October 26, 2009

EPA-HSRB-09-02

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

Subject: June 24-25, 2009 EPA Human Studies Review Board Meeting Report

Dear Dr. Teichman:

The United States Environmental Protection Agency (EPA or Agency) requested the Human Studies Review Board (HSRB) to review three completed studies involving intentional human exposure to the organophosphate pesticide chlorpyrifos. The Agency proposes to rely on these three studies, conducted prior to publication of the EPA's expanded final rule for protection of subjects in human research (40 CFR 26) on February 6, 2006 (71 Federal Register 24, 6137), in actions under the pesticide laws. The Agency asked the HSRB to advise the Agency on a range of scientific and ethics issues regarding how the studies should be assessed against the provisions in 40 CFR sections 26.1701 – 26.1704 of the final human studies rule.

The Agency also requested the HSRB to provide scientific and ethical reviews of one completed and two proposed human studies: a completed ICR, Inc., (ICR) insect repellent efficacy study (ICR A382); a proposed Carroll-Loye Biological Research, Inc. (CLBR) insect repellent efficacy study (LNX-002); and a proposed Agricultural Handler Exposure Task Force, LLC (AHETF) water-soluble packing mixing and loading scenario and protocol (AHE-120).

The enclosed report provides the Board's response to EPA charge questions presented at the June 24-25, 2009 meeting.

Assessment of Completed Research Study MRID 124144: Nolan *et al.* (1982) Chlorpyrifos: Pharmacokinetics in Human Volunteers Following Single Oral and Dermal Doses.

Science

- The Board concluded that data measuring chlorpyrifos at the limit of detection in blood and urine are not useful. The measurements of 3,5,6-trichloro-2-pyridinol (TCP) in urine are generally reliable. It is unclear if the measurement of TCP in blood is useful because of concerns about the ability of the methods used to detect the appropriate TCP conjugate.

- The Board expressed concern over the variability of the erythrocyte cholinesterase activity data and the lack of a control group, but concluded that measurements of cholinesterase activity/inhibition from Nolan *et al.* (1982) were likely to be reliable.

- The Board concluded that the data are reliable but suggested that the Agency be cautious in its use of the erythrocyte cholinesterase data. Since only a single dose level was evaluated, for example, no dose-response data are available from this study.

Ethics

- The Board concurred with the Agency's assessment that there was neither clear and convincing evidence that the study was fundamentally unethical, nor clear and convincing evidence that the study was significantly deficient relative to the ethical standards prevailing at the time the Nolan *et al.* (1982) study was conducted.

Assessment of Completed Research Study MRID 42062701: Honeycutt and DeGeare (1993) Worker Reentry Exposure to Chlorpyrifos in Citrus Treated with Lorsban* 4E Insecticide.

Science

- The Board concluded that the blood and urine measurements of chlorpyrifos and/or TCP from Honeycutt and DeGeare (1993) are likely reliable but of limited value. Board members expressed concerns about: 1) the small sample size; 2) the background chlorpyrifos exposure not being properly accounted for; 3) the likely incompleteness of urine collection; and 4) the high degree of variation seen in the daily measurements.

- The Board concluded that the measurements of cholinesterase activity/inhibition are likely accurate and reliable but raised concerns about their utility. Some of the Board's concerns included: 1) the lack of an untreated control; 2) the reliance on a single post-exposure time point; 3) the small sample size; and 4) the dose received by the subjects varied and was only estimated from dermal dosimetry.

Ethics

- The Board concurred with the Agency's assessment that there neither was clear and convincing evidence that the study was fundamentally unethical, nor clear and convincing evidence that the study was significantly deficient relative to the ethical standards prevailing at the time the Honeycutt and DeGeare (1993) study was conducted.

Assessment of Completed Research Study MRID 44811002: Kisicki *et al.* (1999) A Rising Dose Toxicology Study to Determine the No-Observable Effect-Levels (NOEL) For Erythrocytes Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels.

Science

- The Board raised concerns about the analytic procedures' potential inability to control for the glucuronide-conjugated TCP, and apparent discrepancies in the absorption data when compared with the data from Nolan *et al*. (1982). Thus, the Board questioned the reliability and utility of the blood and urine measurements of chlorpyrifos and/or TCP from Kisicki *et al.* (1999) for risk assessment purposes.

- The Board concluded that these measurements of cholinesterase activity/inhibition likely represent a reliable set of data, but cautioned the Agency about relying on data from the incomplete profile for the one responder at the highest dose level.

- The Board cautioned against relying on the statistical analyses as presented in the report, recommending that these analyses be replicated prior to applying these results to any risk assessment or model development.

- The Board concluded that, in the absence of additional information about the analytic methods used and the accuracy of the TCP measurements, the TCP data is of questionable reliability. If the level of exposure cannot be confirmed as reliable, then by default the erythrocyte data should not be used. The Board thus suggested that the Agency be cautious in its use of the erythrocyte data in light of the variability and analytical concerns raised.

Ethics

- The Board concluded that there neither was clear and convincing evidence that the study was fundamentally unethical, nor clear and convincing evidence that the study was significantly deficient relative to the ethical standards prevailing at the time the Kisicki *et al.* (1999) study was conducted.

Assessment of Completed Research Study MRID 47732701: ICR, Inc. Study A382 – Evaluation of the Efficacy of KBR 3023 (Picaridin; Icaridin) - Based Personal Insect Repellents (20% Cream and 20% Spray) Against Stable Flies in the Laboratory.

Science

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

- The Board recommended correcting several of the statistical analyses present in the report. For example, the standard error for mean protection time and resulting confidence interval needs to be corrected to use the estimated standard error, not the planned standard error. The report should also be corrected to indicate that the data presented are for product failure times and not complete protection times.

Ethics

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

Assessment of Proposed Carroll-Loye Biological Research Study LNX-002: Efficacy Test of KBR 3023 (Picaridin; Icaridin) - Based Personal Insect Repellents (20% Cream and 20% Spray) with Biting Flies Under Field Conditions.

Science

- The Board concurred with the Agency's assessment that this protocol, if modified according to HSRB recommendations and conducted accordingly, will provide scientifically valid results on the efficacy of these two picaridin-based insect repellent formulations against biting flies.

- The Board recommended that the protocol be revised to address the following concerns: 1) the standard of biting used may be insufficiently high to yield valid results and could lead to inappropriately right censored data; 2) the change from the previously-used paradigm of one minute exposure of treated limbs out of each 15 minute period to five minutes in each 30 minute period was not explained or justified; 3) the varied behaviors and aggressiveness of four different species of biting flies to be examined; and 4) accurate calculation of mean complete protection time.

Ethics

- The Board concluded that the protocol submitted for review, if modified in accordance with Agency and HSRB recommendations and conducted accordingly, is likely to meet the applicable requirements of 40 CFR 26, subparts K and L.

Assessment of Proposed AHETF Scenario and Protocol AHE-120: Water-Soluble Packing Mixing and Loading.

Science

- Given the lack of existent reliable and sound data in this area, the Board concurred with the Agency's assessment that this protocol will generate data that are scientifically valid. These data may be useful for assessing the exposure of handlers who mix and load soluble or wettable powder pesticides in water-soluble packaging.

- The Board cautioned that these data are likely to be useful for creating distributions of worker exposure only if worker exposure is found to be proportional to the amount of active ingredient handled (AaiH).

The Board recommended that the AHETF and the Agency acknowledge the limitations of the study design and add appropriate statistical methods or data management approaches to ensure that, once these data are inside the AHED® database, the limitations of the original study design are not forgotten and the data used inappropriately by end-users to generate typical statistical distributions.

Ethics

- The Board concluded that the protocol submitted for review, if modified in accordance with Agency and HSRB recommendations and conducted accordingly, is likely to meet the applicable requirements of 40 CFR 26, subparts K and L. In particular, the Board recommended that the AHETF implement the proposed protocol changes designed to address issues of representativeness language in the informed consent and related documents.

- The Board recommended that the AHETF release individual exposure data only once the study is complete, except in those instances where data collected from individuals suggest an unusually high level of exposure and thus a clear need to mitigate exposure risks.

Sincerely,

Sean Philpott, PhD, MSBioethics
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.

