June x, 2006

EPA-HSRB-06-01

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

Subject: April 4-6, 2006 Meeting EPA Human Studies Review Board Report

Dear Dr. Gray:

The United States Environmental Protection Agency (EPA or Agency) requested the Human Studies Review Board (HSRB) to provide advice on Agency scientific and ethics reviews of completed human studies concerning the following pesticide active ingredients: aldicarb, amitraz, azinphos-methyl, dichlorovos (DDVP), ethephon, methomyl, oxamyl, and sodium cyanide. The studies reviewed included both studies on which the Agency proposes to rely in actions under the pesticide laws and studies that the Agency has decided not to use in its risk assessments, either for ethical or scientific reasons. The enclosed HSRB report addresses the Board's response to EPA charge questions for the Board's consideration at its April 4-6, 2006 meeting.

The HSRB was extremely impressed with the high quality scientific and ethical review brought before the Board. A summary of the Board's conclusions on the scientific and ethical considerations of the human toxicity studies for the eight pesticides under review are provided below.

Aldicarb

Scientific considerations

- The cholinesterase data from the aldicarb human study were reliable for use in the aldicarb single chemical, aggregate risk assessment.
- The cholinesterase data from the aldicarb human toxicity study were reliable for use in the cumulative risk assessment for N-methyl carbamates.

Ethical considerations

- The aldicarb human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.
- There was no clear or convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.
- There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

Methomyl

Scientific Considerations

- The methomyl human study could be appropriately applied to the inter-species risk factor for methomyl and for use in cumulative risk assessment of N-methyl carbamates.

Ethical Considerations

- The methomyl human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.
- There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.
- There was no clear and convincing evidence of significant deficiencies in the ethical procedures could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

Oxamyl

Scientific Considerations

- Although the Board had some questions about the Agency's conclusions regarding lack of sex difference and of the difference between brain and RBC ChE based on only one species, the Board supported the Agency's conclusion that there were no study deficiencies identified that would have affected the outcome of conclusions of this study.

- Considering the high quality of the design and the conduct of the study, the Board agreed that this intentional human dosing study of oxamyl was sufficiently robust to be used for reducing the 10x inter-species (i.e. animal to human) uncertainty factor in the cumulative risk assessment for the N-methyl carbamates.

Ethical Considerations

- The oxamyl human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

- There was no clear or convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

- There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

Azinphos-Methyl

Scientific Considerations

- Data from the 28-day repeat oral dose study of azinphos methyl should not be used in developing a point of departure for extrapolation of risk to workers exposed to azinphos-methyl via the dermal and inhalation routes.

- Data from the 28-day repeat oral dose study of azinphos-methyl cannot be used to inform the inter-species factor in the cumulative risk assessment.

Ethical Considerations

- The AZM human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

- There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

- There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

DDVP

Scientific Considerations

- The DDVP repeat dose human toxicity study was sufficiently robust for developing a point of departure for estimating dermal, incidental oral, and inhalation risk from exposure to DDVP in the single chemical risk assessment.

- The DDVP repeat-dose human toxicity study should not be used to support reducing the default 10X inter-species factor in the cumulative risk assessment of the organophosphate pesticides.

- The HSRB concluded that the other DDVP human toxicity studies available for the Board's consideration should not be used for determining a reduction in the 10X

uncertainty factor to derive reference dose values for DDVP based on animal toxicity endpoints.

Ethical Considerations

- The DDVP repeat dose oral human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

- There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

- There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

Ethephon

Scientific Considerations

- The Board concluded that the scientific quality of both the ethephon 28 day oral toxicity study and the ethephon 16 day human oral toxicity study was not adequate on its own. The 16 day study can be used to inform the ethephon 28 day human oral toxicity study. The Board approved the 28 day study for use in EPA risk assessments, emphasizing that the dose level administered is almost certainly not the lowest dose at which adverse effects are likely to be observed. However, its use in lieu of the animal studies will result in greater protection for exposed human populations.

Ethical Considerations

- Both the 28 and 16 day oral human toxicity studies failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

- There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

- There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

Hydrogen Cyanide

Scientific Considerations

- Data from the amygdalin trial could be used for establishing a point of departure in the acute dietary risk assessment for hydrogen cyanide.

Ethical Considerations

- The hydrogen cyanide human oral toxicity study appeared to meet the specific ethical standards prevalent at the time the research was conducted.

- There was no evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

- There was no evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

Amitraz

Scientific Considerations

- The results from the single oral dose study are informed by the human metabolism study such that the single oral dose study is appropriate for developing a point of departure for acute and chronic dietary risk and short-term oral exposure.

- The combined results from the single oral dose study and the human metabolism study were not appropriate for developing a point of departure for chronic dietary risk, short-term oral exposure, or inhalation risk.

- The majority of the Board concluded that the human dermal study was not appropriate for developing a point of departure for dermal exposures of various durations.

Ethical Considerations

- The amitraz acute oral and dermal human toxicity studies failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

- There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

- There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

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

Sincerely,

Celia Fisher, Ph.D. Chair
EPA Human Studies Review Board

NOTICE

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

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

United States Environmental Protection Agency Human Studies Review Board

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* Not in attendance at April 4-6, 2006 Public Meeting

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INTRODUCTION

On February 6, 2006 (71 FR 24, 6137), EPA published its final rule for protection of subjects in human research. This new rule included creating an independent Human Studies Review Board (HSRB) to provide advice, information and recommendations related to the scientific and ethical issues of such research.

The Food Quality Protection Act (1996) mandated that the Agency reassess pesticide tolerances (i.e., the legal limit of pesticides in food) by August, 2006. The Agency is in the final stages of reassessing all tolerances and has elected for the inaugural April 4-6, 2006 HSRB meeting to focus the first meeting of the Board on those pesticides which are subject to the August, 2006 statutory deadline for tolerance reassessment and which have a human toxicity study being considered for incorporation in quantitative human health risk assessment. The HSRB reviewed toxicity studies involving intentional exposure of human subjects to eight pesticide active ingredients: aldicarb, amitraz, azinphos-methyl (AZM), ethephon, dichlorvos (DDVP), methomyl, oxamyl, and sodium cyanide. Three of these pesticides are members of the *N*-methyl carbamate (NMC) common mechanism group (aldicarb, methomyl, oxamyl); two are members of the organophosphorus pesticides (OP) common mechanism group (AZM, DDVP). The three remaining pesticides (amitraz, ethephon, sodium cyanide) each have toxicity profiles that differ from the other chemicals.

The Agency prepared a variety of documents which provided background information. These included the proposed and final Human Studies Rule, report from the National Academy of Sciences regarding human studies, EPA, Office of Pesticide Programs' (OPP's) policy regarding the use of cholinesterase data in human health risk assessments, and a slide presentation providing a broad overview of the OPP's human health risk assessments. The Agency used the framework provided in Emanuel (2000) as the basis for its ethics reviews. The Agency described the approach for performing scientific review of human studies and for incorporating human toxicity data into risk assessment.

For each of the human studies under consideration, the Agency provided the complete study report as submitted to the Agency. For each chemical, the Agency developed a review of the ethical conduct of the study. Each ethics review identified any deficiencies noted in the conduct of the specific study compared to both current ethical standards and the ethical standards prevailing at the time the research was performed. The Agency has intentionally deferred making a final determination of whether an individual study satisfied the ethical standards for acceptability in 40 CFR sections 26.1704 – 26.1706, pending the advice of the Board.

For most studies, the Agency develops documents, called Data Evaluation Records (DERs), containing a scientific review of the study. DERs contain summaries of the study design, methods and results, describe potential deficiencies, and provide conclusions about the usefulness of the study in risk assessment. In addition to the DERs, the Agency prepared for each chemical a "Weight of Evidence" (WOE) memorandum that discusses the differences and similarities between the human and animal toxic responses to each chemical and characterizes the usefulness of the human toxicity studies for human health risk assessment. The WOE memos

express the Agency's most current scientific conclusions on which the Agency solicited comments from the Board.

The HSRB reviewed both studies on which the Agency proposes to rely on actions under the pesticide laws and studies that the Agency has decided not to use in its risk assessments, either for scientific reasons or because they do not meet the standards in EPA's final human studies rule. 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 EPA's final human studies rule. The Board anticipates having a series of meetings to review pesticide human studies relevant to pending re-registration, tolerance reassessment and new registration decisions, in accordance with the provisions of the final rule. This report transmits the HSRB's comments and recommendations from its inaugural April 4-6, 2006 meeting.

REVIEW PROCESS

On April 4-6, 2006 the Board had a public face-to-face meeting in Arlington, Virginia. Advance notice of the meeting was published in the Federal Register "Human Studies Review Board: Notice of Public Meeting and Proposed Candidates for Membership to the Board (71 FR 46, 12194). At the public meeting, following welcoming remarks from Agency officials, Celia B. Fisher, HRSB Chair, proposed a set of scientific and ethics criteria consistent with the language of 71 FR 24, 6137 to guide Board evaluation of each protocol. The Chair's scientific criteria asked the Board to consider the following two questions: (1) Did the research design and implementation meet scientific standards and (2) Did the data generated by the protocol have implications for the Agency's Weight of the Evidence (WOE) review and when applicable aspects of the risk assessment? The Chair's ethics criteria asked the Board to consider three questions: (1) Did the study fail to fully meet specific ethical standards prevalent at the time the research was conducted; (2) Was the conduct of the study *fundamentally unethical* (i.e., specifically was there clear and convincing evidence that the research was intended to seriously harm participants or failed to obtain informed consent); and (3) Was the conduct of the study *significantly deficient* relative to the ethical standards prevailing at the time (i.e., was there clear and convincing evidence that identified deficiencies could have resulted in serious harm based on knowledge available at the time the study was conducted *or* the information provided to participants could seriously impair informed consent. The Board then heard presentations from EPA on the following topics: (1) introduction (summary of EPA protections for subjects of human research, science and ethics considerations of human subjects research); (2) carbamate pesticides (science and ethics of aldicarb, methomyl and oxamyl human studies); (3) organophosphate pesticides (science and ethics of azinphos methyl and DDVP human studies); (4) other pesticides (science and ethics of ethephon, sodium cyanide and amitraz human studies). The Board also heard oral public comments from the following individuals:

<u>Carbamate pesticides</u>
Mr. Angus Cameron representing BCG Europe
Neil Carmichael, Ph.D. representing Bayer Crop Science
Jennifer Sass, Ph.D. representing Natural Resources Defense Council

Organophosphate Pesticides
Monty Eberhart, Ph.D. and Mr. Dan Van Geothen representing Bayer Crop Science and Mahktesheim Agan
Robert Levine, Ph.D. representing Amvac
Mr. Ian Chart representing Amvac
Laura Plunkett, Ph.D. representing Amvac
Thomas Starr, Ph.D. representing Amvac
Jennifer Sass, Ph.D. representing Natural Resources Defense Council
Ms. Shelley Davis representing the Farmworker Justice Fund

Other Pesticides
Neil Carmichael, Ph.D. representing Bayer Crop Science

Following Agency presentations and public comments, the Board deliberated on the charge questions. For their deliberations, the Board considered the materials presented at the meeting, written public comments and Agency background documents on each individual pesticide (i.e., pesticide human study, Agency data evaluation record (DER) of the pesticide human study, weight of evidence review, risk assessment and ethics review).

CHARGE TO THE BOARD AND BOARD RESPONSE

1. Aldicarb

Charge to the Board

Aldicarb is a *N*-methyl carbamate (NMC) pesticide whose primary toxic effect is neurotoxicity caused by the inhibition of the enzyme acetylcholinesterase, via carbamylation followed by rapid recovery. Aldicarb can, at sufficiently high doses, lead to a variety of clinical signs. The Agency is conducting an acute, aggregate (single chemical, multi-route) risk assessment of aldicarb. In addition, aldicarb is a member of the *N*-methyl carbamate common mechanism group and is thus included in the cumulative (multi-chemical, multi-route) risk assessment for the NMCs.

1.1 Scientific considerations

The Agency's "Weight of the Evidence" (WOE) document and Data Evaluation Records (DERs) for aldicarb describe the study design and results of the aldicarb acute oral, human toxicity study. The WOE document also discusses the Agency's conclusions regarding the usefulness of the human study in the acute, aggregate, single chemical risk assessment and in the cumulative risk assessment for the NMCs. Regarding the aldicarb human study, the Agency has concluded that the study is sufficiently robust for reducing the inter-species (i.e., animal to human) uncertainty factor in the aggregate and the cumulative risk assessments.

The Board was asked to comment on the scientific evidence that supports whether the aldicarb human study is sufficiently robust for reducing the inter-species (i.e., animal to human) uncertainty factor in:
a. single chemical, aggregate risk assessment and
b. cumulative risk assessment.

Board Response to the Charge

Brief Overview of the Study

The study (Wyld et al. 1992) consisted of a double blind, placebo-controlled, single oral dose of aldicarb in orange juice, taken during the course of a meal. A variety of both subjective and objective observations were accumulated, including red blood cell (RBC) cholinesterase activities, blood pressure, and respiratory parameters among others. Several dose levels were investigated and several time points were also investigated.

