit to the sponsors of the studies. However, a benefit to pesticide manufacturers is not considered in the benefit-risk analyses mandated by FIFRA.

The document indicated that risks to subjects in these studies were greater than minimal. The AHETF authors identified six types of risks: heat-related illness, exposure to surrogate chemicals, scripting of field activities, psychological, exposure to detergents used in sampling, and injuries. Each of these risks was discussed in detail, and plans to minimize risks were included in the report. Risk categories described by the AEATF included chemical risks related to use of a surrogate antimicrobial chemical and exposure to alcohol/water face and hand rinse solutions. Physical risks might arise from heat stress or exaggeration of normal activities.

The authors concluded that the risks to study participants are outweighed by the benefit to society in the form of high quality exposure data for use in evaluating pesticide safety.

Critique

The Board's discussion focused primarily on the Agricultural Handler Exposure Task Force (AHETF) document. Most of these comments are applicable to the AEATF document as well. The document states that there will be no direct benefits to participants. However, it is arguable that there could be a direct benefit of feedback on work and safety performance. Study participants will be provided with their own results. Knowledge of one's ranking among workers could have educational or motivational value for a worker. There is also the potential for direct feedback related to safe and unsafe practices. For example, an individual using a ground boom sprayer with a blocked nozzle might remove the nozzle and attempt to dislodge the blockage by blowing through it. This is clearly poor practice, but it does happen. If the study supervisor observed such behavior then it would be sensible for him/her to provide advice to that individual on safe work practices. A second example would be the identification of more widespread poor working practices. In the United Kingdom, studies in seed treatment facilities documented that, contrary to good practice, some operators were using compressed air to clean residues of seed treatment products from application equipment. This obviously created an airborne hazard. The fact that this practice was commonplace indicated a need to communicate the hazard to the whole industry sector. In this particular case, the industry task force involved with the studies produced a poster illustrating the findings that was distributed to seed treatment facilities. The dissemination of this type of feedback as part of stewardship could be seen as a benefit to pesticide handlers in general. How such information will be communicated to participants and the pesticide handler community in general should be described in the protocol.

The discussion of benefits to growers, landowners, or commercial applicators focused on the provision of free pesticide product for use in the study. The Board agreed with the AHETF's conclusion that the magnitude of this benefit is not likely to result in coercion of employees to volunteer to participate in a study. However, the timing and conditions of how this free offer is made should be explained explicitly as a part of each protocol's discussion of recruitment. This would permit the HSRB to evaluate the specifics of the arrangement, and would permit the AHETF to assure some uniformity in how such offers are being made in different studies (clusters) or/and scenarios. However, the Board questioned whether the free pesticide would indeed be considered a benefit, as opposed to a means to encourage growers to participate in the research. In addition the Board questioned whether such a benefit would also be appropriate to study participants (e.g. commercial applicators).

The nature of risks is discussed thoroughly in the governing documents, but the Board concluded that several issues warranted further clarity. The first issue is related to the scripted nature of the AHETF studies, and the possibility that workers may be asked to use equipment that they would not normally use. Lack of familiarity with equipment could increase the risk of injury; for example, a mechanical injury through collapsing hydraulic systems or an electrocution through folded or folding booms coming into close proximity or contact with overhead power cables. The AHETF document indicates that workers need to be "familiar" with the type of equipment to be used. The Board was concerned that familiarity with equipment was not sufficiently described and might be inadequate. In the United Kingdom, for example, the requirement would be that the workers should be "competent". The Board recommended that future AHETF protocols identify equipment use requirements for workers and the steps that will be taken to ensure that workers can operate the equipment unsupervised in a safe manner. This concern was not applicable to the AEATF studies.

The second issue is related to potential heat stress. The AHETF document outlines a strategy that includes encouraging participants to drink water or sports drinks throughout the monitoring period. It is considered bad practice in occupational hygiene to combine working with hazardous substances and drinking (or eating and smoking). The document provides no advice to minimize potential inadvertent exposure during drinking. In fact the document states that hand washes are not necessary. The document's only guidance is for researchers to remind workers just prior to participation about general ways to minimize exposure to chemicals, such as washing their hands before eating and before removing clothing. The Board recommended that the AHETF develop a consistent policy regarding drinking water or sports drinks and personal hygiene. These same recommendations could be applied to AEATF studies, but the risk from heat stress was judged to be much lower for these studies.

The Board commended the AHETF for developing clear stopping rules to minimize the risk of heat-related health concerns based on the National Oceanic and Atmospheric Administration's (NOAA) National Weather Service heat index. In particular, the AHETF has proposed hourly measurement of heat and humidity when the ambient air temperature exceeds 70°F, with increasing vigilance for signs of heat exhaustion and sunstroke on the part of study investigators as the heat index increases. A study would be halted when the heat index exceeds 130°F, as severe heat-related illness is likely with prolonged physical exertion under such conditions. AHETF researchers also recognize that direct exposure to the sun can contribute to

heat-related illness, and have proposed adjusting the heat index accordingly for agricultural handlers working in the direct sun. The Board was very supportive of these efforts, but concluded that further protection of study participants was warranted.

As calculated, the National Weather Service heat index assumes that the person in question is 5' 7" tall, 147 pounds, Caucasian, clothed in long trousers and a short-sleeved shirt, walking at a speed of 3.1 mph in the shade in a breeze of 6 mph, and not dripping with sweat. It is unclear whether or not these characteristics and conditions apply to the agricultural handlers likely to be enrolled in the proposed studies. The whole body dosimeter used for the proposed research, for example, is described in study documents as "long underwear". The Board recommended that the heat index threshold be adjusted to account for the increased amount of clothing that volunteers will wear during study participation. The Board further recommended that AHETF document the expected levels of physical exertion in its protocols, and consider whether a further adjustment to the heat index threshold would be appropriate.

Finally, the Board was concerned with the use of the 130°F heat index value as a threshold. According to the NOAA website, "heatstroke/sunstroke [is] highly likely with continued exposure" under such conditions (http://www.crh.noaa.gov/arx/heatindex.php). It is important to note, however, that the website also states "sunstroke, heat cramps or heat exhaustion [are] likely, and heat stroke [is] possible with prolonged exposure and/or physical activity" with a heat index of 105-129°F. The Board recommended that the AHETF revisit the issue of heat stress, and develop a stopping point that will minimize risk for the study participants.