US ENVIRONMENTAL PROTECTION AGENCY
HUMAN STUDIES REVIEW BOARD

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* Not in attendance at June 24-25, 2009 Public Meeting

INTRODUCTION

From June 24-25, 2009, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB) met to address scientific and ethical issues concerning: three completed human toxicity studies involving one pesticide active ingredient – chlorpyrifos – conducted prior to publication of the EPA's expanded final rule for protection of subjects in human research; and one completed study involving one insect repellent active ingredient – picaridin – conducted after promulgation of the EPA's expanded final human studies rule. In accordance with 40 CFR 26.1602, EPA sought HSRB review of these completed studies. Each of these studies is discussed more fully below.

In addition, the Agency submitted two protocols for conducting new research involving human participants: one study measuring the efficacy of two registered insect repellents containing picaridin against biting flies under field conditions; and one study measuring levels of exposure received by agricultural handlers when mixing and loading pesticides using water-soluble packing under various conditions. In accordance with 40 CFR 26.1601, EPA sought HSRB review of these proposed protocols. Each of these studies are also is discussed more fully below.

REVIEW PROCESS

On June 24-25, 2009, the Board had a public face-to-face meeting in Arlington, Virginia. Advance notice of the meeting was published in the Federal Register as "Human Studies Review Board; Notice of Public Meeting" (74 Federal Register 106, 26861).

During the public meeting, following welcoming remarks from Agency officials, the Board heard presentations from the Agency on the following topics: three completed studies involving intentional human exposure to the organophosphate pesticide chlorpyrifos (Nolan et al. (1982), Honeycutt and DeGeare (1993), and Kisicki *et al.* (1999)); a completed ICR, Inc., (ICR) insect repellent efficacy study (ICR A382); a proposed Carroll-Loye Biological Research, Inc. (CLBR) insect repellent efficacy study (LNX-002); and a proposed Agricultural Handler Exposure Task Force, LLC (AHETF) water-soluble packing mixing and loading scenario and protocol (AHE-120).

The Board also asked clarifying questions of several study sponsors and/or research investigators, including:

Dr. Craig Barrow, Consultant to Dow AgroSciences
Dr. Victor Canez, Technical Chair, Agricultural Handler Exposure Task Force
Dr. Scott Carroll, Principal, Carroll-Loye Biological Research
Dr. Richard H. Collier, Administrative Committee Chair, Agricultural Handler Exposure Task Force
Mr. Bill Gaynor, Staff Member, ICR
Mr. Shawn King, Director of Operations, Carroll-Loye Biological Research
Dr. Ralph Piedmont, Consultant to ICR

Dr. Ken Racke, Regulatory Specialist, Dow AgroSciences
Dr. Ghona Sangha, Toxicology and Regulatory Consultant, LANXESS Corp.

Oral comments were provided by:

Dr. Ghona Sangha, Toxicology and Regulatory Consultant, LANXESS Corp.

No written public comments were provided.

For their deliberations, the Board considered the materials presented at the meeting, oral comments, and Agency background documents (e.g., published literature, sponsor and investigator research reports, study protocols, data evaluation records, and Agency science and ethics reviews of proposed protocols and completed studies). A comprehensive list of background documents is available online at http://www.regulations.gov.

CHARGE TO THE BOARD AND BOARD RESPONSE

Assessment of Completed Research Study MRID 124144: Nolan *et al.* (1982) Chlorpyrifos: Pharmacokinetics in Human Volunteers Following Single Oral and Dermal Doses.

Overview of the Study

The HSRB reviewed Nolan *et al.* (1982) as part of a package of three studies involving human volunteers exposed to chlorpyrifos that were proposed for use by EPA to characterize and help interpret epidemiological and bio-monitoring data for this pesticide. In addition, the data from human participants might be used for setting bounding estimates and developing physiologically based pharmacokinetic (PBPK) models for chlorpyrifos.

Nolan *et al.* (1982) summarize the results of a single dose level study that was designed to compare the fate of chlorpyrifos when administered orally or dermally to humans. Chlorpyrifos is a highly lipophilic compound that is rapidly metabolized to 3,5,6-trichloro-2-pyridinol (TCP) and diethylthiophosphate. TCP is excreted in urine predominantly as a glucuronide conjugate. Blood and urine levels of chlorpyrifos and TCP (unconjugated) were determined after oral (0.5 mg/kg) or dermal (5 mg/kg) administration of chlorpyrifos.

There were a total of six participants in the Nolan *et al.* (1982) study, with one participant ("subject A') included in the evaluation of the fate of chlorpyrifos after oral dosing. However, this participant received dermal dosages of chlorpyrifos at 0.5 mg/kg in a preliminary evaluation and was not used in the final analyses of the dermal fate of chlorpyrifos (n = 5 for the dermal study). Based on the urinary excretion of TCP, it was estimated that approximately 70% of the orally administered dose of chlorpyrifos was absorbed, whereas less than 3% of the dose was absorbed after dermal application.

Science

Although presented separately, the same scientific criteria were used to assess the reliability of the data from all three chlorpyrifos studies. These criteria are:

1. Verification that the administered dose of chlorpyrifos was confirmed in the study;

2. Assurance that sample collection (blood and urine) was reliable and complete;

3. Validation and appropriateness of the analytical methods for quantification of chlorpyrifos and TCP in blood and urine; and

4. Evaluation of the cholinesterase inhibition data (plasma and/or RBC cholinesterase) for sensitivity, accuracy, reproducibility, timing of sample collection and proper sample handling for analysis.

Along with other relevant study issues, an assessment of how the Nolan *et al.* (1982), as well as the other two chlorpyrifos studies presented, met these general criteria along with other relevant study issues are summarized in responses to the Agency's charge questions below.

Charge to the Board

Are the blood and urine measurements of chlorpyrifos and/or TCP from the Nolan *et al.* (1982) oral and dermal studies reliable?

Board Response to the Charge

HSRB Recommendation

The Board concluded that data measuring chlorpyrifos at the limit of detection in blood and urine are not useful. The measurements of TCP in urine are generally reliable. It is unclear if the measurement of TCP in blood is useful because of concerns about the ability of the methods used to detect the appropriate TCP conjugate.

HSRB Detailed Recommendations and Rationale

Since blood and urinary concentrations of chlorpyrifos were essentially non-detectable, there is little confidence in these data. However, urinary levels of TCP, determined after hydrolysis of the glucuronide conjugate, were well above the limit of detection. Additionally, the data suggest relatively high absorption of chlorpyrifos from oral dosing and considerably less exposure from the dermal route, a pattern that is biologically plausible. It is not clear whether the blood levels of TCP are accurate because the extent to which the glucuronide conjugate of TCP circulates in the blood and the extent to which the glucuronide conjugate may be detected in blood. Confirmation of how the blood samples were handled for analysis may help to increase the confidence in the reported blood concentrations of TCP.

Charge to the Board

Are the measurements of cholinesterase activity/inhibition from the Nolan *et al.* (1982) oral and dermal studies reliable?

Board Response to the Charge

HSRB Recommendation

The HSRB expressed some concern over the variability of the erythrocyte cholinesterase activity data and the lack of a control group, but concluded that measurements of cholinesterase activity/inhibition from Nolan *et al.* (1982) were likely to be reliable.

HSRB Detailed Recommendations and Rationale

A major issue with determination of cholinesterase activity is the inter-day and inter-assay variability (typically 10-15%) that may be observed. In the Nolan *et al.* (1982) study, plasma cholinesterase showed marked inhibition (up to approximately 85%) after oral administration of chlorpyrifos, whereas less plasma cholinesterase inhibition was noted after dermal application (up to about 25%). In contrast, no inhibition of erythrocyte cholinesterase was observed with either route of administration.

The HSRB considered the following limitations or weaknesses of the cholinesterase measurements in Nolan *et al*. (1982):

1. There was no untreated group to control for inter-day laboratory variations; the subject's response was based only on a change from their own pretest samples.

2. The inter-day variability for each subject was high, particularly for the erythrocyte cholinesterase activity, thereby limiting the interpretation of the results.

3. Although the method used to measure cholinesterase activity (the micro-Michel method) is an older method, it was probably the most reliable at the time.

4. Since only a single dose level was evaluated, no dose-response data are available from this study.

Nevertheless, the results demonstrated that oral administration of chlorpyrifos caused greater inhibition of plasma cholinesterase than that observed after dermal application, a result that is consistent with higher absorption of chlorpyrifos following oral dosing.