Critique of Study

The strengths of the study were: it was designed with multiple doses so a dose-response relationship could be studied; it was double blind; a large number of parameters that are relevant to anticholinesterase were studied (including both subjective data such as headache and several objective physiological measures such as blood pressure, pulse, pulmonary function and saliva production); there were exclusion criteria; there were frequent measurements of cholinesterase over a 1 day period in addition to pre-treatment measurements; the observations of clinical signs were made by trained observers; both sexes were studied (although there were fewer doses and individuals in the female group); and the cholinesterase depression was both dose- and time-dependent in both males and females.

The weaknesses of the study were: there were typically only 4-8 individuals per dose; there were fewer female subjects and they were only tested at the middle dose levels; there were a large number of reported signs across all groups, including the placebo, making interpretation difficult; the cholinesterase methodology may have not assayed all of the cholinesterase in RBCs; and the first time point of cholinesterase measurement may have been after the time of peak cholinesterase inhibition.

The Board also noted that, with respect to statistical analysis, the study was likely to be under-powered. With the large number of endpoints and the relatively large between-subject variances in almost all endpoints, the number of subjects was probably insufficient to guarantee adequate statistical power. In addition, no adjustments were made for multiple comparisons. Since each endpoint was analyzed separately and because many comparisons were carried out across genders, time points, doses, etc., the overall confidence level is probably much below the nominal 95%. It was also noted that the univariate statistical models that were fitted to the data account for the repeated measures structure of the design, but do not account for the multivariate structure in the measurements. Because endpoints were measured on the same subjects, one could expect meaningful correlations between the various endpoints that could contain additional information about the treatment effects and the effects of covariates. Consequences of not using

multivariate methods on this data set include a decrease in the power of statistical tests and erosion of confidence levels that occur when many models are fitted to many endpoints independently as was done in this study. These considerations throw some doubt on some of the study results that suggest no statistically significant treatment effects.

Nevertheless, while it is clear that the statistical power of the study was low, the data do show a very clear and predictable dose- and time-dependency in the RBC cholinesterase data. The response of the cholinesterase data is what would be expected of a transient carbamate anticholinesterase. Additionally, it would be expected that blood cholinesterase would be the most sensitive endpoint. RBC cholinesterase is well recognized as a sensitive biomarker of exposure to anticholinesterases. Its inhibition is not responsible for clinical signs of anticholinesterase toxicity, so no cause-and-effect relationship should be expected between RBC cholinesterase inhibition and changes in any of the physiological parameters measured. Therefore, it is also reasonable that inhibition of the RBC cholinesterase would occur whereas the other parameters related to clinical signs, such as salivation or blood pressure, would not. Lastly, the occurrence of numerous clinical signs in the placebo group gives credence to the conclusion that the RBC cholinesterase inhibition was a treatment-related effect whereas clinical signs were not.

The Board concluded that the results of the study could be used in the WOE analysis to determine a NOEL for RBC cholinesterase and clinical signs in males and that the RBC cholinesterase demonstrated a dose-dependent and time-dependent pattern of inhibition in both males and females.

HSRB Consensus and Rationale

The Board concluded that the cholinesterase data from the aldicarb human study were reliable for use in the aldicarb single chemical, aggregate risk assessment.

The Agency Data Evaluation Report for Aldicarb (Sette 1992) suggested that the NOAEL and LOAEL were based upon sweating in males. While sweating is a possible clinical sign resulting from cholinesterase inhibition, the responses in the subjects were not consistently dose-related. Although sweating is an objective endpoint, the WOE document indicates that the RBC cholinesterase inhibition would be the more appropriate objective endpoint. There are additional reasons why there is more confidence in the cholinesterase data. First, the blood cholinesterase data, despite their weaknesses, are the most consistent responses to aldicarb exposure, because they follow time- and dose-dependent patterns, as would be expected of a transient anticholinesterase. Second, the cholinesterase data, though probably based on incomplete fractions of the entire RBC cholinesterase population due to the nature of the analysis method, would still be expected to be internally consistent within this study between samples of treated and placebo individuals, and therefore the inhibition levels can probably be believed. There was a dose- and time-to-recovery-related relationship to cholinesterase inhibition in both sexes, so the cholinesterase data are also consistent between the sexes.

The Board concluded that the cholinesterase data from the aldicarb human toxicity study were reliable for use in the cumulative risk assessment for N-methyl carbamates. Aldicarb,

being an N-methyl carbamate, belongs to this common mechanism group which acts via acetylcholinesterase inhibition. Therefore, the endpoint in the cumulative risk assessment must be cholinesterase inhibition. Moreover, the dose-response data from the human study appeared such that BMD and BMDL can be calculated. The HSRB concluded that the aldicarb human study appears to be a scientifically valid study, suitable for use in both the aggregate risk assessment and the cumulative risk assessment.

While the Board concluded that the cholinesterase data could be reliably used in the cumulative risk assessment, it did note some limitations. The animal data were not supplied so the HSRB cannot address the accuracy of the corresponding animal data. Because of the rapid reactivation of carbamylated cholinesterase, it is unclear whether accurate cholinesterase inhibition values were obtained in the human study or that peak inhibition was measured because the earliest time point measured was 1 hour post-dose. The WOE document stated that the human and rat cholinesterase inhibition were comparable at the 0.05 mg/kg dose level; a question arises as to whether this comparison was made at the same point in the inhibition/recovery patterns of both species. However, it should be possible to make extrapolations of the possible peak from animal data on the time course pattern of cholinesterase inhibition and recovery, and the Agency is urged to make certain that BMD and BMDL comparisons between animals and humans are being made on comparable times in the inhibition/recovery pattern.

1.2. Ethical considerations

Charge to the Board

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

In light of the ethics committee's instruction that the lay summary be "greatly expanded," and the fact that the materials used to obtain informed consent listed a limited range of symptoms of carbamate toxicity (excluding some reported as adverse effects in the study), included multiple references to the test material as a drug, and failed to identify dose levels to be administered to male subjects, whether, the materials used to obtain informed consent should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted

Whether the absence from the protocol of discussion of the potential risks to subjects or benefits to society of conducting the proposed research (as required by the 1989 Declaration of Helsinki, Principle # 4, with which the research asserted compliance) should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted;

b. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of this study:

OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical.

Whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Board Response to the Charge

Study Overview

The study was completed on March 11, 1992. The study sponsor was located in France and the performing laboratory was located in Scotland. The protocol documents specifically stated that the research was conducted in compliance with the Principles of Good Clinical Practice as promulgated by the EMEA CPMP in May 1990, and the Declaration of Helsinki (in either the 1983 or 1989 version, as cited in two different portions of the study documents). Although the May 1990 document on good clinical practice was the precursor for the subsequent 1997 document promulgated by the International Conference on Harmonization, the earlier document does not include any specification of the contents of the informed consent form. However, the supporting and supplementary study documents claimed that the research ethics committee providing independent review and oversight of the research functioned according to the standards of the FDA regulations found in 21 CFR 56. These regulations cross-reference the informed consent requirements found in 21 CFR 50 Both FDA regulations were effective on January 27, 1981. As such, the research ethics committee should have applied the FDA regulations from nearly a decade before the conduct of this particular research protocol. It should be noted that the sponsor subsequently claimed that the study also was performed according to the requirements of 40 CFR 26, based on a retrospective analysis published in 2003.

Critique of Study

The Board concurred with the Agency on the factual observations of the strengths and weaknesses of the study, as detailed in Carley (2006a). However, further comments are warranted on (1) the documentation and process of informed consent, and (2) the minimization of risk with respect to the study design.

It is clear that the written documentation for informed consent is not up to the standards found in the above regulations. The Volunteer Information that was given to research subjects as an appendix to the signed informed consent document did not have an adequate discussion of the risks of the research. At the request of the research ethics committee (dated December 24, 1991), the Volunteer Information included the possibility that administration of the test product could result in "gut motility effects (abdominal pains), effect on eye pupil size and muscle weakness"). In addition to the concerns that the risk information was insufficient in the written consent documentation, selected subjects were enrolled a second time. This was not discussed or in the protocol or the informed consent documentation and therefore raises doubts to the Board about the adequacy of the consent process. In effect, the re-dosing of selected subjects suggests that the investigator was unblinded to group assignment, reducing the supposedly double blind study to a single-blind study and raising doubts (unanswered by the documentation) about the sufficiency of informed consent. In particular, the re-use of selected subjects suggests that some study participants were randomized to receive placebo versus active compound while other

subjects were not. As a result, the burden of risks was unlikely to have been distributed evenly among all study participants.

There were several observations that raised concerns about the extent to which the study design minimized risk to research subjects. First, the dosing schedule did not adhere to a strict dose escalation design. As a result, it is possible that subjects dosed at higher levels would experience significant adverse events that could have been anticipated in a more traditional dose escalation design. The Board recognized that the public comments by Bayer CropScience presented during the public comment period at the HSRB meeting suggests that there was previous human testing data available to indicate that such risks would be unlikely. Second, the stopping rule based on a 70% inhibition of cholinesterase activity is fairly liberal (i.e. not protective), given that 70% inhibition would likely result in significant adverse effects. Although no subject in the research actually experienced this level of cholinesterase reduction, the selection of such a stopping rule increases the risk to subjects. Finally, the letter to the subjects' general practitioner does not contain any information about the actual study and the product being administered other than the name of the compound. As such, this minimizes the extent to which the opinion of the general practitioner could protect the research subject.

HSRB Consensus and Rationale

The Board concluded that:

a) The aldicarb human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no clear or convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

Informed Consent: While informed consent was obtained, the informed consent documents did not include adequate discussion of the research risks as documented by Carley (2006a) and did not fully meet ethical standards prevalent at the time the study was conducted. There was a suggestion that additional aspects of risk may have been discussed in the Volunteer Information. Although the Board recognized that the documentation would not meet today's standards, the lack of documentation does not in and of itself demonstrate a significant deficiency that would have seriously impaired the participants' ability to provide informed consent.

Participant risk: The Board concurred that the stopping rule based on 70% reduction in cholinesterase activity raised the possibility of exposing subjects to inappropriate risk. However, the Board acknowledged that data existed at the time to suggest that the doses used in the study

would likely not achieve this level. Thus, there is no evidence that serious harm was intended nor that serious harm could have resulted from the implementation of the study.

The Board concluded that there was no obvious reason why the Agency cannot rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

2. Methomyl

Charge to the Board

Methomyl is a member of the *N*-methyl carbamate (NMC) common mechanism group based on its ability to inhibit acetylcholinesterase via carbamylation. The Agency has previously completed the acute, aggregate (single chemical, multi-route) risk assessment of methomyl. At the present time, the Agency is considering the use of the methomyl acute oral, human toxicity study to inform the inter-species uncertainty factor used in the cumulative risk assessment of the NMCs.

Scientific considerations

The Agency's WOE document and DER for methomyl describe the study design and results of the methomyl acute oral human study. The WOE document also discusses the Agency's conclusions regarding the usefulness of the human study in the cumulative risk assessment for the NMCs. For methomyl, the Agency has concluded that the human toxicity study supports a 10X inter-species uncertainty factor for methomyl in the cumulative risk assessment of the NMCs.

Please comment on the scientific evidence that supports this conclusion.

Board Response to the Charge

Study Overview

A study with methomyl was conducted in human subjects using a double-blind, placebo-controlled, single ascending dose design (McFarlane et al. 1998). The defined goal of the study was to determine an acute no-adverse effect level (NOAEL) based on inhibition of plasma and RBC cholinesterase, and in the original protocol, the doses were projected to be 0.1, 0.3, 0.5, 0.75, 1 and 1.5 mg/kg. Methomyl was administered as a bolus dose in a capsule immediately (about 5 minutes) following a meal, and post-dose blood sampling commenced at 15 minutes after dosing. Collection of blood samples for analysis of plasma and RBC acetylcholinesterase activity was continued for 24 hours after dosing. Plasma and RBC acetylcholinesterase activities were compared to a baseline measurement, determined from two predose samples collected 16 hours and 30 minutes prior to dosing, for each subject. The results were compared to the placebo group. In addition, numerous physiological parameters including ECG, heart rate, pulse, blood pressure, body temperature, clinical chemistry and hematology parameters, determination of

pupil size and salivation were assessed during the entire 24 hour period after dosing. The criterion for not escalating to the next dose was inhibition of RBC cholinesterase exceeding 40%.

Critique of Study

The scientific validity of this study was determined by its comparative strengths and weaknesses. The strengths of this study are determined by the comprehensive analysis of cholinesterase inhibition that was obtained including:

- The design and results established a clear dose-response and time-dependent relationship for inhibition of RBC cholinesterase. This was based on the execution of three dose levels in the ascending dose paradigm. The dosages evaluated were 0.1, 0.2 and 0.3 mg/kg.
- The analysis of the temporal pattern of RBC cholinesterase inhibition was comprehensive and complete. Specifically, blood samples were collected at 15 minutes after dosing, with 15 minute sampling intervals for the first 2 hours, followed by sample collection for evaluation at 2, 3, 4, 6, 8, 12 and 24 hours after dosing. This time course established the peak of cholinesterase inhibition and assured recovery of enzyme activity in all subjects.
- The inclusion of clinical endpoints associated with increased cholinergic stimulation as additional endpoints provided additional relevant measures of potential adverse effects during the course of the 24 hour study.