HSRB Consensus and Rationale

The AHETF and AEATF documents have provided the HSRB with a detailed and thoughtful analysis of expected benefits and risks associated with the conduct of human exposure monitoring. The Board recommended that the particular arrangements for providing the test substance be outlined in the individual protocols. Finally, the Board concurred with the AHETF and AEATF that the database developed from these studies will improve the quality of risk assessments, and that they should be considered a valuable societal benefit, provided that the data collected are accurate, or at the least do not underestimate real-world exposures.

The Board recommended that AHETF pay more careful attention to the issue of safety when asking participants to operate equipment with which they do not normally work. In particular, AHETF should be more explicit about the level of competency expected of workers when operating such equipment. The Board commended AHETF for developing clear guidelines for stopping work based on a heat index. However, the Board concluded that the approach described in the governing documents was not fully protective of workers, and recommended that additional attention be given to this matter, including consideration of a lower heat index threshold for stopping work.

Addressing Potential Sources of Underestimation Bias

Charge to the Board

1) Has EPA appropriately characterized the limitations on the scientific usefulness of a handler database that does not include data characterizing the efficiency of residue removal procedures? If not, what limitations have been overlooked?

Board Response

Introduction

The Agency presented its concerns regarding potential underestimation of dermal exposure. It relied on the work of the January 2007 EPA Scientific Advisory Panel (SAP) report, as well as a review of the scientific literature. The SAP observed that whole body passive dosimetry and biological monitoring comparisons did not seem to indicate a systematic bias. The SAP concluded that bias may exist, but the extent of potential bias between dermal exposure and biological monitoring could not be detected because of the statistical uncertainty inherent in the exposure and biomonitoring data. The SAP also noted that passive dosimetry can generate data that can be used to develop predictive estimates of exposure for a number of different scenarios and activities. The SAP suggested that biological monitoring could be a useful check on passive dosimetry, but declined to suggest that require biological monitoring be included in a protocol. EPA agreed with the overall SAP conclusions and described some disadvantages to biological monitoring including additional cost, logistical considerations (e.g., number of days required for metabolites to clear), and a lack of acceptable biomonitoring methods for many of the surrogate compounds proposed for the AHETF and AEATF studies.

The SAP gave particular attention to potential underestimation bias resulting from the use of hand wash and skin rinse techniques. The scientific literature indicates that hand wash/rinse performance can be influenced by the chemical properties of the pesticide, such as solubility, octanol/water partition coefficient, or formulation type; residence time on the skin before hand rinsing is performed; type of solvent used to rinse the hands (e.g., alcohol, soap and water); concentration of the chemical on the skin (microgram/cm^2); duration of the exposure monitoring period; and nature of the residue (whether exposed to pesticide concentrates, dilute sprays, or field residues). Hand rinse removal efficiency values from several studies involving human subjects ranged from approximately 70 to 90 percent; however, in the case of chlorpyrifos the efficiency was approximately 20 to 40 percent). An unpublished AEATF hand rinse efficiency study reported up to 90 percent efficiency for didecyl dimethyl ammonium, but further details were not provided.

Face/neck wipes were not specifically discussed at the SAP meeting. This method was not among those recommended by the Agency in its 1987 Subdivision U Agency guidelines for pesticide handler exposure studies but this does not mean the EPA would not accept its use. It was also not included in the later (1997) Agency 875 guidelines for occupational and residential exposure assessment. The Agency has concluded that exposure to the head/face and neck is expected to be very low for the majority of exposure scenarios planned by the AHETF. The Agency pointed to an exception in the case of the open-cab airblast application AHETF studies.

The SAP was equivocal about the need to correct the results from hand washing for its efficiency at recovering pesticides from skin. The SAP would accept a rinse validation study if it could decrease the uncertainty in exposure estimates at a reasonable cost, and be done within approved human studies guidelines.

The AHETF argued that no correction was needed for any potential method bias because of reasonable congruence in exposure estimates between studies based on biological monitoring and those using passive dosimetry. AEATF argued that no correction was needed in studies where individuals will not be wearing gloves and that the hand correction factor was reasonable.

The EPA concluded that substantial underestimation by whole body garments was unlikely, and that the most relevant methods to be corrected for potential underestimations were the handwash and the face/neck wipe. For the proposed AHETF studies, the Agency stated that the contribution of hand exposure was expected to be minimal because all subjects will be wearing chemical resistant gloves (CRG) during all operations. The AEATF intends to collect data based on individuals not wearing gloves (consumer products), but for most scenarios the Agency again expects exposure to head, face, and neck to be low. The Agency proposed two options to the task forces: biological monitoring could be included as a check for potential breakthrough or other losses when using surrogate chemicals that have well-established methods; cotton gloves beneath the CRG and hat patches when measuring head, face, and neck exposures could be used in scenarios for which exposures to these body regions might be relatively high.

The Agency concluded that conditions should be established for correcting hand rinse and face/neck wipe exposure values. The EPA proposed a set of conditions for consideration by both task forces; namely, if measured exposures from hands, face, and neck contribute less than 20 percent of total exposure, no action is required; if measured exposure contribution represents between 20 and 60 percent of total exposure, an automatic 50 percent adjustment can be made or a validation study can be submitted; if measured exposure contribution is greater than 60 percent, a validation study is required. Because validation studies involve intentional exposure of human subjects, review of such studies by the HSRB would be required.

Critique

Dermal exposure assessment methods are considered to be of three types: interception, removal and visual. Interception methods use a collection device on the skin to capture chemicals; removal methods use washing or wiping to remove residues from the skin; visual methods use dyes or fluorescent compounds to visualize chemical deposition patterns on skin and clothing. Interception techniques can overestimate exposures because they capture more material than the skin would normally collect. They can also underestimate exposure if breakthrough occurs. Removal techniques typically underestimate exposure, since they can only remove chemicals that have not been adsorbed irreversibly onto or absorbed into the skin. Visual techniques have been most useful in qualitative evaluations of exposure and worker education.

The use of the term "passive dosimetry" to describe the dermal sampling methods proposed by AHETF and AEATF can lead to some confusion. First, the approach relies on both interception (whole body garments) and removal (handwash, face/neck wipe) techniques. It is

difficult to reconcile the very active procedures required to remove chemicals from the skin with the notion of a "passive" measurement method. Second, the term "dosimetry" is a misnomer, as it is exposure rather than dose that is being measured.