Ethics

Charge to the Board

Is there clear and convincing evidence that the conduct of the Nolan *et al.* (1982) study was fundamentally unethical, or significantly deficient relative to the standards of ethical research conduct prevailing when it was conducted?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment (Carley 2009a) that there neither was clear and convincing evidence that the study was fundamentally unethical, nor clear and convincing evidence that the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

HSRB Detailed Recommendations and Rationale

Given that the Nolan *et al.* study was conducted in 1981 and 1982, the Board concurred with the Agency's assessment that the applicable regulatory standard at that time was FIFRA §12(a)(2)(P). In response to questions from the Agency, the study sponsor indicated that participants signed a one-page consent form that gave few details about the study but affirmed that they had read the study protocol (Dow AgroSciences 2009a). There had been two ethical reviews of this protocol, one by the Dow Human Health Research Committee, and a second by the University of Michigan Ethical Review Committee (The Committee to Review Grants for Clinical Research and Investigation Involving Human Beings).

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

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

    a. *Not fundamentally unethical*. With regard to determining whether or not a study is fundamentally unethical, the Board's standard is to decide if the research was intended to seriously harm participants, or if it failed to obtain informed consent, or if it was fundamentally unethical for other reasons.

        - The study was not intended to seriously harm participants. Voluntary informed consent of participants was obtained. Given that there does not appear to be clear and convincing evidence that for any other reasons it might have been fundamentally unethical, the Board concludes that it was not fundamentally unethical.

    b. *Not significantly deficient*. With regard to determining whether a study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted, the Board's standard is to determine whether or not any ethical deficiencies identified could have resulted in serious harm based on knowledge available to researchers at the time the study was conducted, or whether the information provided to study participants could seriously impair informed consent.

        - Based on toxicological data that was available at the time for chlorpyrifos, study participants were unlikely to be at risk for serious side effects given the exposure dose used in this study.

- The Board did not have a copy of the protocol that was given to the prospective participants when obtaining their informed consent, so the HSRB did not know exactly what information the participants received. Given that the study was reviewed by two ethics review boards, however, the Board concluded that there was no clear and convincing evidence that the information provided to participants could seriously impair informed consent. With regard to studies that were conducted prior to the effective date of the Agency's regulations, the Board does wish to convey to the Agency the importance of obtaining as much information about such studies as is reasonably possible. It would have been desirable for the Board to have had access to the actual study protocol.

Assessment of Completed Research Study MRID 42062701: Honeycutt and DeGeare (1993) Worker Reentry Exposure to Chlorpyrifos in Citrus Treated with Lorsban* 4E Insecticide.

Overview of the Study

The study reported by Honeycutt and DeGeare (1993) was a biological monitoring study to determine potential exposure to chlorpyrifos in individuals who reenter a citrus grove after agricultural use of chlorpyrifos. There were 15 participants in the study, representing five citrus pickers and ten citrus pruners (five each in wet and dry conditions, respectively). Pickers entered the test site 43 days after application of chlorpyrifos, whereas pruners re-entered two days after the application of chlorpyrifos. The exposure period was 5-6 hours, and the assessment of chlorpyrifos exposure was achieved with whole body dosimeters, air sampling, leaf punches and quantification of urinary excretion of TCP. The results from Nolan *et al.* (1982) justify monitoring urinary TCP to estimate chlorpyrifos exposure. The report included considerable emphasis on the handling of all samples in the field and a comprehensive summary of all analytical conditions and methods used in determining chlorpyrifos in all study matrices.

Both chlorpyrifos and TCP were analyzed with negative ion-chemical ionization gas chromatography with mass selective detection (GC/MS) and urinary TCP was quantified after acid hydrolysis employing concentrated hydrochloric acid with heating for 1-2 hr followed by derivitization with N-methyl-(N-tertbutyldimethylsilyl)-trifloroacetamide. Analytical recoveries were reported, and the limit of detection was defined as three-times higher than the signal to noise ratio. No limit of quantitation (LOQ) was provided.

Science

Charge to the Board

Are the blood and urine measurements of chlorpyrifos and/or TCP from the Honeycutt and DeGeare (1993) worker biomonitoring study reliable?

Board Response to the Charge

HSRB Recommendation

The Board concluded that the data from Honeycutt and DeGeare (1993) are likely reliable but of limited value. Board members expressed concerns about: 1) the small sample size; 2) the background chlorpyrifos exposure not being properly accounted for; 3) the likely incompleteness of urine collection; and 4) the high degree of variation seen in the daily measurements.

HSRB Detailed Recommendations and Rationale

Relevant weaknesses or limitations concerning study conduct and data reported by Honeycutt and DeGeare (1993) were assessed as follows:

1. The strength of this study is that it provides data on real-world exposures to chlorpyrifos. However, the critical element regarding confirmation of the dosage was not achieved in this study because the purpose of this study was to actually determine the dosage.

2. Urine samples were collected for 24 hrs prior to reentry and across 12-hr time intervals for up to five days after completion of the 5-6 hr work period. However, there is evidence to suggest that urine collections were not complete. This conclusion was based on variability in total urinary creatinine excretion along with several instances in which incomplete sample collection was noted. In total, five of 15 participants had total urinary creatinine levels that were significantly below normal daily reference range for creatinine excretion. In addition, for nine of 15 study participants the coefficient of variability exceeded reported urinary creatinine levels by up to four-fold, suggesting that urine collection was inconsistent at best. In light of these discrepancies, efforts were made to normalize urinary excretion data to a population average for creatinine excretion, but the accuracy and validity of this calculation is unknown, as discussed further below.

3. The control of the participants both prior to and after completion of the exposure was inadequate. One worker (Pruner 9) showed maximum urinary TCP levels on day three of the study suggesting additional exposure to chlorpyrifos after the test exposure period. More importantly, several workers showed detectable levels of urinary TCP in pretest samples, which was suggested to represent a steady state level of TCP resulting from occupational exposure. However, the validity of this conclusion is unknown, and the possibility that the observed levels may have resulted from a more recent exposure to chlorpyrifos was not considered.

4. In light of the uncertainties regarding urine sample collection and subject control, urinary TCP, and hence exposure to chlorpyrifos, were determined using six different methods including no corrections, correction for background TCP excretion, correction for creatinine excretion and combinations of corrections for background and creatinine levels. Across the six different methods used, the range of urinary TCP concentrations was about two-fold for pickers and four-fold for pruners. In

addition to the range of urinary TCP concentrations and the uncertainty regarding which method is most accurate, the small sample size (n = 5 participants per scenario) decreases the confidence of the final reported values.

Thus, although this study provides the potential for assessing chlorpyrifos exposure from a relevant occupational exposure, the uncertainty resulting from incomplete urine sample collection, background exposure to chlorpyrifos, the small sample size, inter-individual and inter-day variability and the use of six different calculations to estimate chlorpyrifos exposure undermine confidence in the reliability of the data.

Charge to the Board

Are the measurements of cholinesterase activity/inhibition from the Honeycutt and DeGeare (1993) worker biomonitoring study reliable?

Board Response to the Charge

HSRB Recommendation

The Board concluded that the laboratory data are likely accurate and reliable but raised concerns about their utility. Some of the Board's concerns included: 1) the lack of an untreated control group; 2) the reliance on a single post-exposure time point; 3) the small sample size; and 4) the dose received by the subjects varied and was only estimated from dermal dosimetry.

HSRB Detailed Recommendations and Rationale

Plasma and blood cholinesterase activities were determined pre-exposure (typically 2 occasions prior to exposure) and 24 hr after working in the citrus grove. The modified Ellman procedure was used to determine cholinesterase activities. Overall, no significant changes in plasma or erythrocyte cholinesterase activities were observed. The following limitations concerning plasma and erythrocyte cholinesterase activities reported by Honeycutt and DeGeare (1993) were identified:

1. Only one post-exposure sample was collected for analysis (24 hr). Additional sampling would have been helpful, as it is not clear whether the 24 hr time point was most appropriate for analysis.

2. Considerable variability was noted across the samples. In six of the workers, the two pre-dose measurements were not within 15% of each other, thereby confounding the accuracy of the baseline activities, and in three of the pruners, post-exposure erythrocyte cholinesterase levels were actually increased by more than 20% over baseline values. Collectively, there is considerable uncertainty in the baseline data.

3. No data from an unexposed group of control subjects was used to control for inter-day variability in the assay. It was noted that the practice of including laboratory controls for intra-assay variability is published, standard practice for this assay.