Several weaknesses of the study were noted, and these weaknesses are also based on aspects relating to its design and conduct and include:

- All subjects were male, with no data obtained from females
- The group size (5/dose level) was small
- The establishment of the baseline RBC cholinesterase level from which all subjects were compared was the mean of 2 determinations, which, given the general pattern of variation, reduces the confidence in the calculation of percent inhibition of the enzyme.

One additional issue concerning the design and execution of this study was the initial proposed dose escalation which ranged from 0.1 to 1.5 mg/kg (0.1, 0.3, 0.5, 0.75, 1, and 1.5 mg/kg) and the criterion to not escalate dose was inhibition of RBC cholinesterase exceeding 40%. There was one instance in which this criterion was not adhered to. In the first session of the study, one subject was dosed with placebo and the other received methomyl at 0.1 mg/kg. The subject receiving methomyl showed evidence of RBC cholinesterase inhibition exceeding 40% (approximately 43%), but this change was determined at 8 hours after dosing. The study sponsor concluded that, based on the general knowledge and understanding of the rapidity of enzyme inhibition observed with the carbamates, this finding was likely to be a spurious result. In review of the data for RBC cholinesterase inhibition for this session, it was also noted that the placebo-treated subject in this session showed a 20% decrease in baseline enzyme activity, thereby suggesting a possible, albeit unconfirmed, issue regarding the accuracy and reliability of the analysis of enzyme activity in these samples. Therefore, the sponsor's conclusion that the effect observed in the subject dosed with 0.1 mg/kg methomyl was most likely to be a spurious

result was considered scientifically reasonable and justifiable, and the decision to escalate the dose in the next session was not in violation of the protocol.

In the second session of the study, the lead subject dosed with methomyl at 0.3 mg/kg also showed a greater than 40% inhibition of RBC cholinesterase. Consequently, the next session completed the five subjects to be used in this group but did not escalate the dose further as per the study protocol. Therefore, the highest dosage evaluated in the study was 0.3 mg/kg, which was much lower than the original intended escalation up to 1.5 mg/kg. To establish a better dose-response relationship, the study was unblinded, and a dosage of 0.2 mg/kg was added to the protocol after which the study was re-blinded and completed.

HSRB Consensus and Rationale

The Board concluded that the methomyl human study could be appropriately applied to the inter-species risk factor for methomyl and for use in cumulative risk assessment of N-methyl carbamates.

The HSRB concluded that data from the study in human subjects exposed to methomyl provided a meaningful assessment of the dose-response relationship and time course for inhibition of RBC cholinesterase in human subjects. The strengths of the study design and conduct outweigh the indicated weaknesses, and the results are scientifically valid. Therefore, the data can be used to inform the assessment of interspecies uncertainty factors for methomyl.

Ethical considerations

Charge to the Board

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

- Whether the investigators' decision to administer a dose to additional subjects in session 3, when one subject receiving that dose in session 2 displayed RBC ChEI greater than 40%, a response that triggered the protocol's anti-escalation provision, should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted;

- Whether the timing of the investigators' report to the ethics committee of the adverse effects observed in one subject during session 2 should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted;

- Whether the failure of the investigators to request approval from the ethics committee for certain amendments to the approved protocol, as required by the protocol, when the changes were administrative and had no effect on the safety of the subjects should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

- Whether the absence from the protocol of discussion of the potential risks to subjects or benefits to society of conducting the proposed research (as required by the Declaration of Helsinki, Principle # 5) should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

2.2.2. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of this study:

- OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical.

- Whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted,

Board Response to the Charge

Brief Overview of the Study

The study was performed by a commercial research organization, Inveresk Clinical Research of Edinburgh, Scotland and sponsored by the Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Company, of Newark, Delaware. The protocol documents specifically state that the research was conducted in compliance with the Principles of Good Clinical Practice promulgated by the International Conference on Harmonization (CMPM/ICH/135/95), and the 1996 version of the Declaration of Helsinki.

Critique of Study

The Board concurred with the Agency's factual observations of the strengths and weaknesses of the study, as detailed in Carley (2006b). However, further comments are warranted on certain events that took place during the conduct of the study.

1. Determination That Certain Data Were Spurious

In session one of the study, one of the subjects exhibited a greater than 40% inhibition in RBC cholinesterase activity at one point, without any signs or symptoms. According to the protocol, such inhibition of cholinesterase was a criterion for not escalating to the next dose level. Because this occurred at 8 hours post dose, study personnel considered this to be a spurious result and went ahead with escalation to the next dose without consulting with the ethics review board (It is somewhat unclear when the results of the assay became available; the observation about this result is described as being noted "retrospectively at the time of reporting, when there was no sample available for retesting."). If this determination of spuriousness had been incorrect, then giving the following subject an escalated dose might have placed that subject at considerable risk.

The Board concluded that the determination of spuriousness was reasonable at the time, given the late timing of the change in cholinesterase levels, and the similar change observed in cholinesterase levels for a subject in session one who received placebo. Nonetheless, the Board determined that a far more appropriate action would have been for the researchers to have first consulted with the ethics review committee about repeating that 0.1 mg/kg dose before proceeding with dose escalation. Moreover, the report of the study results stated that "laboratory tests showing abnormal values for any subject were repeated as often as deemed necessary by the clinical investigator until the test values returned to accepted limits or until an explanation other than compound effect was given." In spite of this, it does not appear that any additional tests were performed; study participants only received scheduled laboratory tests at the 12 hour and 24 hour points. Although there is a possibility that these events might have placed subjects at an increased risk of harm, the Board determined that there was not clear and convincing evidence that this could have resulted in serious harm to subjects, particularly given the information known about the compound being tested and the other protections in place as part of the protocol.

2. Decision to Proceed with Third Session

In session two of the study, one subject who received an escalated dose of 0.3 mg/kg exhibited a greater than 40% inhibition in RBC cholinesterase, together with self-reported clinical symptoms (i.e. headache). As noted above, the protocol indicated that dose escalation would not occur if any subject experienced such a level of inhibition. The protocol was followed in this regard: additional subjects were tested at the 0.3 mg/kg dose, but not at any higher doses. However, the protocol also stated that the study would stop if a subject both had greater than 40% inhibition and also demonstrated signs or symptoms of carbamate toxicity (one of which might be considered to be a headache). Although the Board was concerned that this decision to continue the study and to test additional subjects at the 0.3 mg/kg dose was made without consulting the ethics review committee, it determined that the provisions of the protocol were sufficiently unclear as applied to these facts (particularly regarding what constitute signs and symptoms of toxicity); the actions of the investigators were thus consistent with the protocol as written. In addition, given the protections that were in place as part of the protocol, and the information then known about the compound being studied, the Board concluded that there was not clear and convincing evidence that this decision presented a risk of substantial harm to the subjects. Nonetheless, the Board concluded that a more appropriate course of action would have been for the researchers to have consulted with the ethics review board before proceeding with the study. This conclusion and the desirability of having taken similar actions after the apparently "spurious" result in session one is underscored by public comments presented to the Board that the observation of acetylcholinesterase depression at 0.3 mg/kg was "surprising." There is a danger in explaining away unexpected findings based on prior expectations, and ethics review boards should play an important role in preventing investigators from falling prey to such expectations.

3. Amendment of the Protocol

Following the testing of the subjects at the 0.3 mg/kg level as part of session three, the protocol was amended to allow additional subjects to be tested at the 0.2 mg/kg level. This

change was approved by the chair of the ethics review committee, consistent with procedures for the committee which allowed the chair to approve amendments that did not increase the risk level (i.e., did not involve testing subjects at a dose level above those approved by the convened board). Although the Board did not conclude this was a formal violation of then-existing ethical standards, some members of the Board were concerned that the ethics committee chair's actions were inappropriate and that the protocol change should have been reviewed by the full committee because additional subjects were being exposed to a study compound beyond those contemplated by the unamended protocol.

HSRB Consensus and Rationale

The Board concluded that:

a. The methomyl human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b. There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c. There was no clear and convincing evidence of significant deficiencies in the ethical procedures could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

(1) The Board concluded that the decision to administer a dose to additional subjects in session three could not be determined to be significantly deficient relative to the ethical standards prevailing when the study was conducted because, as discussed above, that decision could be interpreted as consistent with the terms of the protocol and was not likely to expose the subjects to additional serious harm.

(2) The Board concluded that the timing of the investigators' report to the ethics committee regarding what happened to the subject who experienced cholinesterase inhibition during session two should not be considered significantly deficient relative to the ethical standards prevailing when the study was conducted, given the subject's minimal symptoms and the fact that RBC cholinesterase inhibition by itself is not considered an adverse event.

(3) The Board determined that the failure of the investigators to request approval from the ethics committee for certain amendments to the approved protocol, while deficient relative to the ethical standards prevailing at the time the study was conducted, should not be considered significantly deficient, given that these changes were purely administrative.

(4) The Board determined that the absence from the protocol of discussion of the potential risks to subjects or benefits to society of conducting the proposed research (as required by the Declaration of Helsinki, Principle # 5) should not be considered significantly deficient relative to the ethical standards prevailing when the study was conducted. The potential risks and benefits are described in the research subject information sheet/informed consent document.

While not in the protocol itself, they are nonetheless included in the materials provided to the ethics committee (and to the potential research subject). Thus, the ethics committee and the potential research subjects had the opportunity to weigh the risks and benefits.

The Board concluded that there was no obvious reason why the Agency cannot rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

3. Oxamyl

Charge to the Board

Similar to aldicarb and methomyl, oxamyl is a member of the *N*-methyl carbamate (NMC) common mechanism group based on its ability to inhibit acetylcholinesterase via carbamylation and is thus included in the NMC cumulative risk assessment. The Agency has previously completed the acute, aggregate (single chemical, multi-route) risk assessment of oxamyl. The Agency is now considering the use of the oxamyl acute oral, human toxicity study to inform the inter-species uncertainty factor in the cumulative risk assessment of the NMCs.

3.1. Scientific considerations

The Agency's WOE document and DER for oxamyl describe the study design and results of the oxamyl acute oral human toxicity study. The WOE document also discusses the Agency's conclusions regarding the usefulness of the human study in the cumulative risk assessment for the NMCs. For oxamyl, the Agency has concluded that the human toxicity study is sufficiently robust for reducing the 10X inter-species (i.e., animal to human) uncertainty factor in the cumulative risk assessment.

Please comment on the scientific evidence that supports this conclusion.

Board Response to the Charge

Study Overview

The stated primary objective of this randomized, double-blind, ascending oral dose study with oxamyl (McFarlane and Freestone 1999a) was to estimate the no-observable-adverse effect level (NOAEL) for oxamyl in humans after oral administration.

The study used 40 healthy male subjects, aged 19-39. Each was given a single oral dose of oxamyl in a gelatin capsule at doses of 0 (placebo), 0.005, 0.015, 0.03, 0.06, or 0.15 mg/kg BW. With the exception of dose sessions 1 (2 placebos), 2 (1 placebo and 1 at 0.005), 7 (1 placebo and 4 at 0.09), 8 (1 placebo and 1 0.15), and 9 (1 placebo and 4 at 0.15), all other dose sessions had 1 placebo, 4 at current dose, and 1 at the next higher dose. Plasma and RBC cholinesterase activity were assayed at screening and 2 days, 16 hours and 30 minutes prior to dosing and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12,and 24 hours post dose and 7

days post dose. Other clinical parameters indicative of carbamate exposure, such as urinalysis, pupillometry, saliva increase etc were also measured.

No treatment related effects for ECG, heart rate, pulse, blood pressure, respiratory rate, body temperature, hematology, clinical chemistry, urinalysis, or pupillometry were recorded.

At 0.09 mg/kg b.w., 7-12% plasma and RBC ChE inhibition was observed with three of five volunteers exhibiting greater than 20% plasma ChE inhibition. Therefore, 0.06 mg/kg b.w. was considered the NOAEL. The Agency generated BMD and BMDL estimates of 0.083 mg/kg and 0.069 mg/kg, respectively, based on the RBC ChE data from the study

Critique of the Study

The scientific validity of this study was analyzed by weighing its strengths and weaknesses in order to determine if the study was sufficiently robust to be of use in the Agency's risk assessment for oxamyl.

Appropriate endpoints for this study were selected. Acetyl cholinesterase inhibition in the nervous system was viewed as a key molecular event in the mechanism of toxicity of carbamate compounds. Measurements of acetyl cholinesterase inhibition in both RBCs and brain are considered to be biomarkers of exposure for these compounds. In its policy on the se of data on cholinesterase inhibition for risk assessments of organophosphorus and carbamate pesticides (USEPA, 2000), the Agency stated that it will treat cholinesterase inhibition in the blood as a surrogate measure for the peripheral nervous system in animals and for the peripheral and central nervous system in humans.

The animal data for oxamyl were not available to the Board to allow it to make an independent judgment as to whether the methods used to determine acetyl cholinesterase inhibition in the animal studies and the human study were comparable. According to EPA, however, the methods used in the animal studies are comparable to that in the human study.

Previous animal data was used to predict that the starting dose 0.005 mg/kg would have no detectable effect on humans. Oral administration of the compound is the most predictive mode of administration since exposure to residues would come from food consumption. There was no justification for the sample size of 4 for oxamyl for each dose session.