The Board expressed some reservation regarding the SAP and Agency conclusion that passive dosimetry does not underestimate dermal exposure. The conclusion was based primarily on a recent article by Ross et al. (2007). In that article it is stated, in regard to the methods proposed by the AHETF and AEATF, that "the passive dosimetry methods used have never been validated." The article then analyzed 14 concurrent or consecutive passive dosimetry-biomonitoring studies, and reported that this analysis produced "generally similar" estimates of absorbed dose from these two methods. However, when these datasets were examined in detail, this conclusion was not well-supported. For some chemicals the passive dosimetry estimates under-predicted the biomonitoring estimates, while in others the opposite was true (see Table 3 of the article, with reference to chlorpyrifos and atrazine, respectively). Dose estimates based on passive dosimetry measurements were dependent on laboratory studies of dermal absorption. Dose estimates from biomonitoring studies were based on laboratory studies of urinary metabolite excretion. In each case, the laboratory data are characterized by very high variability and uncertainty. Given the variability of these data, the Board was not persuaded that this analysis provided a validation of passive dosimetry measurements.

Many studies of pesticide handlers have demonstrated that hand exposure can be a major contributor to total dermal exposure. The heretofore cited OECD guidance document states, "Monitoring of hand exposure may be the most important measurement in a dermal exposure study. The contribution of the hands to total exposure has been well documented by many investigators, using a variety of methods." The Board supported the Agency's plans to evaluate the relative importance of hand, neck and face exposures for each study submitted by the Task Forces.

The Board concurred with the Agency's concerns regarding potential under-estimation of exposure by the handwash and face/neck wipe methods. No justification has been given to support the validity of AHETF's proposal to use "AOT" (or of the AEATF's proposal to use either propanol or "AOT") in the hand wash or/and face/neck wipe sampling methods to assess dermal exposure. A great deal of information is available suggesting that wash data can significantly underestimate exposure and wipe data can be worse than wash data. The Board recommended that the task forces either generate data supporting the efficiency of removing their surrogate pesticides from skin by washing and by wiping, or accept the automatic adjustments being proposed by the Agency; in fact, the adjustment for wipes could even be increased beyond that being proposed for washing. This recommendation is consistent with the advice provided in the 1997 OECD guidance document: "The best that can be achieved for a hand wash or hand rinse method is a laboratory validation of the efficiency of recovery of material from the hands of human volunteers."

Existing data clearly indicate that adsorption (more than absorption) of certain pesticides can occur within a matter of minutes after the exposure has occurred. For example, data presented in Fenske and Lu (1994) show that several handwashings recovered less than 50% of chlorpyrifos from the skin immediately after exposure, and recovered only about 20% from the

hands one hour after exposure. The handwashing efficiency data summarized in Table 1 of a review by Brouwer *et al.* (2000) range from 23 to 96%, an even wider range than the values summarized in the Agency's presentation. These data indicate that the results from washing can range from negligibly biased to a four-fold underestimation of the true exposure, although the frequent washings indicated within the preliminary protocols may limit the bias to about two-fold.

In contrast to hand washing, much of the hand wipe data presented (such as the 10% mean recovery with a CV of 33% for azinphos-methyl from Fenske *et al.* (1999) and the many chemicals with *circa* 50% mean recovery with a similar CV in Table 2 of the review by Brouwer *et al.* (2000)) indicate that head/neck wipes may be both more biased and more variable than hand washes. The above data support the Agency's proposal that, lacking a validation study of the wash method, a 50% adjustment (multiply the results by 2x) should be applied. The above data support an even larger adjustment (of *circa* 3x) for unvalidated wipe data. In both cases, a validation study for recovery efficiency of wash or wipe samples could be tested *in vitro* and would not require human exposure testing. Some Board members also recommended that the Agency explore the use of modeling to adjust hand exposure, and offered as an example an algorithm based on some of the literature cited by the Agency in the SAP documentation.

HSRB Consensus and Rationale

The Board recommended that the validity of dose estimates based on passive dosimetry be reassessed given the variability of previous laboratory data. Given some uncertainty as to exposure to hands, face and neck for various scenarios, the Board concurred with the Agency's intention to evaluate data provided by the task forces for each scenario to determine the relative contribution from skin residues versus whole body dosimeters. The Board recommended that the Task Forces either generate data supporting the efficiency of removing their surrogate pesticides from skin by washing and by wiping, or accept the automatic adjustments being proposed by the Agency; in fact, the adjustment for wipes could even be increased beyond that being proposed for washing. This recommendation is consistent with the advice provided in the 1997 OECD guidance document: "The best that can be achieved for a hand wash or hand rinse method is a laboratory validation of the efficiency of recovery of material from the hands of human volunteers." In addition, the Board suggested a validation study for recovery efficiency of wash or wipe samples could be tested *in vitro* and not require human exposure testing.

Charge to the Board

2) Has EPA identified the relevant scientific and practical considerations affecting the choice to include biomonitoring, and has EPA appropriately characterized the limitations on the scientific usefulness of the resulting data if no biomonitoring is conducted? If not, what other considerations should bear on a decision to conduct biomonitoring in addition to WBD?

Board Response

The HSRB discussed the perspective that, although inclusion of biomonitoring data in the conduct of the AEATF and AHETF research programs was intellectually satisfying, this was not

an easy task. Furthermore, the difficulties in assessing the utility of biomonitoring in the conduct of the Task Force's programs were evidenced by the response previously provided by the EPA's Scientific Advisory Panel. After lengthy discussion, the HSRB recommended that biomonitoring did not need to be included in the AEATF and AHETF programs. The Board felt that the EPA had not fully characterized the scientific and practical issues and considerations relative to the use of biomonitoring. If anything, the HSRB added to the reasons to not use biomonitoring, with the following points emphasized:

1. The goal of the Task Force programs is to describe exposure from a particular use scenario. Biomonitoring provides data that is chemical-specific rather than scenario-specific. Hence, whole body dosimetry is the most appropriate measure by which to provide an estimate of exposure under a specific use condition.
2. Given the list of surrogate chemicals provided by the Task Forces, it is not clear whether biomonitoring is technically feasible and would provide reliable data. Technical feasibility requires that there are well-established analytical methods in place along with knowledge of the metabolism and kinetics of a given compound in order to accurately assess internal dosimetry. Such studies would also increase the complexity of the design and execution, as they would require pre- and post-exposure sample collection, with the post-exposure time period determined by the kinetic properties of the compound.
3. Biomonitoring is important to understanding, determining and estimating risk. The Task Force programs are focused on establishing exposure so as to assess risk, and any risk assessment would be performed in a chemical-specific manner.