4. The small group size further confounds the interpretation of the highly variable results.

Thus, the HSRB concluded that the experimental evidence suggesting that plasma and erythrocyte cholinesterase activities were not significantly altered in the pickers and pruners exposed to chlorpyrifos in this study were likely to be accurate. However, the Board also cautioned that the reliability of the data was limited because of the issues noted above including analysis at only a single time point after exposure, the small sample size, the high degree of intra- and inter-subject variability and the lack of an untreated control.

Ethics

Charge to the Board

Is there clear and convincing evidence that the conduct of the Honeycutt and DeGeare (1993) study was fundamentally unethical, or significantly deficient relative to the standards of ethical research conduct prevailing when it was conducted?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment (Carley 2009b) that there neither was clear and convincing evidence that the study was fundamentally unethical, nor clear and convincing evidence that the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

HSRB Detailed Recommendations and Rationale

This study was initiated in 1991 in California. As a result, it was subject to the California Code of Regulations, Title 3, Section 6710, as amended September 26, 1988, which required pre-approval of the study by the Director of the California Department of Pesticide Registration, and by an Institutional Review Board approved by the US Department of Health and Human Services. It was also subject to FIFRA § 12(a)(2)(P). The study was reviewed and approved by the Committee on Human Subjects Research at the University of California, San Francisco.

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

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

   a. *Not fundamentally unethical*. With regard to determining whether or not a study is fundamentally unethical, the Board's standard is to decide if the research was intended to

seriously harm participants, or if it failed to obtain informed consent, or if it was fundamentally unethical for other reasons.

- The study was not intended to seriously harm participants. Voluntary informed consent of participants was obtained. Given that there does not appear to be clear and convincing evidence that for any other reasons it might have been fundamentally unethical, the Board concludes that it was not fundamentally unethical.

b. *Not significantly deficient*. With regard to determining whether a study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted, the Board's standard is to determine whether or not any ethical deficiencies identified could have resulted in serious harm based on knowledge available to researchers at the time the study was conducted, or whether the information provided to study participants could seriously impair informed consent.

- Based on toxicological data that was available at the time for chlorpyrifos, study participants were unlikely to be at risk for serious side effects given the exposure dose used in this study.

- Exposure took place while participants were performing activities that were the same as those they regularly performed as part of their usual employment as citrus workers. Accordingly, it does not appear that there were ethical deficiencies that could have resulted in serious harm.

- The volunteers were briefed about the design of the study and the risks relating to participation, and they signed consent forms containing that information. As the Agency noted (Carley 2009b), there was misleading information in some of the forms, such as comments that the residue from the spraying of the pesticide would have disappeared by the time they entered the groves. That misinformation was certainly inappropriate but does not, in and of itself, provide clear and convincing evidence that the informed consent process was seriously impaired.

- The study participants were employed by Mr. Gary Austin of Leffingwell Ag Sales Co., and he recruited them himself. It is unclear to what extent these participants might therefore have been vulnerable to his influence in deciding to enter the study. Nonetheless, this type of arrangement does not appear to be unusual at that time.

- The privacy of participants was compromised by the fact that their full names and Social Security numbers were included in an appendix to the study report. This is inappropriate, but would not appear to constitute clear and convincing evidence of an ethical deficiency that resulted in serious harm to these participants.

Assessment of Completed Research Study MRID 44811002: Kisicki *et al.* (1999) A Rising Dose Toxicology Study to Determine the No-Observable Effect-Levels (NOEL) For

Erythrocytes Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels.

Overview of the Study

This study was a double blind, randomized and placebo-controlled rising dose study designed to determine the no-observable effect level (NOEL) for inhibition of erythrocyte acetylcholinesterase in human volunteers. The dosages used in the study were 0.5, 1 and 2 mg/kg, with the starting dose of 0.5 mg/kg based on the results of Nolan *et al*. (1982) discussed previously. In addition to assessing cholinesterase activity, blood and urine were collected and analyzed for chlorpyrifos and TCP to help define the pharmacokinetic properties in humans. Finally, paraoxonase status of each subject was determined (but will not be discussed here).

Chlorpyrifos was administered orally in a gelatin capsule with 0 (lactose), 0.5 and 1 mg chlorpyrifos/kg administered in Phase I of the study followed by a second phase that include 0 (control) and 2 mg/kg dosages. There were six male and six female participants for each dose level. Subjects were confined to the testing facility overnight prior to treatment through the first 48 hr after dosing. Additional samples were collected thereafter at 24 hr intervals through 168 hr (one week) post dosing. Blood, collected pre-dose (-10 and 0 hr) and at 2, 4, 8, 24, 36, 48, 72, 96, 120, 144 and 168 hr post treatment, was used for determining erythrocyte acetylcholinesterase activity and chlorpyrifos and TCP levels. Voided urine was collected at 12 hr intervals starting 48 hr prior to dosing and through the 168 hr dosing period (with the exception that immediately after dosing urine was collected at 0-6 and 6-12 hr).

Science

Charge to the Board

Are the blood and urine measurements of chlorpyrifos and/or TCP from the Kisicki *et al.* (1999) oral study reliable and appropriate for use in characterizing the results of epidemiological studies with chlorpyrifos?

Board Response to the Charge

HSRB Recommendation

Because of concerns about the analytic procedures' potential inability to control for the glucuronide-conjugated TCP, and apparent discrepancies in the absorption data when compared with the data from Nolan *et al*. (1982), the Board questioned the reliability and utility of the blood and urine measurements of chlorpyrifos and/or TCP from Kisicki *et al.* (1999) for risk assessment purposes.

HSRB Detailed Recommendations and Rationale

With respect to the general elements considered in the evaluation of the scientific reliability of the chlorpyrifos exposure data, this study provided clear documentation of the

administered dose. Chlorpyrifos was added to a gelatin capsule normalized with lactose powder and the weight of the filled capsule was determined. There was one noted discrepancy that one participants in the 2 mg/kg dose group may have received a lower dose (1.63 mg/kg) based on conflicting data on body weight and administered dose data, but overall the documentation of the administered dose was satisfactory.

There was also generally good reliability with respect to sample collections. Participants remained in the testing facility for the first 48 hrs after dosing and 48 of the 60 subjects provided all periodic urine samples. Urine collections were judged to be complete based on creatinine excretion for 19 of the 60 subjects. One participant in the 2-mg/kg-dose group failed to return for sample collection and clinical monitoring after the initial 48 hr post-dosing period.

Chlorpyrifos and TCP were measured in blood and urine with GC/MS detection, and TCP was derivatized with N-methyl-(N-tertbutyldimethylsilyl)-trifloroacetamide. In general, these methods were similar to those described by Honeycutt and DeGeare with the exception that stable labels of chlorpyrifos and TCP (13C and/or 15N-labeled) were used as internal standards in the present analyses.

In reviewing this study, the HSRB identified several limitations or issues that may impact the overall reliability of the data. These included:

1. The amount of chlorpyrifos absorbed after oral administration in the present study was markedly lower than that reported by Nolan *et al*. (1982). A dose of 0.5 mg/kg was common to both studies, with Nolan *et al.* reporting approximately 70%, and Kisicki *et al.* reporting approximately 35% absorbed. The rationale provided by the authors for this large difference was that chlorpyrifos was administered using lactose tablets and gelatin capsules in the Nolan *et al.* and Kisicki *et al.* studies, respectively. Kisicki *et al.* suggested that oral absorption is slowed by the dissolution of the gelatin capsule with a concurrent reduction in the total amount absorbed. Although the Board agreed that absorption of chlorpyrifos from the gelatin capsule might be slower than that from a lactose tablet, there was some skepticism that the difference in dosage form would yield the large discrepancy in total percent absorbed in the two studies.

2. In the methodological details provided for the analysis of urinary levels of TCP, there was no indication that urine samples were subjected to acid hydrolysis required to liberate the glucuronide conjugate of TCP, which is the major urinary metabolite of chlorpyrifos. This is an important distinction, as both Nolan *et al.* (1982) and Honeycutt and DeGeare (1993) indicated that urine samples were treated with concentrated sulfuric or hydrochloric acid and heated in order to hydrolyze the glucuronide conjugate and liberate TCP which is then derivatized (in the methods used by Honeycutt and DeGeare and Kisicki *et al.*). In the analytical portion of the Kisicki *et al.* study (Brzak 2000), there is a clear designation that blood samples were treated with concentrated hydrochloric acid prior to extraction, but no similar details are provided for the analysis of the urine samples. Although it seems unlikely that this important (and necessary) step was omitted, the lack of explicit indication of this

critical analytical step raises some doubt that it was done. If urine samples were not properly hydrolyzed prior to analysis, then the validity and reliability of these data are uncertain. Furthermore, since urinary TCP is used to estimate oral absorption of chlorpyrifos, it is possible that differences in sample handling may explain or at least contribute to the lower percent absorption reported by Kisicki *et al*.