Forty healthy males of similar age and weight were used in this study. A weakness of this study is that no female subjects were used in this study and there was no indication that any human female data exists from other studies. However, the Agency advised the Board that, based upon its evaluation of the animal data, no sex differences would be expected.

The specific schedule for dosing was described in protocol. It was an ascending double blind, placebo controlled study. Dose escalation did not occur if there were clinically significant symptoms/signs of toxicity. Stopping rules were equal to or greater than 40% inhibition of RBC cholinesterase at a single time point or 25% at two consecutive time points. If there were associated symptoms/signs of carbamate toxicity, no further dose escalation would occur.

A repeated measures analysis of variance was used to analyze the percentage change from baseline in BRC and plasma cholinesterase activity, pupillometry and saliva collection. At each time point of measurements, a test for linear trend with dose was performed using a linear contrast. If the test for linear trend was significant at the 5% significance level, the pairwise comparisons at each timepoint were not adjusted for multiple comparisons. Otherwise, a Bonferroni adjustment was applied to the pairwise test at the 0.83% significance level.

At 0.09 mg/kg b.w., 7-12% plasma and RBC ChE inhibition was observed with three of five volunteers exhibiting greater than 20% plasma ChE inhibition. Therefore, 0.06 mg/kg b.w. was considered the NOAEL. The Agency generated BMD and BMDL estimates of 0.083 mg/kg and 0.069 mg/kg, respectively, based on the RBC ChE data from the study

The Board highlighted the strengths of the study as follows:

(a) The study design, including both the increasing dose levels and the time point of observations/measurements, was very rich in information content to allow evaluation of dose-response and estimation of the NOAEL and the dose-escalation in a step-wise fashion was scientifically sound considering the uncertainties in the risk.

(b) The study had a very clearly defined dose-escalation scheme with properly justified stopping rules. The schedule of observation and measurements were frequent enough to give sufficient information on the time-course effect of oxamyl on subjects.

(c) The statistical methods used for analysis, including the adjustment for multiple comparisons between placebo and active doses when there was no statistical significance in the linear trend test, were quite adequate for the study.

The Board highlighted the weaknesses of the study as follows:

(a) There appears to be no specific justification for the sample size of four for oxamyl for each dose session and no traditional statistical test of effect size regarding hypotheses. Because the study was exploratory versus confirmatory, the lack of justification was not critical. Nevertheless it would have been helpful to know what effect size could be detected between the placebo and the active dose groups given the sample size of the study.

(b) Because of lack of justification for the sample size, the lack of statistical significance in the observed difference between placebo and active doses could not be interpreted appropriately. Specifically the statement in the Agency Data Evaluation Report (Moyer and Milznez 2001) that "RBC cholinesterase activity was statistically similar to placebo at all time points in the 0.005, 0.015, 0.03, and 0.06 mg/kg dose groups" was not justified as the study was not designed to test for statistical similarity.

(c) The Agency concluded that "there is no indication from the available rat data for either oxamyl or methomyl to suggest a sex difference." The animal data were not supplied to

the Board and therefore the Board could not independently verify this conclusion. However the Board was not uncomfortable with accepting EPA's conclusions on this matter.

The Agency also concluded that "Given the similarity between brain and RBC ChE in rats, the Agency is not aware of any biological or physiological reason that human brain ChE would be more sensitive than the rat brain." Again without seeing the animal data, the Board could not verify this conclusion about rats, but the conclusion about human brain and RBC cholinesterase is reasonable scientifically

HSRB Consensus and Rationale

Although the Board had some questions about the Agency's conclusions regarding lack of sex difference and of the difference between brain and RBC ChE based on only one species, the Board supported the Agency's conclusion that there were no study deficiencies identified that would have affected the outcome of conclusions of this study.

Considering the high quality of the design and the conduct of the study, the Board agreed that this intentional human dosing study of oxamyl was sufficiently robust to be used for reducing the 10x inter-species (i.e. animal to human) uncertainty factor in the cumulative risk assessment for the N-methyl carbamates.

3.2. Ethical considerations

Charge to the Board

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

- Whether inclusion in the protocol submitted to the ethics committee of a factually inaccurate statement regarding unavailability of data on accidental or incidental exposure to oxamyl should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted;

- Whether the absence from the protocol of any discussion of the potential risks to subjects or benefits to society of conducting the proposed research (as required by the Declaration of Helsinki, Principle # 5) should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

b. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of [this/each] study:

- OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical.

- Whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Board Response to the Charge

Study Overview

The study was performed by a contract research organization, Inveresk Clinical Research of Edinburgh, Scotland and sponsored by the Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Company, of Newark, Delaware. The protocol documents specifically state that the research was conducted in compliance with the Principles of Good Clinical Practice promulgated by the International Conference on Harmonization (CMPM/ICH/135/95), and the 1996 version of the Declaration of Helsinki. Comments provided by du Pont Crop Protection (Dupont 2006), also assert compliance with the 1996 version of the Declaration of Helsinki.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in Carley 2006b). However, further comments are warranted regarding: 1) whether the exclusion of data on accidental or incidental exposure to oxamyl meets the legal definition of research misconduct prevalent at the time the study was conducted; 2) whether the escalating-dose oral-exposure protocol used was designed to minimize risks to study participants; and 3) whether the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent.

1) Exclusion of Available Data on Accidental or Incidental Exposure

Page 15 of the original study protocol provided to the independent ethics review committee established by Inveresk Clinical Research included the following statement: "No data on accidental or incidental exposure of people to oxamyl is available" (McFarlane and Freestone 1999). This statement is false. Prior to the start of the Randomised Double Blind Ascending Oral Dose Study with Oxamyl in late 1998, Du Pont submitted eleven adverse event reports involving exposure of humans to oxamyl for inclusion in the EPA's Office of Pesticide Programs (OPP) Incident Data System (IDS). Between 1982 and 1994, the State of California Department of Food and Agriculture's Pesticide Illness Surveillance Program recorded an additional 61 incidents, including 30 cases involving occupational exposure to oxamyl alone. In comments provided to the EPA on March 31, 2006, Du Pont Crop Protection stated that "most of the accidents or incidents reported in these databases lack confirmation of the product involved or the amount of product involved in the exposure. Also, some reports lack information on symptoms or details of the exposure. Therefore, none of this data was useful for designing the study." (DuPont 2006). This latter statement may be true, but the discounting of the relevance of adverse event data from accidental or incidental exposures is ill-advised and borders on the unprofessional. This may have been an appropriate decision but the data, evaluation and assessment should have been discussed in the protocol and the consent document. Furthermore,

the statement in the original study protocol remains untrue. The question before the Board, therefore, was whether or not this misstatement met the legal definition of research misconduct.

The Agency has accepted the federal policy on research misconduct (Classification No.: 3120.5; Approval Date: 03/18/2003; Review Date: 03/18/2006), as defined in 42 CFR 93.103:

Research misconduct means fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results.

(a) Fabrication is making up data or results and recording or reporting them.

(b) Falsification is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.

(c) Plagiarism is the appropriation of another person's ideas, processes, results, or words without giving appropriate credit.

(d) Research misconduct does not include honest error or differences of opinion.

Although the failure to include the incidental exposure data may meet this definition of falsification, a finding of research misconduct made under 42 CFR 93.104 requires that: 1) there be a significant departure from accepted practices of the relevant research community; 2) the misconduct be committed intentionally, knowingly, or recklessly; and 3) the allegation be proven by a preponderance of the evidence. Under the standards outlined in 42 CFR 93.103-104, given the limitations in the studies cited by Du Pont, and lack of evidence that the absence of information was intentional, knowing, or reckless, it is the opinion of the Board that this omission does not meet the evidentiary threshold necessary for a finding of research misconduct based on falsification.

2) Minimization of Risks to Study Participants

Reasonable exposure levels appear to have been chosen using available animal data. Although human exposure data from the EPA OPP IDS and California's Pesticide Illness Surveillance Program also may have been useful in designing the study so as to minimize risk to research subjects, there is no clear and convincing evidence of willful or blatant disregard for participant safety. Adherence to a strict dose escalation protocol, with a stopping rule based on 40% inhibition of RBC cholinesterase, reduced the likelihood that study subjects would experience unanticipated adverse clinical events. Based on the knowledge available to investigators at the time the study was conducted, this double-blind ascending-dose oral exposure trial appears to have been designed to adequately minimize potential risks to study participants.

3) Voluntary Informed Consent

It is clear that the written documentation for informed consent failed to meet the standards outlined in Principles of Good Clinical Practice (CMPM/ICH/135/95), and the 1996 version of the Declaration of Helsinki.

The information sheet that was given to research subjects as an appendix to the signed informed consent document lacked adequate discussion of the potential risks of participating in the research study. Several examples of what Board members consider inadequate discussion of potential risks are illustrated below:

Data on incidents involving accidental oxamyl exposure were available through both the EPA's OPP IDS and the California Pesticide Illness Surveillance Program, as previously discussed. These data included reports of adverse clinical events, including detailed reports of systemic effects, eye effects and dermatological effects resulting from exposure to oxamyl. These data were not provided to study participants.

The background information provided to study participants enrolling in both the original and amended study included the statement that "results from this study would further confirm that the use of oxamyl for crop protection would not pose an unreasonable risk to human health." (McFarlane and Freestone 1999). Although the informed consent document clearly states that this is the first study involving intentional exposure of human subjects, particularly given the incident data discussed above, this statement seems to be written in order to downplay any fears that study participants might have with regard to participation.

Study participants enrolling in the amended study, designed to look for clinical or biological outcomes associated with exposure to 0.15 mg/kg body weight of oxamyl, were informed that "no significant side effects [were] noticed in any of [the previous lower] dose levels." (McFarlane and Freestone 1999). Previous trial participants receiving 0.09 mg/kg body weight of oxamyl exhibited 4-24% and 12-22% RBC and plasma cholinesterase inhibition. The study investigators concluded that these observed levels of cholinesterase inhibition did not qualify as adverse events because of the variability in the data, but they also state that the level of inhibition observed in study participants receiving 0.09 mg/kg body weight of oxamyl achieved statistical significance. Because the consent form described cholinesterase inhibition as a side effect of oxamyl exposure, it can be argued that this information should have been provided to subjects participating in the 0.15 mg/kg dose study. The information sheet provided to volunteers, however, specifically describes cholinesterase inhibition as one of the side effects of oxamyl exposure.

The letter to the subjects' general practitioner contained no information about the actual study and the product being administered other than the name of the compound. It thus minimized the extent to which the opinion of the general practitioner could protect the research subject.

In hindsight, it is clear that the documentation provided failed to rigorously meet the standards of voluntary informed consent applicable to studies conducted in Scotland in 1998-1999. However, there was no clear and convincing evidence that these deficiencies knowingly and seriously impaired the informed consent process.

HSRB Consensus and Rationale

The Board concluded that:

a) The oxamyl human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no clear or convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

Although the study failed to meet the specific ethical standards prevalent at the time the research was conducted, including those cited in the study protocol itself, the Board concurred with the assessment of the Agency that there was no clear and convincing evidence that the conduct of the research was fundamentally unethical in that it was not intended to harm subjects nor failed entirely to obtain informed consent.

It is believed that potential risks to study participants were inadequately addressed in the documents provided to research participants at enrollment, thus impairing the informed consent process. Nevertheless, the Board believed that there was no clear and convincing evidence to suggest that the study investigators failed to obtain voluntary informed consent from all research participants.

The Board was of the opinion that the deficiencies noted above are unlikely to have resulted in serious harm to study participants, based on the knowledge available to the investigators at the time.

The Board concluded that there is no obvious reason why the Agency cannot rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

4. Azinphos-Methyl

Charge to the Board

Azinphos methyl (AZM) is an organophosphate pesticide (OP). Consistent with other OPs, AZM elicits neurotoxicity through the inhibition of the enzyme, acetylcholinesterase, via phosphorylation of the active site. At sufficiently high doses, exposure to AZM can lead to a variety of clinical signs. The Agency is developing an assessment to estimate risk to workers from exposure to AZM. In addition, AZM is a member of the OP common mechanism group and is thus included in the cumulative risk assessment for the OPs.

Scientific considerations

The Agency's WOE document and DER for AZM describe the study design and results of the AZM repeat dose, oral, human toxicity study. The WOE document also discusses the Agency's conclusions regarding the usefulness of the human study in the worker risk assessment and in the cumulative risk assessment for the OPs. For AZM, the Agency has concluded that the human toxicity study is appropriate for developing a point of departure for extrapolation of risk to workers exposed to AZM via the dermal and inhalation routes. For the cumulative risk assessment, the Agency has determined that because no cholinesterase inhibition was seen in the human toxicity study, it is not possible to evaluate whether steady state had been reached in humans at 28 days of exposure. Thus, the Agency has concluded that the AZM repeat dose, oral, toxicity study is not sufficiently robust for informing the inter-species factor in the cumulative risk assessment of the OPs.

Please comment on the scientific evidence that supports the conclusions for:

a. the use of the human toxicity study to develop a point of departure for extrapolation of risk to workers in the worker risk assessment and

b. the determination that the human toxicity study cannot be used to inform the inter-species factor in the cumulative risk assessment.