Additional points raised during Board discussion included observations that requiring concurrent biomonitoring would impose additional burdens on participants and would severely restrict study participants to those with no recent prior or immediately subsequent exposure to the chemical, a restriction that has the potential to seriously bias the results. Inclusion of biomonitoring would also restrict the range of surrogate chemicals that have sufficiently sensitive metabolites to be useful at the low levels expected in these studies. Furthermore, the variability implicit in back-calculating any detectable biomonitoring data to dermal dose (necessary for use in the data base) is likely to add as much uncertainty as clarity to the conclusions.

HSRB Consensus and Rationale

The HSRB was comfortable with not including concurrent biomonitoring in the protocols. In fact, the Board recommended that the use of additional monitoring units was more appropriate than the inclusion of biomonitoring in these programs.

QA and QC Controls

Charge to the Board

Do the Task Forces' Standard Operating Procedures appear adequate to ensure that the data resulting from the proposed research will be of high quality? If not, what other Quality Assurance or Quality Control procedures need to be addressed?

Board Response

The HSRB noted that the volume of Standard Operating Procedures (SOPs) provided by the Task Forces represents a significant effort to develop the infrastructure required to develop the procedures that govern this work. To this point, it was noted that several of the governing documents provided to the Board contained information that was relevant to the SOPs, and should be added as appropriate. Overall, the SOPs outlining the overall administration, report generation and quality assurance (QA) oversight seem reasonably complete. The HSRB reviewers noted two major areas that should be expanded and/or revised for additional clarity, namely the SOPs that focused on data quality and sample integrity and compliance. Specific recommendations were as follows:

1. The SOPs need to define what represents a "good sample." What general guidance will be provided to define sample quality? How long after completion of work is a sample collected? What tolerances are allowed in targeted airflow or environmental conditions? What conditions determine whether a sample is to be "weathered" and how will "weathering" be performed?
2. On-site spiking of samples is intended to be used for analytical standardization and reliability. No details were provided on how such samples were generated, and how they were handled to simulate actual exposure conditions.
3. The roles of the study director and the principal investigator are unclear and should be expanded.
4. There was a recognized need for training in the execution of these studies along with information detailing how compliance to the protocol would be established.
5. The SOPs should provide for the means by which incidents relating to lack of compliance or possible negligent conduct can be reported.

HSRB Consensus and Rationale

Overall, the Standard Operating Procedures (SOPs) outlining the overall administration, report generation and quality assurance (QA) oversight seems reasonably complete. The Board noted two major areas that should be expanded and/or revised for additional clarity, namely the SOPs that focused on data quality and sample integrity and compliance.

Design of scenario-level sampling strategies

Charge to the Board

With regard to the AHETF and AEATF plans to conduct their proposed handler research using purposive diversity sampling strategies:

1) Has EPA identified the relevant scientific and practical considerations affecting the choice of a strategy for sample selection? If not, what other considerations should bear on the choice?

Board Response to the Charge

The Task Forces have proposed study designs focused on scenario-level sampling. The target population was considered to be the set of all possible handler-days in which scenario-specific tasks would be performed. It was estimated by the AHETF that this would include approximately 1.1 million handlers and approximately 2 million handler-days. The Agency considered two approaches for gathering a probability sample: a simple random sample and a complex probability sample. For example, the National Health and Nutrition Examination Survey (NHANES) has used complex probability sampling to sample a representative U.S. population. Considerations associated with complex probability sampling for the Task Force exposure monitoring programs include likely high cost, the absence of a sampling frame, and the likelihood of significant selection bias.

In light of these issues, the Agency and the task forces have considered two alternative sampling strategies: purposive representative sampling and purposive diversity sampling (PDS). Purposive representative sampling captures a small sample of handler-days that is a "miniature" of the target population, with respect to important factors concerning the range and extent of exposure, while PDS captures a small sample of handler-days that are diverse with respect to factors related to the range and extent of exposure. The task forces have proposed PDS as the strategy more likely to reflect a broad range of heterogeneous conditions. PDS can be diversified on the amount of active ingredient handled, the individual (MU), location and time, and other factors (such as equipment type, crops, rates, and micro-location). Site selection will emphasize more common conditions and the task forces will be required to provide a rationale and/or justification for selection of sites or site conditions based on diversity criteria.

The Task Forces' statistical consultants have argued that PDS permits a non-random sample to perform at least as well as a small, same-sized probability sample. It provides greater assurance of obtaining a sample that reflects a broad range of conditions, and makes it less likely that high end or low end exposure conditions would be missed. Augmenting scenario data with new clusters in the future would be straightforward, and conditions of interest would be easier to target. Nonetheless, it was acknowledged that PDS is not a probability-based sample and can only be used to establish a surrogate distribution of exposures. A surrogate distribution cannot be equated to the actual distribution in a target population using pure statistical sampling theory; however, PDS can capture major aspects of an actual distribution. The Task Forces assert that the results using this type of sample are not expected to be substantially different from those derived using a small, same-sized cluster random sample. Therefore the Task Forces argue PDS should also be considered adequate for practical regulatory purposes.

The EPA's Scientific Advisory Panel (SAP) has expressed concern with the proposed purposive nature of sample selection because PDS assumes underlying random selection can be used to estimate sample sizes. In Appendix C of its January 2007 report, the SAP provided a discussion of potential for bias and an alternative stratified approach. The SAP expressed concern that use of a non-probability sample would essentially preclude consideration of appropriate weighting to estimate distributional parameters including means, standard deviations, upper percentiles, etc. Thus, the SAP recommended an informal approach for identifying top factors and for assigning probability weights to approximate frequencies. In response to the SAP concerns, the task forces have outlined the constraints regarding available data and resources.