3. The total mass balance (total recovery of the administered chlorpyrifos) was not determined. Inclusion of total recovery could increase the confidence in the estimated percent of absorption in the study and possibly clarify whether there was an issue with the TCP analysis conducted by Kisicki *et al*.

4. There were several questions regarding the designation and use of alternate participants in the study. The basis for participant replacement and verification of body weight and dose administered to the alternate participants were not adequately provided.

Because of concerns about the analytic procedures' potential inability to control for the glucuronide-conjugated TCP, and apparent discrepancies in the absorption data when compared with the data from Nolan *et al*. (1982), the Board thus questioned the reliability and utility of the blood and urine measurements of chlorpyrifos and/or TCP from Kisicki *et al.* (1999) for risk assessment purposes.

Charge to the Board

Are the measurements of cholinesterase activity/inhibition from the Kisicki *et al.* (1999) oral study reliable?

Board Response to the Charge

HSRB Recommendation

The Board concluded that these measurements likely represent a reliable set of data, but cautioned the Agency about relying on data from the incomplete profile for the one responder at the highest dose level. The Board also cautioned against relying on the statistical analyses as presented in the report.

HSRB Detailed Recommendations and Rationale

In this study, erythrocyte acetylcholinesterase activities were determined pre-dose (2 samples) and 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 after dosing (Ellman method). Plasma cholinesterase activity was not measured. The results showed a relatively high degree of variability, and samples collected at 96 hr (Phase I; 0, 0.5 and 1 mg/kg) were judged to be low relative to all other treatment times. One participant in the 1-mg/kg dose group showed a 24.5% decrease in activity at the 96 hr time point. However, given the decreased activity observed in the entire group of samples collected at 96 hr along with the fact that peak inhibition would be

expected to occur before 96 hr, this result was not considered to be a real finding. Only one participant in the 2-mg/kg-dose group showed a reduction in erythrocyte cholinesterase activity in the study, with a nadir (72% of her baseline level) observed 12 hr after treatment. However, this volunteer did not return for blood collections after the initial 48 hr period, resulting in an incomplete profile. This participant did not appear to show adverse clinical signs associated with cholinesterase inhibition. Based on the reduction in erythrocyte cholinesterase activity in this subject, the NOEL for enzyme inhibition was considered to be 1 mg/kg.

In reviewing this study, the Board identified several limitations or issues that may impact the overall reliability of the data. These included:

1. There was generally poor documentation provided for the grouping or batch processing of the blood samples. Given the recognized inter-day and inter-assay variability associated with the Ellman method, this is a critical weakness. The study records were noted to be insufficient to confirm that samples were batched correctly to allow the proper utilization of the unexposed control data to correct for day-to-day or batch-to-batch variations in the mean lab results. This limitation is particularly important to detect or to quantify accurately small changes within an exposed population.

2. The interpretation of the results is centered on the single individual who showed more than a 25% inhibition of erythrocyte cholinesterase activity at the 2-mg/kg-dose level. The conclusions regarding both the duration and magnitude of inhibition depend upon the inclusion of this participant, and the lack of follow-up in this participant beyond 48 hr thereby limits the interpretation of the results.

3. The reported statistical analyses of these data are highly problematic, and the results in Appendix 3 were judged to be clearly wrong. Specifically, it was noted that a univariate repeated measures ANOVA was performed (Kisicki *et al*. 1999, 25), and Appendix 3 summarizes the results of such analyses (Kisicki *et al*. 1999, 151-158, 184-188, 206-211). Although it is not possible to replicate these analyses, the report suggests that the variable being analyzed is the Normalized Percent of Baseline (Kisicki *et al*. 1999, 24). The report indicates that the Group Mean Square (Kisicki *et al*. 1999, 157) is 19.1207 while the Error Mean Square is 52,835,395,914.644. It is virtually impossible that these two values can be this different for real data. Furthermore, the corresponding Individual Hour Error Mean Squares from the corresponding mixed model analysis range from about 14.39 to 48.26. These are much more reasonable values. The report (Kisicki *et al*. 1999, 25) also indicates that mixed effects models were used where gender and treatment were considered to be fixed factors, and that time was considered to be a random continuous covariate. According to the mixed model analyses reported in Appendix 3, time is treated as a continuous covariate, but not as a random continuous covariate. Overall, the Board concluded that the statistical analyses are likely incorrect, and there should be some effort to perform correct analyses before relying on these data.

4. Plasma cholinesterase activity was not determined in the study. It is recognized that the erythrocyte cholinesterase activity is more biologically relevant for assessing potential adverse effect of chlorpyrifos, and as such, the lack of data on plasma cholinesterase is not a serious limitation. However, given the issues raised concerning sample handling, batch processing and statistical evaluations, inclusion of plasma cholinesterase may have helped to assess the overall reliability of the data.

The Board thus concluded that the measurements of erythrocyte acetylcholinesterase activities were generally reliable. However, Board members cautioned that the incomplete profile obtained from the only subject who showed inhibition at the 2 m/kg dose level limited the utility of the data. Moreover, the Board recommended that, although the data might be reliable, the statistical analyses in general and particularly for the data in Appendix 3 should be replicated prior to applying these results to any risk assessment or model development.

Ethics

Charge to the Board

Is there clear and convincing evidence that the conduct of the Kisicki *et al.* (1999) study was fundamentally unethical, or significantly deficient relative to the standards of ethical research conduct prevailing when it was conducted?

Board Response to the Charge

HSRB Recommendation

The Board concluded that there neither was clear and convincing evidence that the study was fundamentally unethical, nor clear and convincing evidence that the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

HSRB Detailed Recommendations and Rationale

According to documents supplied by the sponsor (Juberg and Mattsson 2009a, Juberg and Mattsson 2009b), the study was conducted in accordance with US Food and Drug Administration rules relating to the protection of human subjects, as codified at 21 CFR parts 50 and 56, and in accordance with the International Guidelines for Human Testing promulgated in the Declaration of Helsinki (as amended in 1996). In addition, the study was subject to FIFRA § 12(a)(2)(P). The protocol and related study documents were reviewed and approved by the MDS Harris Institutional Review Board. The consent of all participants was obtained and documented through the use of a consent form approved by that IRB.

1. The Board concurred with most of the conclusions and factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's initial Ethics Review (Carley 2009c). The Board disagreed, however, with the Agency's initial conclusion that the study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted. After considering additional supplementary information provided to the

Agency on June 23, 2009 and described in detail below (Kisicki 2009), the Board found no clear and convincing evidence that the study was fundamentally unethical or significantly deficient in its design and conduct. Following the public meeting, additional supplementary material (including copies of MDS Harris Laboratories screening and consent logs) was submitted to the Agency for review. Although the Board shares the Agency's concerns that these supplementary documents still raise concerns about the recruitment and consent process (Carley 2009e), the information contained therein do not meet the evidentiary standard of clear and convincing evidence that the study was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time. It is possible, for example, that the deficiencies noted in the logs are the result of sloppy record keeping rather than a serious impaired participant recruitment, consent and screening process.

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

   a. *Not fundamentally unethical*. With regard to determining whether or not a study is fundamentally unethical, the Board's standard is to decide if the research was intended to seriously harm participants, or if it failed to obtain informed consent, or if it was fundamentally unethical for other reasons.

      - The study was not intended to seriously harm participants. Voluntary informed consent of participants was obtained. Given that there does not appear to be clear and convincing evidence that for any other reasons it might have been fundamentally unethical, the Board concludes that it was not fundamentally unethical.

   b. *Not significantly deficient*. With regard to determining whether a study was significantly deficient relative to the ethical standards prevailing at the time the research was conducted, the Board's standard is to determine whether or not any ethical deficiencies identified could have resulted in serious harm based on knowledge available to researchers at the time the study was conducted, or whether the information provided to study participants could seriously impair informed consent.

      - Based on toxicological data that was available at the time for chlorpyrifos, study participants were unlikely to be at risk for serious side effects given the exposure dose used in this study.