Board Response to the Charge

Brief Overview of the Study

In a repeat dose oral human toxicity study, conducted in 1999 by the Inveresk Laboratory (McFarlane & Freestone, 1999b), four males were dosed with a placebo (lactose) and eight males were dosed with 0.25 mg/kg/day of AZM for 28 consecutive days. The sole dose of 0.25 mg/kg was chosen based on the results of subchronic rat study and an earlier study with human subjects that indicated this would be a NOAEL for inhibition of plasma and RBC cholinesterase. Previous human studies were conducted in 1972 and 1974 and regulatory agencies reluctant to rely on the data "due to deficiencies in documentation, specifically the availability of raw data and the lack of GLP compliance."

Dosing was orally by capsule and the subjects were housed in a clinic throughout the dosing period. The subjects were monitored for reactions and samples of blood were taken at predose (eight times) and daily throughout the dosing period. On some days, a blood sample was taken both prior to administering the dose and four hours later. Both plasma cholinesterase (ChE) and RBC acetylcholinesterase (AChE) were measured. In addition, hematology, clinical chemistry, and urinalysis were evaluated as well as blood pressure and electrocardiogram (ECG). There were no reactions to treatment and blood pressure and ECG were not affected. Eight of the 12 volunteers were noted to have symptoms of a viral infection and some were treated with paracetamol. The postdosing group mean data were compared three ways in an effort to see if AZM caused inhibition of either plasma ChE or RBC AChE. None of the comparisons indicated inhibition of either measure. Also, samples taken four hours after dosing did not indicate

inhibition. It was concluded that a dose of 0.25 mg/kg/day for 28 days did not result in either clinical symptoms or inhibition of either plasma ChE or RBC AChE in human males, so that this dose could be considered a NOAEL.

Critique of the Study

One strength of the study was its design as a randomized, double blind study including a placebo group. The clear inclusion and exclusion criteria for subject selection appeared to have been chosen to reduce the likelihood of confounding. The subjects were in good health, were not using prescription or illicit drugs, were non-smokers, and were not agricultural workers or pesticide applicators who might have experienced OP exposure within the preceding month. Subjects resided in the clinic from the day before study start to 24 hours after the last dose was administered, received a standardized diet, and were under medical and nursing supervision throughout.

The assessment of critical outcomes was comprehensive, involving a general clinical exam, measurement of systolic and diastolic blood pressure, the administration of an ECG, and various blood and urine studies. Multiple measurements were made of plasma ChE and RBC AChE, namely on 8 pre-dose days then daily 4 hours after dosing. Moreover, on 12 post-dosing days, two blood samples were taken, one before the dose and one 4 hours afterwards.

Possible group differences in plasma ChE and RBC AChE were evaluated in several ways, both in grouped and individual data)

a) Comparison of the day-to-day ChE activities within the AZM group to the same day values in the control group

b) Comparison of same day predosing ChE activities with inhibition 4 hours after dosing

c) Comparison of the day-to-day ChE activities values within the AZM group to predosing baseline value.

Several weaknesses were identified in the study. First, the subjects were all male, however there have been no consistent sex differences in sensitivity observed in animal models. The sample size was not large, involving only 12 adult males (four in the placebo group, eight in the AZM group), raising a concern that the statistical power might have been inadequate to detect a modest degree of inhibition in the AZM group.

The standard deviations of the ChE levels were large, as great as 20% for plasma ChE and 15% for RBC ChE, reducing confidence in the consistency of these endpoints.

A variety of symptoms were reported over the course of the study, with the majority occurring in subjects administered AZM. The conclusion drawn in the Agency's Data Evaluation Record (Doherty 1999) that none of the symptoms likely reflected effects of AZM is not verifiable, nor is it credible.

Certain aspects of the statistical analyses are problematic. For the analyses of percentage change each day from baseline (predosing) plasma and RBC cholinesterase activity in the subjects receiving AZM, the choice of repeated measures ANOVA as the statistical approach

was appropriate given the structure of the data. This would have allowed each subject to be used as his own control, but this does not appear to be the way the analyses were done. The pooled baseline RBC and plasma ChE values were used as covariates rather than individual subject values. This essentially ignores the value of the individual levels in increasing statistical power by taking account of pre-dosing inter-individual differences in ChE levels. The other two types of analyses conducted were not limited by this deficiency, but the analysis of within-subject change from baseline is potentially the most sensitive analysis in this study.

In proposing to use the results of this study for dermal and inhalation risk assessment, the Agency applied a dermal absorption factor of 42% based on a rat study and an inhalation absorption factor of 100%. Bayer Crop Science commented that data are available supporting a lower estimate of human dermal absorption (21.5%) but these data were not available for evaluation by the Board. Another concern was whether the effects of a single oral dose administered daily provide a reasonable estimate of the effects of the continuous dosing via all routes of exposure that would be experienced by agricultural workers.

The major concern regarding this human study was it was designed to demonstrate a NOAEL. No LOAEL could be established to validate the NOAEL because only one dose was used. The fact that inhibition was not observed at the single dose used raised concern about sensitivity of the methods used. The Agency was reassured by the laboratory's observation of plasma and RBC cholinesterase inhibition in other studies of carbamates, including aldicarb, methomyl, and oxamyl. Nevertheless, it is not possible to be sure if the absence of an effect in this study was because the methods used were, for some reason, not up to the standards of those used in the other studies, and therefore less sensitive.

HSRB Consensus and Rationale

The Board concluded that:

a. Data from the 28-day repeat oral dose study of AZM should not be used in developing a point of departure for extrapolation of risk to workers exposed to AZM via the dermal and inhalation routes.

b. Data from the 28-day repeat oral dose study of AZM cannot be used to inform the inter-species factor in the cumulative risk assessment.

Despite the study's strengths, the HSRB had only limited confidence in the inferences that can be drawn from this study, in large part because only one AZM dose was used. In order to use these data, one must assume that the lab would have detected inhibition had it been present at the single dose administered. The conclusions drawn could be correct, but this cannot be determined with certainty. The conclusion that this study is not appropriate for developing a point of departure for either the worker risk assessment or the cumulative risk assessment is consistent with the recommendation of the NAS committee (NAS 2001) that the Agency should not consider data from NOEL-only studies**.**

Ethical considerations

Charge to the Board

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

Whether the informed consent materials – which refer to "the company" and "supervising doctor" without further identification, and contain no discussion of who would benefit from the research – should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and,

Whether the absence from the protocol of any discussion of the potential risks to subjects or benefits to society of conducting the proposed research (as required by the 1996 Declaration of Helsinki, Principle # 5, with which the research asserted compliance) should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

b. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of [this/each] study:

OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical.

Whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Brief Overview of the Study

As noted above, the study was conducted in 1999 by a contract research organization, Inveresk Clinical Research of Edinburgh, Scotland, at its Elphinstone Research Centre in Tranent. The study sponsor was the Bayer Corporation, Agricultural Division, of Stilwell, Kansas. The protocol documents specifically state that the research was conducted in compliance with the guideline for Good Clinical Practice, and in accordance with the guidelines of the Declaration of Helsinki, 1964, as amended through 1996.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in Carley (2006d). However, further comments are warranted with regard to whether the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent.

It is clear that the written documentation for informed consent failed to meet fully the standards contained in the 1996 version of the Declaration of Helsinki.

In particular, the information sheet that was given to research subjects as an appendix to the signed informed consent document contained the following statement under the subheading

Aim: "The aim of the study is to establish a recommended daily intake following long term dietary exposure and to determine the most appropriate way of assessing workers exposure." By using the phrase "recommended daily intake," this statement would appear to suggest that it is actually desirable to consume a daily minimum amount of the study compound. This sentence is thus misleading. However, other portions of the information sheet made it clear that the study compound was a pesticide, and that consuming it is not good for people, thus leading the Board to conclude that this one sentence by itself does not provide clear and convincing evidence that the consent process as a whole was significantly deficient relative to the ethical standards prevailing at the time the study was conducted.

HSRB Consensus and Rationale

The Board concluded that:

a) The azinphos-methyl human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

The Board determined that the informed consent materials – which refer to "the company" and "supervising doctor", without further identification, and which contain no discussion of who would benefit from the research – did not fully comply with the ethical standards at the time. However, the Board did not find clear and convincing evidence that the level of non-compliance should be considered significantly deficient relative to those then-prevailing ethical standards. With regard to the use of the terms "the company" and "supervising doctor," this type of language, though highly undesirable, is unfortunately not uncommon, even at the present. With regard to the benefit issue, the Board determined that the consent materials, while far from ideal, did in fact somewhat address this issue. They indicate, for example, that the study may lead to further information about the risk that this compound poses to workers and consumers.

The Board determined that the absence from the protocol of discussion of the potential risks to subjects or benefits to society of conducting the proposed research (as required by the Declaration of Helsinki, Principle # 5) should not be considered significantly deficient relative to the ethical standards prevailing when the study was conducted. The potential risks and benefits are described in the research subject information sheet/informed consent document. While not in the protocol itself, they are nonetheless included in the materials provided to the ethics committee (and to the potential research subject). Thus the ethics committee and the potential research subjects had the opportunity to weigh the risks and benefits.

The Board concluded that there was no obvious reason why the Agency cannot rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

5. DDVP

Charge to the Board

Like AZM, DDVP is an organophosphate pesticide (OP) which elicits neurotoxicity through the inhibition of acetylcholinesterase, via phosphorylation of the active site. The Agency is conducting an aggregate (single chemical, multi-route, multi-duration) risk assessment of DDVP. In addition, DDVP is a member of the OP common mechanism group and is thus included in the cumulative (multi-chemical, multi-route) risk assessment for the OPs.

5.1. Scientific considerations

a. The Agency's WOE document and DER for DDVP describe the study design and results of the DDVP repeat dose oral human study. The WOE document also discusses the Agency's conclusions regarding the usefulness of this study in the aggregate risk assessment and in the cumulative risk assessment for the OPs. For the single chemical risk assessment, the Agency has concluded that the human study is sufficiently robust for developing a point of departure for estimating dermal, incidental oral, and inhalation risk from exposure to DDVP in the single chemical risk assessment. For the cumulative risk assessment, the Agency has determined that results of the DDVP multi-dose human toxicity study do not support reducing the default 10X inter-species factor in the cumulative risk assessment of the OPs.

Please comment on the scientific evidence that supports the conclusions for:

a) the Agency's conclusions for use of the human study for developing a point of departure for estimating risk in the single chemical, aggregate risk assessment and

b) the Agency's determination that the human study cannot be used to reduce the interspecies factor in the cumulative risk assessment.

Board Response to the Charge

Study Overview

A single blind, randomized placebo-controlled oral study was conducted with DDVP in which six healthy male volunteers were administered a daily dose of DDVP at approximately 0.1mg/kg/day and three volunteers were administered a placebo (Gledhill 1997). DDVP was dissolved in corn oil and administered daily in a gelatin capsule to fasted subjects (subjects did not eat after midnight prior to each morning dose) for a total of 21 days. Blood samples were collected for analysis of red blood cell (RBC) cholinesterase activity. For each subject, the baseline level of RBC cholinesterase activity was established from seven pre-dose samples, and a

single blood sample was collected approximately 24 hours after dosing (just prior to dosing on the following day) on days 1, 2, 4, 7, 9, 11, 14, 16 and 18 of the study. No blood samples were collected on the last three days of the study (days 19-21). After cessation of dosing, subjects returned approximately 1 week later (on or about day 25) for a follow-up analysis of RBC cholinesterase activity. The criterion for withdrawal from the study was a inhibition of RBC cholinesterase activity of 20% or more on a given day's analysis followed by a further decrease in activity in the next successive sample. A linear mixed model was fitted to the cholinesterase activity and significant differences were found within subjects and also between groups (treatment versus placebo subjects) for pre-dose and post-dose cholinesterase activity.

Critique of Study

The scientific validity of the repeated dose study for DDVP was determined by the relative strengths and weakness of the design and conduct of the study.

The strengths of the study were considered to be as follows:

- The repeat dosing paradigm affords the opportunity to more critically evaluate the sustained nature of RBC cholinesterase inhibition associated with OPs.
- The analysis of RBC cholinesterase activity is fairly robust, given the assessment of predose baseline values on 7 separate occasions. The baseline values were fairly consistent both within and between subjects (16,000 – 21,000 IU).
- A low, but statistically significant effect was observed in RBC cholinesterase inhibition.

There are, however, numerous weaknesses of the study that are relevant to the utility of these data in establishing a point of departure for use in single chemical, aggregate and cumulative risk assessments for DDVP. These weaknesses included:

- A single dosage was used, preventing establishment of dose-response relationships.

- The sample size was small and included only male volunteers. In this regard, it is not clear whether the study was properly powered, given that no sample size calculations seem to have been used in order to arrive at the number of volunteers used in the study.

- RBC cholinesterase inhibition was determined only at 24 hours post dosing. Although organophosphates are known to show prolonged inhibition of cholinesterase activity, it was not clear whether the peak of RBC cholinesterase was established.