The Agency plans to evaluate the data and documentation that will be submitted by the task forces to support their approach. However, given the unique aspects of this monitoring program and its relatively small size, the Agency continues to believe that PDS is adequately representative of the target population and can be used to develop exposure assessments of occupational handler populations.

Critique

The Board commended the Agency and the task forces for the work they have conducted over the past year to develop a viable sampling strategy for pesticide handler and consumer exposures. However, the Board had a number of questions regarding the sampling strategy, and in particular the selection of purposive diversity sampling as the foundation for these studies. The more practical aspects of the sampling strategy are addressed in this section of the Board's report, while certain scientific aspects are addressed below in response to specific charge questions put forth by the Agency.

A central consideration in the sampling strategy design is the choice of key variables that will define the scenarios and the particular tasks carried out by workers within these scenarios. The Board was not clear as to the criteria by which these variables would be selected. One variable that appeared central to the proposed study design was the amount of active ingredient handled. The Agency currently normalizes exposure based on this variable. The task forces would like to collect data sufficient to test whether or not this is a sound scientific practice. Thus, the study design calls for collecting data over a wide range of values for this variable. It was not clear to the Board whether this particular scientific question should be an important driver of the study design. There may be other factors equally worthy of study. For example in the AHETF study crop, type of equipment, or mixing and loading procedures. One variable – farm size -- was of particular concern to the Board based on practical experience. Farm size tends to be correlated with many other factors that can influence exposure, such as different sizes of equipment used, different training procedures, behaviors, and application details. Larger farms tend to have larger and more modern equipment with more technical/engineering controls, make more timely applications, and use more innovative application practices. The Board also indicated that the level of training of study participants was an important variable to consider in the study design. In the United States, pesticide handlers do not need to be certified to mix, load and apply pesticides in many situations; instead they work under the supervision of a certified applicator. There are important differences in training by a supervisor versus certification training. Specific to the AHETF protocols, the Board concluded that any attempt at diversity sampling should include an appropriate number of non-certified applicators.

For both the AHETF and AEATF studies the Board recommended that prior to data collection the Agency ensure that the critical variables associated with exposure are ranked, accompanied by an appropriate rationale and justification for the ranking. This ranking would then inform the study design in terms of how sampling sites and individual participants are selected.

HSRB Consensus and Rationale

The Board acknowledged the great complexity of study design development, given the many variables associated with exposure and the practical constraints that arise in the conduct of human exposure studies. The Board commended the Agency and the task forces for their efforts in developing a detailed discussion of sampling strategies. The Board remained concerned that the number of variables is large, and that the relative importance of these variables has not yet been defined adequately.

For both the AHETF and AEATF studies, the Board recommended that prior to data collection, the Agency ensure that the critical variables associated with exposure are ranked, accompanied by an appropriate rationale and justification for this ranking. This ranking would then inform the study design in terms of how sampling sites and individual participants are selected.

Charge to the Board

2) Does the HSRB agree with EPA that the Task Forces should provide scenario-specific information about the availability of data to identify significant variables (other than AaiH) potentially influencing exposure and about the feasibility of developing a sampling strategy to address those variables quantitatively? If not, what additional information is needed?

Board Response to the Charge

To be scientifically assessed, and for the Board to provide its scientific advice, scenario-specific information needs to be provided. This information would necessarily include significant variables that would potentially influence exposure and its effect, in addition to AaiH, and a feasible sampling strategy, that would be essential to meet the scientific criteria to provide reliable and useful data. Such would reaffirm the EPA Scientific Advisory Panel's recommendation that all major factors of importance be included in each scenario-specific study to be conducted. The information could be provided briefly for the Board to consider, not necessarily to the extent provided by the AEATF example. It could be presented as one would a study design for the scenario-specific study to be evaluated (with appropriate references) and would contain in such design not only the specifics of the population (including its size) to be studied but also the list of variables and how they were to be collected and analyzed. It could also briefly respond to the scientific criteria proposed previously by the Board.

Critical to the Board's evaluation and to the representativeness and usefulness of the exposure data collected and provided, would be the information as to the relationship between the scenario-specific exposure assessment and the representative exposure in such scenarios in the target population.

In terms of the relevant variables, one could group them into those that are essential or less so, and whether they would significantly affect the exposure (or exposure scenario and data collection) or might be important to have. Description of the target population and the subjects selected are essential, including inclusion and exclusion criteria. Description of the primary measuring instruments and how they are to be used is essential. Because environmental

conditions are significant determinants of exposure, essential environmental variables (e.g., site description, temperature and humidity) would have to be measured, and others (e.g., wind speed, microclimatic conditions [including, presence of significant factors that affect airflow]) might or might not be necessary. The subjects' type of external clothing could be considered essential, and the subjects' work history with the type of application and pesticide would be important, whereas minor differences between the subjects would not; gender and age would be necessary. It might be important to record, if possible, the subjects' health status and any physical deformities that might influence how they handle the pesticide and perform the application required. It would be worth noting by observation and recording significant features of the handling and application. Of course, prior experience and documented studies of this nature will illustrate what was and should have been critically measured.

Known variability in similar data previously collected, including inter- and inter-subject variability in exposures, would even help design the study and determine the type and size of the population to be studied. Finally, and repetitiously, how one would analyze these variables is important to know.

HSRB Conclusion and Rationale

The Board recommended that the following information was necessary for it to provide its scientific advice on scenario-specific information:

- Scenario specific information detailing variables that might influence exposure and its effect;
- A feasible sampling strategy including specifics of population to be tested (including its size), a list of relevant variables and how they would be collected and analyzed;
- Information on relationship between scenario-specific exposure assessment and the representative exposure in such scenarios in the target population;
- Essential environmental variables including site description, temperature, humidity, wind levels as well as subjects' external clothing, work history and type of pesticide application;
- Relevant data on inter-subject variability;
- Data analysis plan.

Charge to the Board

3) Has EPA appropriately characterized the limitations on the scientific usefulness of the resulting data attributable to the choice of the sampling strategy? If not, what has EPA overlooked?

Board Response

The AHETF and AEATF reports make a persuasive argument in favor of purposive sampling. The reports are very well written and the issue of sampling has clearly received much attention. The task forces should be commended for a thorough and rather sophisticated analysis.

Many of their points are well taken. The limitations of the proposed sampling strategy have not been fully discussed.