      - Participants underwent some screening tests (urine and blood sampling) prior to signing the written consent form. According to materials provided to the EPA and made public on the day of the Board's discussion of this study (Kisicki 2009), volunteers were given a copy of the approved consent form and required to listen to an audiotape about the study before they agreed to undergo the screening procedures. Later documents submitted to the Agency (Radke 2009), however, raise questions as to whether all potential study participants or alternates underwent voluntary informed consent prior to screening. Although the failure to have potential participants and alternates sign an informed consent form prior to screening could be a violation of

applicable FDA regulations, given these circumstances it does not appear that the informed consent process was serious impaired.

- There were various sentences in the informed consent form that appear inappropriate, such as characterizing the study compound as one that improves performance on tests of mental functioning. In addition, portions of that form were written at an inappropriately high reading level. Taken as a whole, however, the informed consent document does appear to have provided prospective subjects with an accurate and understandable picture of the likely risks from participation – which were very low. Thus, it does not appear that the form, as written, seriously impaired the informed consent process.

Assessment of Completed Research Study MRID 47732701: ICR, Inc. Study A382 – Evaluation of the Efficacy of KBR 3023 (Picaridin; Icaridin) - Based Personal Insect Repellents (20% Cream and 20% Spray) Against Stable Flies in the Laboratory.

Overview of the Study

A382 was a laboratory study conducted by ICR to assess the efficacy of two formulations containing the repellent picaridin against laboratory-reared stable flies. The study followed the protocol previously reviewed by the HSRB and recommended for acceptance with some suggested modifications.

The study used a dosimetry phase for dose determination. The initial study had to be aborted because the stable flies did not show sufficient biting pressure for a valid repellency test. Adjustments were subsequently made in the husbandry of the flies to insure that they were sufficiently hungry to bite the test subjects in the testing period, and the data from the second test, along with the dosimetry results, were presented. Complete protection times were calculated.

Science

Charge to the Board

Is the ICR study A382 sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy of the tested formulations against stable flies in the laboratory?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment (Sweeney 2009) that this study provides scientifically valid results to assess the repellent efficacy against stable flies of the formulations tested. However, some of the data presentation requires revision.

HSRB Detailed Recommendations and Rationale

ICR was responsive to Board concerns and recommendations of the previous Board review (EPA HSRB 2008). These responses included the use of a dosimetry phase for dose determination and the inclusion of individuals from racial minorities in the test population. The study also used the time to first bite as the criterion, and not the time to first confirmed bite, which was also a Board recommendation for participant protection. The study included 12 participants (seven females and five males). One female participant served as an untreated control to verify landing pressure. The total number of study participants is higher than the EPA guidelines recommend. Study documents (Gaynor 2009, Gaynor *et al.* 2009) indicated that, according to ICR's database, landings underestimate bites. Thus, bites were selected as the endpoint despite earlier Board recommendations concerning participant safety (EPA HSRB 2008). Nevertheless, in order to obtain scientifically valid data useful for regulatory purposes, the use of first bite, unconfirmed by a subsequent bite, is acceptable. The Margins of Exposure (MoEs) were adequate for protection of participants from the potential toxicity of the repellent.

Statistical analyses were generally appropriate. However, the standard error for mean protection time and resulting confidence interval need to be corrected to use the estimated standard error, not the planned standard error. It would also be helpful to present the range of values observed in the data. For study planning, basing sample size calculations on an estimated standard deviation from stable fly data would have been more appropriate than using a published estimate from mosquito data. Lastly, Table 4 in the submitted report should be corrected to indicate that the data presented are for product failure times and not complete protection times.

Ethics

Charge to the Board

Does available information support a determination that study ICR A382 was conducted in substantial compliance with subparts K and L 40 CFR Part 26?

Board response to the Charge

HSRB recommendation

The Board concurred with the Agency's assessment (Carley 2009d) that the study submitted for review was conducted in substantial compliance with subparts K and L of 40 CFR 26.

HSRB Detailed Recommendations and Rationale

The documents provided by ICR (Gaynor 2009, Gaynor *et al.* 2009) state that each study was conducted in compliance the requirements of the US EPA Good Laboratory Practice (GLP) Regulations for Pesticide Programs (40 CFR 160). Additional regulations – 40 CFR 26 subparts K and L; and FIFRA § 12(a)(2)(P – are also applicable. The study was reviewed and approved

by a commercial human subjects review committee, Essex Institutional Review Board, Inc. (EIRB, Inc.) of Lebanon, NJ.

1. The Board concurred with the conclusions and factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's Ethics Review (Carley 2009d). The completed study met all applicable ethical requirements for research involving human participants, in accordance with the following criteria as developed by the Board:

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

        - Minor and pregnant or lactating women were excluded from participation, with pregnancy either confirmed by over-the-counter pregnancy testing on the day of study or by opt-out. The potential stigma resulting from study exclusion due to pregnancy was also appropriately minimized.

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

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

        - The study uses first bite as an endpoint. Stable fly bites, however, usually cause only minor symptoms and are readily treated with over-the-counter antipruritic lotions.

        - The study was conducted with laboratory-raised flies free of known pathogens. This latter point, in particular, resulted in the one notable change to the protocol. At its initial review, the HSRB recommended that the stable flies be raised on 10% sucrose rather than bovine blood meals, thus minimizing the risk of potential zoonoses (EPA HSRB 2008). As a result, the first planned test of picaridin as a repellent for stable flies failed. Specifically, the trial had to be aborted due to insufficient landing pressure. Upon consulting with an entomologist, ICR researchers concluded that the stable flies "[overfed] on the 10% sucrose solution, which then interfered with their normal inclination to land and take a blood meal" (Gaynor *et al.* 2009). This was addressed by: 1) changing the feeding regimen to dry sugar cubes; 2) increasing the numbers of stable flies used per cage; and 3) increasing the length of participant exposure to stable flies to determine attractiveness. These changes were submitted to EIRB, Inc. as protocol amendments and approved prior to implementation. This protocol change was unlikely to have increased the risk of study participants or compromised the informed consent process.

    b. *Voluntary and informed consent of all participants*

- The study protocol included several mechanisms designed to minimized coercive recruitment and enrollment.

- Monetary compensation was not so high as to unduly influence participants.

c. *Equitable study participant selection and recruitment*

- In response to HSRB concerns, ICR modified their recruitment strategy to identify study participants more representative of the racial and ethnic distribution of likely repellent users.

2. The Board also recommended that, for future intentional dosing studies, the sponsor work with the IRB of record to address some of the gaps of submitted information noted in the EPA's Ethics Review (Carley 2009d).

   a. Several Board members raised concerns about the quality of IRB review and the qualifications of IRB members to review insect repellent protocols, given the failure of EIRB, Inc. to provide the sponsor or the Agency with a description of IRB member qualifications and detailed meeting minutes that offer a description of IRB deliberations and debate of intentional exposure protocols. It is unlikely that these deficiencies placed study participants at risk or compromised the informed consent process, but they do represent a deviation from the expectations of 40 CFR 26.

   b. The sponsor and IRB of record should ensure that all telephone scripts and other recruitment materials, and informed consent documents be reviewed prior to study initiation to ensure that the information contained in each is accurate. For example, the telephone script submitted to the IRB for review and approval failed to note two significant changes to the study design: the change on the duration of attractiveness testing; and the change to pregnancy screening protocol that allowed women who are physically incapable of having children (i.e., post-menopausal or surgically sterilized) to opt-out of over-the-counter pregnancy testing. This deviation, however, was unlikely to have compromised the informed consent process as potential study participants were informed of these protocol changes later when formally consented at the ICR facility.

Assessment of Proposed Carroll-Loye Biological Research Study LNX-002: Efficacy Test of KBR 3023 (Picaridin; Icaridin) - Based Personal Insect Repellents (20% Cream and 20% Spray) with Biting Flies Under Field Conditions.

Overview of the Study

The protocol describes a study to test the efficacy of two formulations (lotion and spray) of 20% picaridin in the field against at least one of 4 species of biting flies. Dosimetry data recently accumulated in a previous study (LNX-001) would be used for dose selection. One habitat is proposed. Ten test subjects and two untreated controls would be tested in a suitable environment that had adequate biting pressure of biting flies but relatively few mosquitoes.