- The sponsor did not include the analysis of plasma cholinesterase measurements in the study. The reason for this omission is not clear, and it was noted that virtually every toxicological and exposure assessment study that has focused on cholinesterase inhibition due to organphosphorus insecticides includes measurement of both plasma (serum) cholinesterase and RBC cholinesterase. The combination of these measurements has been used routinely for risk assessments by numerous regulatory agencies, including the EPA, as well as by clinicians who diagnose pesticide poisonings. Current worker surveillance programs in some states (California and Washington) require measurement of both plasma and erythrocyte cholinesterase, and both measurements are used in combination

to initiate workplace investigations and remove workers from pesticide-related job activities until enzyme activities return to baseline.

- Sampling was incomplete because the last day of analysis of enzyme inhibition was day 18 but dosing continued until day 21. It is not clear whether any additional decrease in enzyme activity would have been observed on days 19-21. In the sampling schedule outlined above, the maximum time gap between samples during the dosing period (days 1-21) was three days (between days 11 and 14). Logically then, an additional sample would have been taken on day 21 or 22, the time in the study when maximum effects might be observed. In this regard, two subjects presented with inhibition values of 22 and 23%, exceeding the 20% cut-off for subsequent analysis and consideration for withdrawal on day 18 of the study. The lack of additional measurements in these subjects during the remainder of the dosing period is scientifically inadequate and ethically troublesome.

- It is neither clear nor convincing that steady state inhibition of RBC cholinesterase activity was achieved in the study. The data suggest that the level of enzyme inhibition was still increasing, particularly in the time period from 11-18 days, with a difference of 16% in activity on Day 18 compared to pre-dose values. Dosing was stopped on Day 21, with no further analysis until approximately 4 days after cessation of dosing. Although the difference in activity between day 18 post-dose and the average of the pre-dose measurements was deemed to be "not-biologically significant" by the study sponsor, the possibility that RBC cholinesterase inhibition may have reached or exceeded 20% by the end of the study cannot be excluded.

- There was a lack of appropriate follow-up in all subjects in this study. As noted earlier, two subjects presented with inhibition values of 22 and 23%, exceeding the 20% cut-off for subsequent analysis and consideration for withdrawal on Day 18. However, there were no additional analyses of RBC cholinesterase activity in these individuals on days 19-21 of dosing. Additionally, a blood sample was collected within 4 to 10 days following completion of the 21-day dosing period for all subjects. The mean inhibition of the enzyme observed at seven days following cessation of dosing was 83%, suggesting little if any recovery of enzyme at this time, but these data were not analyzed for statistical significance. Furthermore, one subject ended his participation in the study (day 25) with RBC cholinesterase inhibition greater than 20%. In all cases, there was no additional follow-up of the subjects after the single post-dose analysis of cholinesterase activity.

HSRB Consensus and Rationale

The Board concluded that:

a) The DDVP repeat dose human toxicity study was sufficiently robust for developing a point of departure for estimating dermal, incidental oral, and inhalation risk from exposure to DDVP in the single chemical risk assessment.

In specific response to the Agency's charge question, the HSRB concluded that there are numerous technical limitations to the data obtained in the study. In addition to the weaknesses of the study design and execution outlined above, the HSRB concluded that the evaluation of only a single dose level of DDVP along with the omission of plasma cholinesterase measurements greatly limit value of this study in terms of producing new knowledge that can be used in the regulatory process and in medical diagnosis. Furthermore, study investigators have an obligation to design a study that provides appropriate oversight of subjects until indications of the effects of the administered dose are no longer present, and the Board considered that the design of this study which continued intentional dosing without collection of blood samples for cholinesterase analysis was not defensible scientifically. Following considerable discussion, the HSRB concluded that the dosage evaluated in the repeat dosing human study can be used as a LOAEL for the single chemical aggregate risk assessment for DDVP. Although a study using a single dose level is not ideal for establishing a LOAEL, there was general consensus that RBC cholinesterase is a well-characterized endpoint for compounds that inhibit acetylcholinesterase activity and therefore, because the decreased activity in RBC cholinesterase activity observed in this study was at or near the limit of what could be distinguished from baseline values, it was unlikely that a lower dose would produce a measurable effect in RBC cholinesterase activity. However, the HSRB strongly recommended that, in most cases, studies in human subjects designed to define the NOAEL or LOAEL for a compound should include more than a single dose level.

b) Results of the DDVP repeat-dose human toxicity study should not be used to support reducing the default 10X inter-species factor in the cumulative risk assessment of the organophosphate pesticides.

Although the data obtained in this study can be used to establish a point of departure for the single chemical aggregate risk assessment, the consensus of the HSRB was that the scientific limitations of the study design do not justify its use in the cumulative risk assessment. In particular, the lack of sample collection through to the completion of dosing, the lack of a clear demonstration that steady state inhibition of RBC cholinesterase inhibition had been achieved, and the lack of any dose-response data limit the overall utility of the human data. Accordingly, the HSRB recommended that the default interspecies uncertainty factor should be applied for the cumulative risk assessment for DDVP.

Charge to the Board

b. The Agency has concluded that other human studies made available to the Board do not provide sufficient scientifically sound information to warrant any reduction in the 10X inter-species uncertainty factor used to derive reference dose values for DDVP based on animal toxicity endpoints.

Please comment on the scientific evidence that supports these conclusions.

Board Response to the Charge

HSRB Consensus and Rationale

The HSRB concluded that the other DDVP human toxicity studies available for the Board's consideration should not be used for determining a reduction in the 10X uncertainty factor to derive reference dose values for DDVP based on animal toxicity endpoints.

The Board's deliberations for this question focused on the acceptability or not of these human studies for the Agency's intended purposes, rather than on the evaluation of the magnitude of the interspecies uncertainty factor. Since the results of animal studies or benchmark dose modeling were not provided to the Board, the review was restricted to the evaluation of the acceptability of the human studies for the intended purposes. The Board concluded these other human studies did not warrant use in determining the inter-species uncertainty factor because of policy considerations (e.g., exposures involving infants, children, pregnant women) or due to uncertainty associated with the study itself (e.g., inappropriate duration, questionable route, exposure data of limited utility, lack of time course data on critical effect). Board concerns regarding these other human studies are provided in Table 1.

While the studies reviewed are not useable either because they are unacceptable due to policy considerations or due to uncertainty associated with the study itself, the Board also suggested that the Agency should take a critical hard look at those studies that are ethically acceptable (e.g., the ones that did not include exposures of children and pregnant women), to the extent to which they can be informative about the magnitude of the interspecies uncertainty factor. In making such evaluations, the Agency should consider taking into account: (1) applicability of Haber's law, (2) nature of the toxic moiety, and (3) the relative importance of interspecies differences in pharmacokinetics and sensitivity. Further, consideration should be given to the manner in which the data from the different species are pooled and analyzed (e.g., continuous response versus maximal response).

Table 1. Evaluation of Specific DDVP Human Studies Provided to the HSRB

Reference	Observations/Concerns
Glendhill Acute toxicity study	Time course measurements of cholinesterase
Cavagna (1970)	Conducted in newborns
Slomka (1981)	Exposure through plastic bead (polyvinyl resin) formulation; rate of absorption not known; data provided as ranges
Cervoni (1969)	Efficacy trial; measurement of cholinesterase inhibition not the main focus; no assurance of consistent methods of measurement of inhibition
Leary (1974)	Exposure via insecticide strips; possibility children were involved, but composition of families not given; inhalation route – caveat of dose calculations; no information on consent of subjects
Smith (1972)	Performed in altitude chamber; short term inhalation study; no information on consent of subjects
Cavagna (1969)	Exposure via insecticide strips included pregnant women, infants and children in a hospital
Pena-Chavarria (1969)	Exposure of patients (most of them anemic, treated for parasite infection); not designed to identify NOAEL or LOAEL
Stein (1966)	Insufficient information on the time course of cholinesterase inhibition; doses not quantitated
Funckes (1963)	Study population included children, some below six years; time course evaluation on inhibition insufficient
Witter (1961)	Simulated aircraft cabin; one hour exposures; time course information lacking
Gratz (1963	Inadequate information to evaluate dose and NOAEL; inadequate information about how many people exposed at each concentration; single application-based exposures

5.2. Ethical considerations

Charge to the Board

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

Whether references to the test material as a drug and other statements that could indicate the study constituted medical research, that appear in the materials used to obtain informed consent should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted;

Whether the administration of the test material for three additional days without monitoring subjects' cholinesterase levels following the detection of cholinesterase inhibition greater than 20 % in some subjects should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

Whether the lack of medical surveillance of subjects, following the termination of dosing, to establish the subjects' cholinesterase levels returned to normal should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

b. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of the Gledhill repeated dose study:

OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical; and

Whether there is clear and convincing evidence that the conduct of the Gledhill repeat dose study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Board Response to the Charge

Brief Overview of the Study

As noted above, the study was conducted in 1996 by the Zeneca Central Toxicology Laboratory, Cheshire, United Kingdom. The study sponsor was the Amvac Chemical Corporation. The protocol documents specifically state that the research was conducted in compliance with the guideline of the Declaration of Helsinki, 1964, as amended through 1989.

Critique of Study

The Board concurred with the Agency's factual observations of the strengths and weaknesses of the study, as detailed in Carley (2006e). However, further comments are warranted with regard to: 1) whether the provisions in the protocol for monitoring the subjects adequately protected them, and 2) whether the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent.

1. Monitoring of Subjects While Receiving Study Compound

The protocol permitted subjects to continue to be administered the study compound for three days following the detection of RBC cholinesterase inhibition of greater than 20%. Given

that study subjects were not monitored during most of the study (and were not full-time residents at the study laboratory), it was possible that they might develop clinically significant symptoms during this period and no one would be available to detect that and to treat them. The Board concluded that this aspect of the study design was inadequate. However, given that there had been a prior similar study at triple the dose used in this study, and that study had demonstrated minimal symptoms, the Board could not conclude that there was clear and convincing evidence that this element of the study design could have resulted in serious harm based on the knowledge available to the investigators at the time.

2. Monitoring of Subjects at End of Study

The protocol also provided that, at the end of the study, inhibition levels would not be retested for periods as long as seven days even though some subjects had RBC cholinesterase inhibition of greater than 20%. During this period of time, due to the long-acting nature of the study compound, subjects' cholinesterase inhibition levels could have been increasing, and thus symptoms might have developed and, theoretically have become serious at a time when they were being infrequently monitored. The Board determined that this aspect of the study design was inadequate. However, as noted above, given that there had been *a prior* similar study at triple the dose used in this study, and that study had demonstrated minimal symptoms, the Board could not conclude that there was clear and convincing evidence that this element of the study design could have resulted in serious harm based on the knowledge available to the investigators at the time.

The inadequate monitoring during and at the end of the study undermines the scientific value of the study. The fact that the research subjects were unsupervised during the study, and the ascertainment of adverse event data was through self-report, renders the data establishing a NOAEL suspect. The risk of a "false negative" conclusion is quite high. In addition, the failure to achieve a steady state in the inhibition of acetyl cholinesterase activity may render these data inadequate as well, and of limited utility. This fact, also noted in the Board's scientific review, can negatively influence the prospective benefit to risk ratio.

3. Informed Consent

It is clear that the written documentation for informed consent failed to fully meet the standards outlined of the 1989 version of the Declaration of Helsinki.

In particular, the information sheet that was given to research subjects as an appendix to the signed informed consent document under the subheading Possible Adverse Events noted that if "any symptoms possibly due to enzyme blocking did occur, they could rapidly be reversed by a specific antidote." Although that statement is a correct statement of the scientific issue, it failed to take account the fact that this study did not involve subjects remaining under 24 hour supervision. For most of the time, the subjects would not be under any supervision. Given that enzyme inhibition could build up over time due to the long-acting nature of the compound being studied, symptoms could likely occur at a time when there was no one available to administer the antidote. Telling the subjects that an antidote could rapidly reverse symptoms without explaining

that there were circumstances in which that antidote would not be readily available was not an adequate way to inform subjects of the true nature of this risk.

In hindsight, it is clear that the documentation provided fails to rigorously meet the standards of voluntary informed consent applicable to studies conducted in the United Kingdom in 1996. However, there was not clear and convincing evidence that these deficiencies knowingly and seriously impaired the informed consent process.

HSRB Consensus and Rationale

The Board concluded that:

a) The DDVP repeat dose oral human toxicity study failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

The Board determined that there was not clear and convincing evidence that references to the test material as a drug and other statements that could indicate the study constituted medical research, that appear in the materials used to obtain informed consent should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted. These statements are highly undesirable, and should not be used as part of modern-day practice in writing such consent materials. Nonetheless, it was unfortunately not uncommon for such wording to have been used in consent materials in the past. In addition, other wording in the consent materials clearly advised subjects that this was a study involving consuming an insecticide. The Board, however, specifically rejects the arguments of the study sponsor that it was appropriate to use the "drug" terminology because the consent materials were generic materials used for a variety of studies. It also rejects the study sponsor's argument that because the test material is sometimes used as a drug, it was appropriate to use that terminology in this study even though this study in no way related to its use as a drug.

For the reasons discussed above, the Board determined that there was not clear and convincing evidence that the administration of the test material for three additional days without monitoring subjects' cholinesterase levels following the detection of cholinesterase inhibition greater than 20 % in some subjects could have led to serious harm. There the study should not be considered significantly deficient relative to the ethical standards prevailing when the study was conducted.