No one knows what factors affect exposure. It appears that not only are there myriad factors but that their importance is not clear. The only way to protect against potentially significant biases introduced by unknown or unaccounted for factors is through randomization.

Purposive sampling has a definite role to play in *qualitative* research and in fact is most common in applied social sciences applications. The goal in qualitative studies is often to explore and describe a universe. In quantitative studies, in contrast, the goal is typically to obtain a point estimate of some quantity and also a measure of the **uncertainty** around that estimate. Because purposive sampling includes no random mechanism, the machinery of probability theory is not available to the researcher and thus an estimate of the uncertainty around point estimates cannot be computed. As a consequence, results from a purposive sample **cannot be generalized beyond the sample** except on faith. An example of faith is the discussion in the reports that state that the estimated exposure distributions obtained from the purposive samples will approximate the true exposure distributions at least in the major attributes.

It is true that purposive sampling or its close relative, judgment sampling, is sometimes justifiable when the sample is very small. However, this is true if in addition, the universe is also small and its characteristics are known to the investigator. One argument given against the use of a probability sample in the report is that the sampling frame (the universe) is unknown and difficult to characterize. Unfortunately, this fact also diminishes considerably the advantages of purposive sampling even in small samples (see, e.g., Jessen, 1978, *Statistical Survey Techniques* one of the standard reference survey sampling books).

A basic question arises: "how small does the sample have to be before non-random selection is a better option?" The answer is "very small." It has been argued (again, see Jessen and others) that with sample sizes as small as about 8 or 10, the advantages of the non-random sampling mechanism vanish.

The relevant sample size for comparing sampling designs is the effective sample size and not the number of MUs in a cluster (5) as is presented in the review documents. For a 5 x 5 sampling matric and an assumed ICC of 0.3, the effective sample size is 1 (see Snijders and Bosker, pg. 23). A sample of 11, while small, is large enough to eliminate or at least decrease the advantage of purposed sampling over probability sampling (see Jesssen, 1978, Chapter 1).

In most cases of judgment or purposive selection, a bias will occur. The bias can be negligible or very large, depending on the person actually carrying out the selection. Regretfully, the bias will always be unknown. This is not obvious from the very nice simulations shown in Appendix B of the AHETF report. From some of those simulation studies, it would appear that the biases associated with purposive sampling can be small. This is true if we have a large number of purposive samplers and average over them (central limit theorem). However, a single sampler (even over repeated sampling exercises) can introduce very large biases that are very difficult (or impossible) to quantify or anticipate.

Reliability (defined as the closeness of each observation to its own average over repeated trials) is typically higher in purposive samples than in random samples of similar size. Accuracy (also known as validity, a combination of bias and sampling error and a measure of closeness of an estimator to the targeted value) however is difficult to forecast and can be quite low. Because non-random samples provide no means for computing accuracy, the value of such samples is rather questionable.

One major argument against at least a quasi-random selection of MUs appears to be the cost of selection. However the Board believed the argument is not convincing. Major expenses would be incurred if MUs were to be randomly selected from the entire universe of MUs for a given scenario. But if a stratification step is carried out purposively (as proposed here) the random selection of MUs within cluster should not significantly add to the cost. Suppose that the five clusters are selected purposively. Given a cluster and relative to the actual cost of collecting the dosimetry data, it should be possible for example to first randomly select firms and within firms, randomly select handler-days. Not everyone will agree to participate and not everyone might be handling the desired products on the selected dates, but all that will require is a few additional visits or phone calls to the selected firms.

Random sampling is no more impractical and may be no more costly within the context of costs of the whole study than PDS. Irrespective of response rate, random sampling provides more information regarding the representative of participating sites or operations.

To assist the Agency, the Board recommended the following approach:

- Purposively select locations.
- Within location, list operations.
- Roughly stratify producers by crop and by size.
- Randomly select operations and within operations, randomly select operators. Observe them next time they apply the chemical of interest.

HSRB Consensus and Rationale

The major limitation of non-random sampling is that it provides no means for estimating the error associated with any estimate based on the sample. The exposure distributions based on this type of sample might or might not be anywhere close to the true exposure distributions and there is no way to tell if the results are representative or not. If the estimated exposure distributions will be used for by EPA, it is important to base those estimates on samples that at least approximate a random sample and that permit obtaining data-driven estimates of uncertainty around quantities of interest. Error estimates and other estimates relevant to determination quantities in the AEATF and AHETF reports are based on strong and un-testable assumptions (NRC 1994, 1991, and 1983).

Statistical justification for number of clusters and monitoring units

Charge to the Board

What additional information, if any, would the HSRB need to assess the adequacy of the justification for the number of clusters and number of MUs in specific AHETF and AEATF study proposals?

Board Response to the Charge

The primary objective of the AHETF and AEATF II human exposure monitoring programs is to collect sufficient data for each handler scenario to characterize the distribution of the exposure level, both dermal and inhalation. In other words, both programs are interested in knowing the statistical distribution of the exposure level within an acceptable bound (K) for their relative accuracy. The fold relative accuracy (fRA) measures how far the sample estimate is from the true parameter in a relative sense. The sample size estimation for both programs is based on the same justification of a 3-fold accuracy (K=3), i.e. fRA is less than or equal to 3, for the parameter of interest.

In both programs, the purposive sampling is used to select clusters. However, the sampling of MUs within clusters will be random. Therefore it requires a set of assumptions for the "surrogate sampling model":

1. Observed exposures are viewed as arising (at least approximately) from a random sample of clusters and then from a random sample of MUs within each cluster.
2. The sampling distribution of normalized exposures within and between clusters is, at least approximately, lognormal.

The second assumption is reasonable, and even if it is violated, its impact should be minimal. However, the first assumption is problematic. Depending on how the clusters and the MUs within clusters are selected, bias can be introduced in a way that cannot be corrected. Therefore the sample size justification based on Monte Carlo simulations has its limits.

Under these assumptions, the sample size is estimated based on the nested variance component model for the normalized exposure level. In order to determine the relative accuracy of the estimates of the parameters associated with the statistical distribution for the exposure level, one needs as design parameters reasonable estimates for the geometric standard deviation (GSD) of the exposure level and the "intra-cluster" correlation coefficient, i.e. intraclass correlation coefficient (ICC) due to cluster sampling.