Repellent-treated skin would be exposed for 5 minutes in each 30-minute interval until repellent failure. The endpoint would be the "Lite with Intent to Bite" (LIBe), and the criterion for data to calculate complete protection time would be first confirmed LIBe.

Science

Charge to the Board

If the proposed field repellency study protocol LNX-002 is revised as suggested in EPA's review and if the research is performed as described: Is the research likely to generate scientifically reliable data, useful for assessing the efficacy of the tested materials in repelling biting flies in the field?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment (Carley and Sweeney 2009) that this protocol will provide scientifically valid results on the efficacy of two picaridin formulations against biting flies, with modification as noted below. Additional Board review of the protocol is not required prior to study implementation.

HSRB Detailed Recommendations and Rationale

The protocol submitted for review had many similarities to previously reviewed CLBR protocols and completed studies involving mosquitoes. The format of the protocol description was revised to provide greater clarity. The study protocol and associated documents incorporated many of the Board's previous comments and recommendations. The endpoint was LIBe, which the Board has previously expressed preference for because of the lesser risk to study participants than bites.

Agency reviewers identified two concerns: 1) the standard of biting of one LIBe in five minutes was not well justified and may be insufficiently high to yield valid results and could lead to inappropriately right censored data; and 2) a change of the previously-used paradigm of one minute exposure of treated limbs to insects out of each 15 minute period to five minutes in each 30 minute period was not explained or justified (Carley and Sweeney 2009). The Board concurred with both of these concerns, and recommended that they be addressed in the revised protocol as possible.

Additional Board recommendations concerned two issues: 1) the particular species on which data would be accumulated; and 2) the calculation of complete protection time.

With respect to the species tested, although four types of biting flies were proposed as possible insects to be monitored, it was pointed out that these four species display varied behaviors and aggressiveness. When questioned, the Agency indicated that it already had some useful data on some species of biting flies – such as stable flies – accumulated in the laboratory.

The Agency thus expressed interest in obtaining data from other species that cannot be readily studied in laboratory tests. The Board thus recommended that the study be conducted only if black flies (preferably) and/or biting midges were present in sufficient numbers at the field site. Accumulated data should be acquired on one or both of these species, as well as any other species of biting flies that may also be present. If black flies or biting midges are not available in sufficient numbers and with sufficient biting pressure at the field site, other types of biting flies should not be considered acceptable substitutes.

Echoing earlier concerns about the types of calculations and statistical analyses that will be conducted on these insect repellency data, the Board recommended that the protocol be amended to explain better how mean complete protection time will be calculated accurately using the appropriate types of statistical analyses. Mean protection time versus the duration of the study should be clarified, particularly as it affects the prevalence of censored data. The study's duration should be sufficiently long to ensure that the repellent will fail for a substantial portion of study participants, thereby limiting the occurrence of right-censored data. The protocol should also be revised to clarify how the analysis will proceed in the presence of censored data (using Maximum-likelihood or Kaplan-Meier methods).

In the context of this and other insect repellency studies, the Agency is again urged to update its guidance to sponsors so that they can conduct tests and analyze the resultant data in a useful and accurate manner.

Ethics

Charge to the Board

If the proposed field repellency study protocol LNX-002 is revised as suggested in EPA's review and if the research is performed as described: Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board response to the Charge

HSRB recommendation

The Board concluded that the protocol submitted for review, if modified in accordance with EPA (Carley and Sweeney 2009) and HSRB recommendations, is likely to meet the applicable requirements of 40 CFR 26, subparts K and L.

HSRB Detailed Recommendations and Rationale

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 the US EPA's 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) (Carroll 2009). The requirements of FIFRA §12(a)(2)(P) also apply. The protocol was reviewed

and approved by an independent human subjects review committee, Independent Investigational Review Board, Inc. (IIRB, Inc.), of Plantation, FL prior to submission.

1. The Board concurred with the conclusions and factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's Ethics Review (Carley and Sweeney 2009). The proposed study is likely to meet the applicable ethical requirements for research involving human participants, in accordance with the following criteria:

    a. *Acceptable risk-benefit ratio*. The risks as noted in the study protocol are five-fold: 1) allergic reaction to test materials themselves; 2) exposure to biting arthropods; 3) possible exposure to arthropod-borne diseases; 4) physical stress from the test conditions; and 5) psychological stress and/or breach of confidentiality for pregnancy test results. These risks are minimized appropriately and are justified by the potential societal benefits, particularly data on the efficacy of these new formations as personal insect repellents.

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

        - The study is designed to minimize the likelihood of biting fly and mosquito bites, through the use of: LIBes rather than actual confirmed bites as a study endpoint; pre-bite removal and joint observation; clear stopping rules; limited exposure periods. Study participants will be trained in proper insect observation and handling techniques.

        - Biting fly and mosquito bites, should they occur, are usually mild and easily treated with over-the-counter steroidal creams. The study will also exclude participants who have a history of severe skin reactions to such bites.

        - To minimize the risk that study participants will be exposed to pathogens like West Nile Virus – not transmitted by the biting flies in question but via other arthropods that may be present at the field sites – the study 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 two weeks. The study is also planned for a location where mosquitoes are not abundant and at a time of year in which most arthropod-borne pathogens are not usually detected. Finally, mosquitoes that land with the intent to bite will be collected and subjected to multiplex RT-PCR assays for several known arthropod-borne pathogens—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.

        - The potential risks to participants from environmental stress are minimized by the provision of a climate controlled rest area, food, water and medical supplies, and by careful monitoring for signs of dehydration, heat stress and hypothermia. Appropriate stopping rules and medical management procedures are in place.

- Minor and pregnant or lactating women are excluded from participation, with pregnancy either confirmed by over-the-counter pregnancy testing on the day of study or by opt-out. The potential stigma resulting from study exclusion due to pregnancy is also appropriately minimized.

b. *Voluntary and informed consent of all participants*

- The study protocol includes several mechanisms designed to minimize coercive recruitment and enrollment.

- Monetary compensation is not so high as to unduly influence participants.

c. *Equitable selection of study participants*

- The majority of research participants will be recruited from the University of California at Davis student population. Study participants are likely to represent the appropriate ethnic and racial diversity of individuals in and around the University, but the use of this convenience sample may limit the broad applicability of the study results to the general population. The investigators in the protocol have noted this fact.

2. The Board recommended that the study protocol be modified to address the few concerns noted in the EPA's Ethics Review (Carley and Sweeney 2009). In addition, the Board recommended that the investigators clarify of what "3rd party" medical coverage means, as listed in the current informed consent document.

Assessment of Proposed AHETF Scenario and Protocol AHE-120: Water-Soluble Packing Mixing and Loading.

Overview of the Study

This proposal presents an agricultural handler exposure scenario involving mixing/loading of pesticides enclosed in water-soluble packets (WSP). The protocol calls for study participants to mix and load one of two WSP-enclosed surrogate pesticides (acephate and carbaryl) into a variety of tanks containing water in a variety of agricultural spraying operations. A total of 25 participants (described in the protocol as "Monitoring Units" [MUs]) will be observed; 5 volunteers each from five different growing regions will be enrolled using a purposive sampling method.

Dermal exposure will be measured by a whole body dosimeter (WBD) worn beneath the subject's outer clothing. Hand wash and face/neck wipe samples will also be collected prior to, during, and after completion of pesticide loading and mixing procedures. Airborne concentrations of the surrogate will be monitored in the participant's breathing zone using an OSHA Versatile Sampler (OVS) tube sample collector connected to a personal sampling pump. Additional measures will also record environmental conditions at the time of monitoring, and observers will make field notes, photographs and videos of participant activity throughout the

monitoring event.

The results of sample analysis under the mixing/loading of water-soluble packets scenario, will be posted to the AHED® database, where they will be available to the EPA and other regulatory agencies for statistical analysis. The proposed documentation will report a confidence-interval-based approach to determine the relative accuracy for the arithmetic mean and 95th percentile of unit exposures. The Agency proposes to use these data to estimate daily dermal and inhalation exposures of agricultural handlers who are mixing/loading pesticides in water-soluble packets under a variety of mixing and loading scenarios.

Science

Charge to the Board

If the proposed mix/load WSP field study protocol AHE120 is revised as suggested in EPA's review and if the research is performed as described: Is the research likely to generate scientifically reliable data, useful for assessing the exposure of handlers who mix and load soluble or wettable powder pesticides in water-soluble packaging?