(3) For the reasons discussed above, the Board determined that there was not clear and convincing evidence that following the termination of dosing, the lack of medical surveillance to

establish the subjects' cholinesterase levels returned to normal could have led to serious harm. Therefore the study should not be considered significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board concluded that the Agency can rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

6. Ethephon

Charge to the Board

Ethephon is an organophosphorus compound that, upon absorption into plants, forms ethylene gas which is an important component of the plant hormone complex. The Agency is conducting an aggregate (single chemical, multi-route) risk assessment of ethephon.

Scientific consideration

The Agency's WOE document and DERs for ethephon describe the study design and results of the ethephon repeat dose, oral, human toxicity studies. The WOE document also discusses the Agency's conclusions regarding the usefulness of the human studies in the aggregate, single chemical risk assessment. The Agency has concluded that the 28-day human study is sufficiently robust to establish a point of departure for extrapolating acute and chronic dietary risk.

Please comment on the scientific evidence that supports this conclusion.

Board Response to the Charge

Brief Overview of the Study

According to the Agency's weight of evidence document (WOE) (Kent 2006) , "ethephon is an organophosphorus compound that, upon absorption into plants, forms ethylene gas which is an important component of the plant hormone complex. The production of ethylene by plants occurs naturally as crops mature but it can be slow during periods of unfavorable weather. Ethylene generated from ethephon is absorbed by the plant tissues and moderates the growth process. It is not similar in structure to other organophosphate pesticides. Ethephon registrants have submitted many toxicity studies to support the continued pesticidal use of the chemical, and among these studies are two involving direct dosing of human subjects that OPP is considering using to establish endpoints to assess risk from exposure to ethephon. The WOE document compares the strengths and weaknesses of the human and animal toxicity studies and discusses how the human studies fit in with the animal studies, i.e., are the human data consistent with animal data in terms of types of effects and effect levels or are there notable differences between animals and humans."

According to the WOE document, "as a strong acid, ethephon is corrosive to the skin and eyes (Category I). In acute and subchronic neurotoxicity studies in rats, there were signs of neurotoxicity at doses of 500 mg/kg and above (acute) and 300 mg/kg (subchronic). In dogs there were no clinical signs of neurotoxicity evident at 75 mg/kg/day although plasma and RBC cholinesterase were inhibited in animals at doses as low as 7.5 mg/kg/day."

There are two human studies under consideration, both involve direct dosing of human subjects and are repeated dose oral studies, one for 28 days duration (Reese 1972) and the other for 16 days (Weir 1977).

28 Day Oral Toxicity Study

In a non-guideline oral toxicity human study (Reese 1972), ethephon was administered by capsule in a powdered formulation (10% a.i. weight/weight) of hydrated silica and starch to human adults (5/sex) at an average dose of 1.8 mg a.i./kg/day or placebo 0 mg/kg/day (3/sex) for 28 consecutive days. Administration of the test material or the placebo was given orally by capsule divided into three daily doses. Each subject received 2 capsules postprandially for the first two dosing periods; the third dose was given at the end of the work day in order to simulate as closely as possible ingestion of the material as a crop residue. The capsules were dispensed daily (5 days/week) by an assistant who observed the subjects while they took each capsule and recorded solicited comments related to the ingestion of the material during the first 8 hours. The subjects and the assistant did not know which capsules were placebo or the test material. Each Friday, the subjects received a supply of capsules to be taken during the two day weekend. All subjects were monitored regularly for adverse effects. Hematology measurements, clinical chemistry and urinalysis were conducted initially and on Days 7, 14, 21, 28 and 2 weeks following the last administration. ChE (plasma and RBC) determinations were conducted using the ?pH/hr method (modified Michel method) initially, and on Days 1, 2, 7, 14, 28 and 2 weeks after the last administration.

Critique of 28 Day Oral Toxicity Study MRID 00036510

The ethephon subjects, though exhibiting normal physical appearance and behavior, developed symptoms of diarrhea and increased urination and bowel movement. Three females and one male experienced a sudden onset of diarrhea or an urgency of bowel movement of 1-4 days duration during the first week of the compound administration; the one male also experienced loose stools for 2 weeks. Another male complained of stomach cramps or gas for the duration of the study. One subject in the ethephon group was aymptomatic. Five subjects experienced increased urgency and frequency of urination, 4 of whom experienced this symptom throughout the study. Two subjects experienced decreased appetite while a third (with diarrhea) experienced increased appetite during the first 2 days of the study. It was stated that the symptoms were "consistent with exposure to a cholinesterase inhibitor." The WOE document stated "the clinical signs observed in the study, diarrhea and increased frequency of urination, are consistent with a cholinergic mode of toxicity, but other signs associated with a cholinesterase inhibitor such as pinpoint pupils (miosis), sweating, runny nose, tearing, salivation, respiratory distress and muscle fasciculation (twitching), were not seen [sic]; [and] the test subjects reported symptoms as they considered appropriate, and it is difficult to interpret what the self-reported

symptoms mean in terms of a biological response." In fact, it is unknown if symptoms or signs associated with a cholinesterase inhibitor were seen; they may not have been monitored or specific requests for their reported made by the investigators. However, these symptoms, probably related to irritant effects, are important results.

The Agency's Data Evaluation Record (Khasawinah) and the WOE documents both stated "None of the placebo subjects had similar complaints." This is probably not correct since according to the investigators at least two of these controls were symptomatic as well some test and control subjects also exhibited some atypical lymphocytes of unknown origin.

The WOE document states "Unexpectedly, plasma and RBC ChE activities were similar or slightly higher than initial values in the test subjects. ([The] details of the ChE determinations were not provided.)" However, the ChE levels were not related to the diarrhea occurring in the subjects upon examination of the time of symptom occurrence and ChE data provided. The WOE document states "No compound effects regarding hematology, clinical chemistries and urinalysis were reported", except the atypical lymphocytes of some.

It was concluded by the investigators of this study that the daily dose of 1.8 mg/kg/day is the LOAEL for the oral ingestion of ethephon in human subjects. However, that is quite uncertain since lower doses were not given.

16 Day Oral Toxicity Study

In the other non-guideline oral toxicity human study (Weir 1977), ethephon was administered by capsule in a powdered formulation (2.5% a.i. weight/weight) of hydrated silica and starch to human adults (10/sex) at an average dose of 0.5 mg a.i./kg/day or placebo 0 mg/kg/day (6 males and 4 females) for 16 consecutive days followed by a 29 day recovery period (phase three). The dosing phase (phase two) was preceded with a 6 day period (phase one) where all subjects received daily placebos. Administration of the test material or the placebo was given orally by capsule divided into three daily doses. Each subject received two capsules postprandially for the first two dosing periods; the third dose was given at the end of the work day in order to simulate as closely as possible ingestion of the material as a crop residue. The capsules were dispensed daily (5 days/week) by an assistant who observed the subjects while they took each capsule and recorded solicited comments related to the ingestion of the material for 8 hours. The subjects and the assistant did not know which capsules were placebo or the test material in of the study phases. Each Friday, the subjects received a supply of capsules to be taken during the two day weekend. All subjects were monitored regularly for adverse effects. Hematology measurements and clinical chemistry were conducted on blood samples collected during phases I, II and III while urinalysis was conducted on samples collected during phase II. ChE (plasma and RBC) determinations were conducted using the ?pH/hr method (modified Michel method) on blood collected during phases I, II, and III.

Critique of 16 day Oral Toxicity Study

No clinical symptoms were reported, but it is not known if this was an omission as the report does not state that there were no symptoms or signs. Hematological, clinical chemistry

values and urinalysis parameters were comparable to the placebo controls. The updated executive summary of the Agency Data Evaluation Record (Khasawinah) for the 16-day human study stated: "Plasma and RBC ChE activities were significantly inhibited in both placebo and test groups. Therefore, this finding makes any conclusions regarding the effects attributable to ethephon administration impossible. This is in contradiction to the study authors overall conclusion that a daily ethephon oral dosage of 0.5 mg/kg/day produces significant plasma ChE inhibition, which is reversible in 15 days." The study report states "Plasma cholinesterase was significantly inhibited for the placebo group at 4, 8, 12, and 16 days during the dosage period. In the test group, plasma cholinesterase was also depressed (greater than the placebo group) at 4, 8, 12, and 16 days during the dosage period. Recovery does occur as indicated by the 15 and 29 post dosage values. RBC cholinesterase inhibition was similar for both placebo and test groups (slightly depressed in both groups) on days 4, 12, and 16 of the dosage period and day 15 of the recovery period." Investigators also say that the test group plasma values were significantly different than the placebo group values.

It appears that either the method of testing cholinesterase was poor, or, less likely, something else was depressing plasma cholinesterase in this latter study while increasing it in the former study. Possibly, as the ethephon risk assessment team concluded, measures of blood cholinesterase, either plasma or RBC, were not appropriate for endpoint selection for ethephon.

According to the WOE document (Kent 2006) "The OPP/HED RfD Peer Review Committee determined on February 10, 1994 that the reference dose (RfD) should be based on the 28-day study in human subjects (MRID 00036510). Clinical signs of toxicity were observed at 1.8 mg/kg/day (only dose tested) and included diarrhea, urgency of bowel movements, urinary urgency and stomach cramps."

A Report from EPA, Office of Pesticide Programs, Health Effects Division, Hazard Identification and Assessment Review Committee (HIARC) dated 4/1/98 states in reference to the 28-day human study: "This human study is considered to be of better quality and the findings more reliable than the 16-day human study previously used to define the RfD." Since 1994, the HIARC has repeatedly (8/9/94, 4/1/98, 7/15/02 and 11/5/02) selected the 28-day human study to assess all relevant exposure scenarios, and at the last HIARC meeting, a weight-of-evidence comment regarding endpoint selection was included: "HIARC had considered experimental animal studies on ethephon and concluded this human study was most appropriate for selection of the endpoint of toxicity for risk assessment. The clinical signs of toxicity in this human study occurred at dose levels that were much lower than seen in experimental animals." "Despite the limitations of the study, and the equivocal nature of the reported findings, the HED ethephon risk assessment team believes that, in the absence of data ruling out the observed effects as inconsequential, the 28-day human study is the most appropriate study to use for assessing both the acute and chronic dietary risks of ethephon."

The WOE document stated: "A number of conclusions relevant to endpoint selection can be drawn from the database of ethephon oral toxicity studies in humans and animals: (1) ethephon is neurotoxic in animal studies only at high doses (300 mg/kg and above), (2) ethephon inhibits blood cholinesterases, in particular plasma cholinesterase in animals and humans at relatively low doses, (3) brain acetylcholinesterase is insensitive to ethephon in adult

animals. Accordingly, the team then debated whether to use effects in animals (LOAEL approximately 80 mg/kg/day) or in humans (LOAEL of 1.8 mg/kg/day) for endpoint selection, and opted as a matter of prudence to base endpoint selection on effects observed in humans." The Board concluded that this is much lower than that expected from the animal studies, however, the second study informs and provides the level of uncertainty to accept this LOAEL and use it as a POD.

The WOE document concluded by stating: "The point of departure for the dietary assessments use of a LOAEL was applied to the POD to obtain a reference dose of 0.06 mg/kg/day for assessment of both acute and chronic dietary risks was 1.8 mg/kg/day (with an uncertainty factor of 10x for intraspecies variability and a 3x factor for use of a LOAEL …)"

HSRB Consensus and Rationale

The ethephon 16 day human oral toxicity study (second study) had no value on its own, but it did inform the ethephon 28 day human oral toxicity study (first study); it allows one to consider the results of the first study in light of the information gained there from. The Board approved the first study for use in EPA risk assessments, emphasizing that the scientific quality is not adequate on its own, and that the dose level administered was almost certainly not the lowest dose at which adverse effects are likely to be observed. However, its use in lieu of the animal studies, will result in greater protection for exposed human populations.

6.2. Ethical considerations

Charge to the Board

In its ethics review of this research, EPA documented that the study reports contained very little information concerning the ethical conduct of the research and that the available information raised no ethical concerns. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of each study:

OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical; and

whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Board Response to the Charge

Brief Overview of the Study

Two studies involving repeat high- and low-dose oral exposure of human subjects to ethephon (ethrel) were evaluated. Both studies were performed by Litton Bionetics, Inc. of Bethesda, Maryland. The studies were sponsored by Amchem Products, Inc., of Ambler, Pennsylvania. Both studies were conducted prior to publication of U.S. regulations (drafted 1974, adopted by DHHS and FDA in 1981) or the Common Rule (adopted by EPA in 1991), so

the regulatory standards established by 45 CFR 46 with respect to conduct of research involving human subjects cannot be applied in this retrospective evaluation. The low-dose study, however, was performed after the 1972 Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) went into effect, and is subject to the regulations contained therein. In particular, FIFRA Section 12(a)(2)(P) states that "[i]t shall be unlawful for any person … to use any pesticide in tests on human beings unless such human beings (i) are fully informed of the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable therefrom, and (ii) freely volunteer to participate in the test." Both studies were also are expected to be in compliance with the ethical standards for research involving human subjects established by the 1964 version of the Declaration of Helsinki.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in the EPA's Initial Ethics Review of Ethephon Human Study, dated March 17, 2006. However, further comments are warranted regarding: 1) whether the repeat high-dose oral-exposure protocols used were designed to minimize risks to study participants; and 2) whether the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent.