Nested lognormal variance component assumptions were used in a surrogate-sampling model to determine the sample sizes necessary to achieve a 3-fold relative accuracy of distributional parameter estimates. Reasonable values for the GSD and the ICC of exposure normalized by the amount of ai handled were obtained from an analysis of existing data.

The Board provides specific comments to each task force proposal as noted below

AHETF: The GSD and ICC were estimated respectively as 3.8 and 0.26 for normalized dermal exposure and 4.2 and 0.37 for inhalation exposure from the AHETF monitoring data

based on the nested variance component model. For planning purposes, a GSD of 4.0 and ICC of 0.3 seems reasonable defaults for both dermal and inhalation exposure. Simulation analyses indicate that N_c=5 clusters with N_m=5 MUs per cluster will achieve the desired benchmark goal and is more cost-effective than other feasible configurations. As long as a cluster size of 5 is not exceeded, the same total number of MUs (N=25) will also achieve this same level of relative accuracy even if the number of MUs per cluster varies slightly.

AEATF II: A GSD of 2.86 was derived from four dermal exposure monitoring studies (three by the Chemical Manufacturers Association in wipe, mop and aerosol-hands setting and one by the Pesticide Handlers Exposure Database in aerosol), a coefficient of variation 1.42 was derived from the log-scale standard deviation under the assumption of a lognormal distribution, and an ICC between 0 (independence among observations within a cluster) and 0.3 (a moderate dependence) was assumed. Simulation analyses indicate that N_c=3 clusters with N_m=6 MUs per cluster will achieve the desired benchmark goal and is more cost-effective than other feasible configurations.

The recommended sample size of five clusters with five MUs per cluster for the AHETF program and three clusters with six MUs per cluster for the AEATF II program is considered a 'default' or 'standard' configuration only. It strictly applies only to scenarios without existing data and when the default variability is GSD=4 or 2.86 and ICC=0.3, respectively, for the AHETF and AEATF II program, and benchmark accuracy (K=3) is considered reasonable. In other cases, the simulation techniques can be used to develop optimal sampling plans for each scenario it addresses.

The AHETF and AEATF II human exposure monitoring programs have done an outstanding job of considering the effects of both the numbers of clusters and the numbers of MUs within each cluster.

Given the assumptions made regarding the surrogate sampling, the Governing Documents from the AHETF and the AEATF II human exposure monitoring programs provide a very thorough justification for the sample size in terms of the number of clusters and the number of MUs per cluster in specific AHETF and AEATF II study proposals. The sample size justification includes determination of feasible values of N_c and N_m, optimal configuration of N_c and N_m based on relative cost of sampling cluster *vs* MUs, sensitivity of the relative accuracy bound to the GSD and ICC, and the impact of unequal number of MUs per cluster which may be expected when MUs drop out. It also recognizes its limitations and suggests scenario-specific simulation studies to estimate adequate sample size.

AHETF's initial conclusion that a total of 25 MUs is needed provided that there is no more than 5 MUs per cluster appears valid and likely to be very useful. However, the choice of only three clusters by the AEATF seems risky and it should be increased if at all possible. Three clusters will only give 2 degrees of freedom for estimating the cluster variance component, and making statistical inferences based on samples of size 3 would be considered less than desirable. It seems that the major cost associated with the protocols for the AEATF database is with analyzing the measurement data, and using only three clusters is a matter of convenience. The

AEATF II program should give consideration to increasing the number of monitoring units to 25 with no more than 5 MUs per cluster, which results in at least five clusters per scenario

HSRB Consensus and Rationale

If the purposive sampling method is selected as a surrogate for a probability sampling, no additional information seems to be needed for the HSRB to assess the adequacy of the justification for the number of clusters and the number of MUs in specific AHETF and AEATF II study proposals (however, as noted previously, the Board raised serious concerns about the purposive sampling strategy

As the sample size justification for the AEATF II program is based on the ICC estimate from the AHETF program, it is recommended that the AEATF II program update the proposed sample size based on their study in the future to verify whether the ICC estimates agree with that from the AHETF program.

Within-Worker variability

Charge to the Board

Has EPA appropriately characterized the limitations on the scientific usefulness of a database that does not include repeated measures? If not, what limitations has EPA overlooked?

Board Response

A database that does not include repeated measures will have limited usefulness in the context of some analysis goals but not in the context of others.

With a dataset that includes one observation per person it is not possible to obtain an estimate of the within-person variance in exposure. This variance reflects the variability in exposures of a handler across different days, even if using the same product and the same application equipment and can be quite large. The impact of ignoring the within-person variance is directly proportional to the relative sizes of the within to the between person variance in exposure.

If the objective of the study is to obtain an estimate of the *mean* exposure of workers in a given scenario, or to obtain an estimate of the on-day distribution of exposure, then a single observation per person will suffice. This is true even when the quantity of interest is a median, a geometric mean or in general, any distributional attribute associated with the center of the distribution. We refer to this as the distribution of *usual exposures* under a given scenario. Ideally, this usual exposure distribution would be estimated from the mean handler exposures computed from observing each handler during a large number of randomly selected days. This is clearly an impractical approach. Alternatively, it can also be estimated from a database that includes at least one replicate observation on at least a randomly selected sub-sample by fitting the appropriate random effects model (see, e.g., Nusser, Carriquiry, Dodd and Fuller, *Journal of the American Statistical Association,* 1996; Carriquiry, *Public Health Nutrition,* 1999;

Carriquiry, *Journal of Nutrition,* 2003). This approach has been recommended by both the US National Academy of Sciences (2002) and by the World Health Organization (2006) for estimating the distributions of usual exposures to components in food and drinking water.

The estimated distribution that is based on a single measurement,has a variance that includes the between-person variance and the within-person (or day-to-day) variance. The latter is problematic and inflates the overall variance of the exposure distribution by an amount that can be quite significant. As a consequence, upper-tail quantiles of the exposure distribution tend to be overestimated. We refer to this estimate as the one-day estimate of the distribution.

From a regulatory stand-point, using the one-day distribution in lieu of the usual exposure distribution tends to be conservative. That is, estimated percentiles such as the 95th percentile will typically be larger than what they would be if estimated using the distribution with the correct variance. In this sense, the one-day distribution is *protective*. If the sample of MUs in each scenario were large enough to allow estimation of the one-day distribution, then using the one-day distribution as a proxy for the usual exposure distribution would be acceptable.