Board Response to the Charge

HSRB Recommendation

Given the lack of existent reliable and sound data in this area, the Board concurred with the Agency's assessment (Evans and Sherman 2009) that this protocol will generate data that are scientifically valid and that *may* be useful for assessing the exposure of handlers who mix and load soluble or wettable powder pesticides in water-soluble packaging. The Board cautioned that these data are likely to be useful for creating distributions of worker exposure only if worker exposure is found to be proportional to the amount of active ingredient handled (AaiH).

The Board also recommended a number of protocol modifications, as listed below. Additional Board review of the protocol is not required prior to study implementation.

HSRB Detailed Recommendations and Rationale

The Board concluded that the proposed monitoring and quality assurance and control methods appear adequate, particularly in the supplemental SOPs provided by the AHETF in response to the Agency's initial science and ethics review (Collier 2009b; Evans and Sherman 2009). The protocol and supplemental SOPs adequately address a number of key scientific issues, including: the scientific objective, the quantification of the test materials, the data collection and compilation methods and summary of test results, the justification for selection of the test substances, and the QA/QC requirements. The Board also commended the AHETF and the Agency for taking the time to plan, test, and revise different scenarios and approaches, and for pilot testing some aspects of these and related protocols. The protocol presented to the Board was well thought out and written as a result.

The Board did raise a number of concerns, however, about the perceived inadequacies in the study design, including issues of sample size and the use of inappropriate statistical analyses. The Board raised many of these issues previously when it reviewed earlier AHETF protocols. Both the AHETF and the Agency will need to acknowledge the limitations of the study design that occur in the AHETF handling scenarios and either change their goals accordingly, or add appropriate statistical methods or data management approaches to extrapolate from the data obtained from these limited scenarios to the wider regulatory purposes that these data will be used for. Some Board members expressed the concern, for example, once these data are inside the AHED® database, some users may overlook the limitations of the original study design and use the data to generate the typical statistical distributions in error.

Illustrating this point, the Board raised concerns about the types of statistical analyses proposed for the water-soluble packing scenario presented here. The proposed study will rely on a purposive sampling strategy, for example, but the researchers propose to treat the monitoring data as if "it were collected as a two-stage random sample from an infinite population" (Collier 2009a). The Board questioned the validity of this approach, noting that the data will be collected from a non-random non-population based sample. There is no statistical theory that can be applied to non-random samples of this type. Thus, the statistical analyses proposed, including mixed model approaches, are not valid.

The Board also raised concerns about the key objective of the study – namely, to determine the relationship between measured worker exposure and AaiH. In particular, the Board disagreed with the Agency's assessment that "past studies have shown that AaiH is strongly associated with exposure and is a meta-factor associated with differences in equipment and spraying practices" (Evans and Sherman 2009). To expect a linear relationship between AaiH and worker exposure seems logical; one should expect that a consistently small fraction of the amount of pesticide that a worker handles would be deposited onto their skin. Data previously presented by the AHETF to the Agency's FIFRA Scientific Advisory Panel (SAP), however, did not demonstrate a linear relationship between worker exposures of the amount of active ingredient handled. There are a number of factors that may explain why there is not a clear linear relationship between measured worker exposure and AaiH, including ecological, engineering, and statistical factors. As submitted, the AHETF protocol will not be able to distinguish between these different factors nor allow researchers to determine the true relationship between exposure and AaiH. For example, there are a number of uncontrolled ecological variables that may influence worker exposure, including: environmental conditions, the types of equipment used, the types of crops treated, grower preferences, etc. Indeed, some workers will likely experience mixing and loading conditions that are atypical for normal use of the active ingredient being monitored. Myriad sources of natural variation are likely to have a marked impact on worker exposure. Given the relatively small sample size of each monitoring cluster in this exposure scenario, these sources of natural variation will likely introduce considerable estimation bias into the final data.

Finally, current consensus is that estimates of the geometric mean, the arithmetic mean, and the 95th percentile need to be accurate within three-fold of the actual population value. The current protocol includes no methods to validate the actual population data and determine whether the resulting estimates fall within this necessary range.

Ethics

Charge to the Board

If the proposed mix/load WSP field study protocol AHE120 is revised as suggested in EPA's review and if the research is performed as described: Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board response to the Charge

HSRB recommendation

The Board concluded that the protocol submitted for review, if modified in accordance with EPA (Evans and Sherman 2009) and HSRB recommendations, is likely to meet the applicable requirements of 40 CFR 26, subparts K and L.

HSRB Detailed Recommendations and Rationale

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 the US EPA's GLP Standards described at 40 CFR 160 (Collier 2009a). The requirements of FIFRA §12(a)(2)(P) and, where applicable, the California State EPA Department of Pesticide Regulation study monitoring (California Code of Regulations Title 3, Section 6710) also apply. The protocol was reviewed and approved by an independent human subjects review committee, IIRB, Inc. of Plantation, FL prior to submission.

1. The Board concurred with the conclusions and factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's Ethics Review (Evans and Sherman 2009). The proposed study is likely to meet the applicable ethical requirements for research involving human participants, in accordance with the following criteria:

    a. *Acceptable risk-benefit ratio*. The risks as noted in the study protocol are five-fold: 1) heat-related illness; 2) accidental exposure to the surrogate chemicals; 3) injury associated with scripted field activities; 4) allergic reaction to surfactants using for hand washing; and 5) psychological stress and/or breach of confidentiality for pregnancy test results. These risks are minimized appropriately and are justified by the potential societal benefits, particularly data on occupational exposure of agricultural workers to pesticides during mixing and loading activities.

        - The greatest risk to participants is that of heat-related illness, given that the participants will be required to wear two layers of clothing during the scenario activities. This risk is lessened but not eliminated by the application of appropriate stopping rules (including cessation of all monitoring activities when the ambient heat-index exceeds 105°F) and frequent monitoring of participants. Participants will be

given frequent breaks, access to ample amounts of water or sports drinks, and educated about the dangers and symptoms of heat-related illness. Appropriate medical management procedures are also in place.

- The surrogate materials consist of two common pesticides, acephate and carbaryl, both of which have been extensively tested. The participants will only be exposed to concentrations of the surrogate compound at accepted exposure thresholds.

- Participants will be selected from volunteers with experience handling these or similar compounds in WSP mixing and loading scenarios. Thus, all of the participants will have extensive experience in using these or similar products, and thus unlikely to misuse them in a way that might increase their likelihood of being accidentally exposed.

- Participants will be reminded about safe handling practices and procedures, wear appropriate PPE, and will be monitored for any accidental or unintended product exposure.

- Allergic reactions to the surfactants used in hand washing are usually mild and easily treated with over-the-counter steroidal creams. The study will exclude participants who have a history of severe skin reactions to such detergents.

- Minor and pregnant or lactating women are excluded from participation, with pregnancy either confirmed by over-the-counter pregnancy testing on the day of study or by opt-out. The potential stigma resulting from study exclusion due to pregnancy is also appropriately minimized.

b. *Voluntary and informed consent of all participants*

- There is the possibility that the participants in this study might represent particularly vulnerable populations, susceptible to coercion and undue influence. The study protocol, however, includes several mechanisms designed to minimize coercive recruitment and enrollment.

- Monetary compensation is not so high as to unduly influence participants.

- Spanish translations of the informed consent documents, informational packets, and recruitment flyers were provided. Researchers will be working with local Spanish-speaking community members to ensure that the appropriate regional dialect of Spanish is used.

c. *Equitable selection of study participants*

- The study is designed to recruit an appropriately diverse population of participants who represent skilled agricultural workers in the 5 study locations.

- Community representatives and advocates are appropriately involved in the recruitment and enrollment of study participants.

2. The Board recommended that the study protocol be modified to address the few concerns noted in the EPA's Ethics Review (Evans and Sherman 2009). In addition, the Board also addressed three concerns of the Agency and the sponsors (Collier 2009c) with respect to representativeness, language, and release of individual exposure data.

    - The Board recommended that the AHETF implement the proposed protocol changes designed to address issues of representativeness.

    - As noted above, the Board concluded that the proposed protocol changes designed to address concerns of language in the informed consent and related documents are likely to yield translations that are written in the appropriate regional dialect of Spanish.

    - The Board commended the Task Force for wanting to release individual exposure data to participants promptly but recommended that these data only be released once the study is complete, except in those instances where data collected from individuals suggest an unusually high level of exposure and thus a clear need to mitigate exposure risks.

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