The reports by the AHETF and AEATF argue strongly against collecting within-person repeated observations. The two main arguments are the following:

1. Cost: given the already rather small sample sizes per scenario, collecting replicate observations would imply increasing the number of measurements since reducing the number of MUs is not reasonable.
2. The distribution of mean exposures can be analytically derived given information about the one-day distribution of exposures.

It is difficult to argue with issues of cost. While any experiment involving replication would indeed be more costly, it is possible to minimize the additional cost by using an efficient design..

The second argument, however, is not convincing. First, it is not at all a given that the log-normal is the correct probability model for exposures under all possible scenarios. Even if the log-normal model was the appropriate model, the report confuses the distribution of within-person means with the distribution of the mean of a log-normal. Those two distributions are in general not the same unless we are willing to believe that the within-worker exposure distribution (i.e., the distribution of exposures in a worker observed during a very large number of days) is also log-normal. Thus, without imposing the log-normal model at the worker level as well, the Board was not sure it is possible to analytically derive the distribution of the conditional expectation of daily exposure given handler (which is the distribution of interest).

The task forces propose "borrowing" an estimate of the intra-class correlation in order the "correct" the one-day distribution so that it will better approximate the usual exposure distribution. In principle, using an external estimate of the within-person variance (or of the ICC) might be reasonable, but the actual estimate needs to be carefully selected and very well justified. Is it reasonable to expect, for example, that the within-person variance in exposure will be the same across scenarios?

The limitations introduced by the lack of replication and the assumed model are compounded by the very small sample sizes. Without imposing the log-normal model on the data it would not even be possible to obtain a point estimate of the 95th percentile (at least a reliable one) let alone get an estimate of its standard error. In addition to allowing for estimation of the two relevant variance components, replicate observations would increase the sample size even after accounting for correlation between observations collected from the same person.

HSRB Conclusion and Rationale

The lack of choosing a within-person design is limiting if the Agency wishes to obtain data on usual exposures. However, if the Agency wishes to collect data on one-day exposures, their approach is not limiting, but the Board recommends that as many handler as possible be observed as costs would allow.

Subject recruitment and enrollment issues

Charge to the Board

1) Does the Board agree that the Governing Documents and associated SOPs of the AHETF and AEATF research programs include comprehensive and appropriate protections for human subjects of the research? If not, what has been overlooked?

Board Response

The Board was very impressed with the quality of the work done, and the amount of effort that has clearly gone into producing these documents. The Board had only a few suggestions:

The documents might provide greater clarity regarding input from organizations that represent the subjects (such as farm labor organizations or advocates). For example, at page 94 of 468 of the AHETF materials, it indicated that such organizations "might" be invited to provide input with regard to a particular study. It could be helpful to provide some guidance regarding those situations in which the participation of such organizations would be especially important. In addition, such organizations could also provide useful advice with regard to the Governing Documents and associated SOPs themselves, and not merely limited to particular protocols.

The sample consent form that was provided would benefit from increased attention to the reading level. In a variety of places (e.g., in discussing how compounds will be applied to "vertical" and "horizontal" surfaces), the language could be rewritten at a lower reading level (e.g., by including examples referring to floors, walls, and blinds), particularly given the likely educational background of many of the subjects.

With regard to pregnancy testing of subjects, the AEATF and AHETF documents appear to take different approaches. The former group tests only subjects under age 50, while the latter group tests all female subjects. It would seem appropriate to use the same standard in both types of studies, or else to provide a justification for the difference.

Also on the issue of pregnancy testing, the documents mention that if a pregnancy test is positive, the records relating to that person would be discarded. It would be better to use a word such as "shredded," to make it clearer that the documents will be destroyed, not merely put aside or thrown out where they could be accessed by others. .

Given the language, economic and educational characteristics of many of the prospective subjects, it would be appropriate for the documents to consider additional specific measures that might better protect a worker's right to say no. For example, protocols might be designed so that substantially less than 100% of an employer's workers can participate in the study (e.g., only 5 out of 10). By taking this measure, it would decrease the likelihood that an employer could determine which employees chose not to participate, because the employer could not be sure that some of them were merely unable to participate due to the limit on participation.

HSRB Consensus and Rationale

The Board agreed that the Governing Documents and associated SOPs do include comprehensive and appropriate protections for human subjects.

Charge to the Board

2) In singling out the handling of language differences as an area requiring further refinement, has EPA overlooked other areas in need of revision? If so, what?

Board Response

The Board reaffirmed the importance of making all paper and electronic documents distributed to study volunteers available in both English and Spanish. The Board also expressed strong support for the need for both Spanish- and English-speaking study staff to be available at study sites to answer any questions that research volunteers may have regarding the study or their participation in the study. Spanish- and English-speaking staff should also be available during other study interactions with research volunteers, including data-collection encounters and via telephone lines that are made available to subjects who may have questions about their participation in the study or rights as a research volunteer. The Board expressed concerns about reliance on translators of convenience, such as co-workers and others who may lack sufficient familiarity with the study.

The Board expressed support for the Agency's proposal to have impartial third-party witnesses observe the consent process when a research subject is unable to read relevant study documents. As specific studies are proposed, however, it will be important for investigators to describe the procedures to be employed in recruiting these witnesses. It would be inappropriate, for example, to ask translators to serve as witnesses (as suggested in the materials reviewed by the Board), because one of the main purposes of employing a witness is to ensure that the communication of study-related materials is adequate (and the translator would be conflicted with regard to that assessment). If feasible, the Agency may wish to consider using impartial

"consent monitors" or "research subject advocates" as witnesses, as is increasingly done in certain clinical studies.

The Board also returned to an issue raised at the last Board meeting. At that earlier meeting the Board suggested that in many areas of the U.S., agricultural workers often speak neither English nor Spanish but instead speak another language, such as one of several indigenous languages of Northern Mexico. The Agency may wish to consider this possibility (and its implications for sample bias and just distribution of research benefits and risks) in deciding whether to restrict eligibility to English- and Spanish-speaking subjects.

HSRB Consensus and Rationale

The Board agreed that the handling of language differences is an area requiring further refinement and is appropriate to protections for human subjects. Related issues include mechanisms to ensure understanding and voluntariness in the consent process.

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