1) Minimization of Risks to Study Participants

The two repeat high- and low-dose oral-exposure studies were performed in 1971 and 1973, respectively. The high-dose study involved repeated dosing, over a 28-day period, of 12 subjects (six men and six women) with 1.8 mg/kg body weight of ethephon per day by oral ingestion. Four subjects (two men and two women) received placebo control. The study documents provide no clear justification for the selection of the 1.8 mg/kg body weight dose, other than passing reference to "a preliminary dose range study … carried out in two human subjects. (Litton 1972).

On weekdays, clinical symptoms were monitored continuously for eight hours following ingestion of the test compound; after hours and on weekends, subjects were requested to self-report any adverse clinical symptoms. There was no mention in the documents reviewed by the Board that the investigators made provision for observation or rescue during these off hours. Laboratory analyses, including analysis of blood and urine samples, were performed weekly during the study and also two weeks after last exposure. A large number of study subjects receiving the active compound exhibited clinical symptoms clearly associated with ethephon-exposure, including diarrhea and other gastrointestinal disorders (4 participants), increased frequency and urgency of urination (5 participants), and persistent abdominal pain (1 participant). Laboratory analyses also revealed transient elevations in blood glucose and increased inhibition of plasma cholinesterase in subjects receiving ethephon. No stopping criteria were listed in the final report, nor was there evidence that the investigators considered halting the trial even after large numbers of study participants began to report adverse clinical symptoms.

The low-dose study involved repeated dosing, over a 16-day period, of 20 subjects (10 men and 10 women) with 0.5 mg/kg body weight of ethephon per day by oral ingestion. Ten

subjects (six men and four women) received placebo control. The study documents again provide no clear justification for the selection of the 0.5 mg/kg body weight dose, other than indirect reference to the high-dose study described above.

As before, clinical symptoms were monitored continuously for eight hours and self-reported after hours and on weekends. Laboratory analyses were performed on days 1, 9, and 16, and two weeks following last dose. No adverse clinical events were reported. Surprisingly, the report even fails to mention that no adverse clinical events were seen, leading several Board members to suspect that adverse event data was not rigorously collected.

2) Voluntary Informed Consent

Neither study contained sufficient information for the Board to adequately determine whether or not the informed consent process used to enroll study participants met the standards outlined in FIFRA Section 12(a)(2)(P) and the 1964 version of the Declaration of Helsinki. Each of the final study reports provided to the Board contained only but the briefest of statement as to the nature of the voluntary informed consent process:

"Sixteen human volunteers judged to be in good health as ascertained by pre-physical interview, physical examination and selected clinical laboratory evaluations were chosen for the study. All subjects were thoroughly briefed on the nature and present use of the test product, the animal toxicity data, and the potential pharmacological side effects."

"All subjects were thoroughly informed on the nature and present use of the test substance, the animal toxicity data, potential pharmacologic side effects, and the results of the prior study in human volunteers. Based on this briefing, consent documents were obtained from all subjects."

Although the 1973 low-dose study does contain reference to written consent documents, these were unavailable for review.

Despite the paucity of documentation, several Board members expressed concern over what they consider to be potential violations of accepted standards of informed consent:

- Circumstantial evidence suggests that employees of Litton Bionetics, Inc., may have been enrolled as study participants. For example, there was mention of four study participants "working in the same building." The possible participation of employees in a laboratory-directed research project raised issues of coercion and voluntariness.
- In the high-dose study, a large number of study subjects receiving active compound exhibited clinical symptoms clearly associated with ethephon-exposure, including diarrhea and other gastrointestinal disorders, increased frequency and urgency of urination, and persistent abdominal pain. Not a single study participant, however, withdrew from the study over the 28-day monitoring period, regardless of the severity of illness. This again raised questions about the voluntary nature of participation in research.

HSRB Consensus and Rationale

The Board concluded that:

a) The both the 28 and 16 day oral human toxicity studies failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no clear and convincing evidence that the research is fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no clear and convincing evidence of significant deficiencies in the ethical procedures could that have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

Although these two studies appear to lack justification for the dose selection, as well as clear stopping criteria to prevent serious harm to research participants, the Board does not believe that there was clear and convincing evidence that these studies could have resulted in serious harm based on the knowledge available to the investigators at the time.

There were indications of informed consent deficiencies. Nevertheless, given the paucity of information provided there was no evidence that the voluntary informed consent process used in these two studies failed to meet the few regulatory and ethical standards applicable to research conducted in 1971-1973.

The Board concluded that there was no obvious reason why the Agency cannot rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

7. Hydrogen Cyanide

Charge to the Board

When sodium cyanide is used as a fumigant, hydrogen cyanide is generated by acidification. Because residues of HCN may remain on fumigated citrus, the Agency is conducting an acute dietary risk assessment of hydrogen cyanide.

7.1. Scientific considerations

The Agency's WOE document describes a lack of data appropriate for developing an acute dietary risk assessment for hydrogen cyanide. The WOE and DER present the results from a clinical trial with amygdalin and the usefulness of this clinical trial in the acute dietary risk assessment for hydrogen cyanide. The Agency has concluded that the clinical trial is appropriate for establishing a point of departure in the acute dietary risk assessment for hydrogen cyanide.

Please comment on the scientific evidence that supports this conclusion.

Board Response to the Charge

Brief Overview of the Study

The clinical trial of amygdalin for treatment of cancer (Moertel et al., 1982), which included the preliminary pharmacology and toxicology study (Moertel et al., 1981), involved the administration, under controlled circumstances, of known amounts of amygdalin to patients with terminal cancers for which no known treatments were available. Amygdalin was administered intravenously for 21 days (4.5 g/m^2), followed by oral maintenance therapy of 500 mg (three times per day), plus a "metabolic therapy" consisting of diet, pancreatic enzymes, and vitamins. Most patients were administered the standard dose regimen, but a small number received a high dose regimen.

Clinical signs and symptoms consistent with cyanide toxicity (nausea, vomiting, headache, dizziness, mental obtundation) were observed in some patients following a single 500 mg oral dose of amygdalin. The conclusions regarding toxicity were based largely on several patients who developed such symptoms when they took two 500 mg doses too close together or ate foods such as raw almonds that are rich in ß-glucosidase, an enzyme that causes the release of cyanide from amygdalin. In such patients, the symptoms typically resolved when oral amygdalin was discontinued, the dose was reduced, or the patients stopped eating the critical foods. In the pharmacology and toxicology study, the responses of two patients challenged with almonds provided further support for the conclusion that the symptoms reflected cyanide toxicity. In some patients, the appearance of symptoms was associated with relatively high blood cyanide levels, which in some instances approached levels observed in human fatalities (>3 µg/mL). A dose of 500 mg was interpreted as a minimal effect level but not as a NOAEL.

Critique of Study

The trial had several strengths. The sample size was large, including 178 patients. Blood-cyanide levels were assessed several times, at completion of intravenous (i.v.) treatment, 48 hours after initiation of oral treatment, and at every subsequent evaluation. Measurements were made two hours after the first morning dose of oral amygdalin, when the maximum elevation in cyanide was anticipated. The trial employed a good operational definition of the clinical outcome of interest in the trial, which was tumor progression.

The trial also had several weaknesses. First, it was not blinded and did not include a placebo group. Second, insofar as the primary aim was to assess the effectiveness of amygdalin as a treatment for advanced cancer, the assessment of potential cyanide toxicities was not as systematic as the assessment of tumor progression. As in any clinical trial of drug efficacy, side effects were recorded, however, these side effects were examined in relation to whole-blood cyanide levels. Oral dosing with amygdalin did not begin until 21 days of i.v. treatment had been completed. It is unlikely that this introduced a bias, however. In contrast to delivery by oral route, amygdalin delivered i.v. is largely excreted unchanged in urine without conversion to

cyanide. Whole blood cyanide levels were undetectable following i.v. dosing, making it likely that the rise observed following oral dosing was not confounded by the prior i.v. dosing. Third, patients who participated in the study were terminally ill with cancer. Although the inclusion criteria required that patients be in "good general condition" and ambulatory, they are, nevertheless, likely to represent a particularly sensitive subgroup, thus providing a conservative estimate of the hydrogen cyanide dose at which toxicities occur. Fourth, all clinical signs and symptoms of toxicity (nausea, vomiting, headache, dizziness, mental obtundation, and dermatitis) were observed with nearly equivalent frequencies during i.v. and oral dosing. Many of these signs and symptoms might be increased in terminal cancer patients anyway, and thus unrelated to amygdalin or cyanide. Moreover, if these were indications of toxicity related solely to elevations in blood cyanide, they would not be expected to have occurred with equal frequency during i.v. and oral dosing. The data from the natural experiments within the trial, such as when the doses of individual patients who developed symptoms were changed or the patients were challenged, provides the most persuasive evidence of an increased risk of cyanide toxicity in patients administered amygdalin.

HSRB Consensus and Rationale

The Board concluded that data from the amygdalin trial could be used for establishing a point of departure in the acute dietary risk assessment for hydrogen cyanide.

Despite its limitations, this study provides the best data we are likely to ever have on which to establish a POD for this purpose. Given the severity of the effect, the steepness of the dose-response relationship, and the apparent inter-individual differences in response to a given dose, it would be imprudent to undertake an intentional dosing study of healthy humans in order to establish a LOAEL and NOAEL for hydrogen cyanide.

7.2 Ethical considerations

Charge to the Board

In its ethics review of this research, EPA did not identify any deficiencies with respect to the ethical conduct of this research. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of this study:

OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical; and

whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Board Response to the Charge

Brief Overview of the Study

Two related studies are being evaluated. Both were sponsored by the National Cancer Institute. The first study was a pharmacologic and toxicological study involving six subjects which took place at the Mayo Clinic (Rochester, MN), with the results being published in the Journal of the American Medical Association in 1981 (the Board assumed the study was conducted 1979-1980). Although the published report does not describe the ethical standards to which the researchers adhered, the Board assumed that as a federally-funded study, the research would have been subject to the then-current version of the federal DHHS [DHEW] regulations (45 CFR Part 46) that would later become the Common Rule. The second study was essentially a Phase II follow-up to the first study, involving 178 subjects who were enrolled at four sites (Mayo Comprehensive Cancer Center, Mayo Clinic, Rochester, MN; UCLA Johnson Comprehensive Cancer Center, Los Angeles, CA; Memorial Sloan-Kettering Cancer Center, New York, NY; and University of Arizona Cancer Center, Tucson, AZ). The results of that second study were published in the New England Journal of Medicine in 1982. That article also does not specify the specific ethical rules under which the study was conducted, and this Board again assumes that it would have been subject to the then-current version of the predecessor regulations (DHHS [DHEW], 45 CFR Part 46) to the current Common Rule.

Critique of Study

The Board concurred with the factual observations about the study, as detailed in Carley (2006h).

In particular, the Board concurred with the key observation regarding the difference between this study and the other studies that the Board has heretofore been asked to review. This study involved testing a compound for its safety and efficacy in treating a medical problem. As such, the ethical evaluation of this study was significantly different from that of a study involving exposing subjects to a pesticide. A major consideration in evaluating the ethics of such a study involved determining whether the two arms of the study were in clinical equipoise, a concept that was only in its earliest stages at the time of this study. Nonetheless, it does appear that this study would meet even the modern-day understanding of that concept. All of the subjects were persons with histologically-proven cancer for which no standard treatment was known to be available. The subjects received either a standard dose or a high dose of amygdalin (Laetrile). Prior to this study being conducted, there was substantial uncertainty regarding whether this compound might indeed be a safe and effective treatment for many forms of cancer. As stated in the New England Journal of Medicine article, it had "eclipsed any other unorthodox therapy ever used for any disease in our time." Thus, conducting this study served to answer an important medical question of that time period.

Moreover, the published report of the second study indicated compliance with all of the procedural protections that would be required today. It notes that all "patients were fully informed about the experimental and unorthodox nature of the treatment program as well as any possible alternative treatment available to them. A signed form giving informed consent, approved by the Human Subjects Committee at each of the four participating centers, was obtained from each patient." (The actual forms were not available for review by the Board.) The article further noted that "the methods of this trial were entirely comparable to those employed in

studies of any new agent being developed and tested for cancer treatment through more traditional channels."

HSRB Consensus and Rationale

The Board concluded that:

a) The hydrogen cyanide human oral toxicity study appeared to meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted) nor that information provided to participants seriously impaired their informed consent.

Although the Board based its determinations only on the two published articles, it did not find any ethical flaws in the conduct of these two studies. The article described appropriate informed consent procedures. The risks and potential benefits of the dosing were appropriate for a medical treatment study for this particular cancer population.

The Board concluded that there was no obvious reason why the Agency cannot rely on the results of this study, as appropriate under current pesticide laws, given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing when the study was conducted.

8. Amitraz

Charge to the Board

Exposure to amitraz can result in neurotoxicity as evidenced by clinical signs such as ataxia, ptosis, emesis, labored respiration, muscular weakness, tremors, hypothermia and bradycardia. The Agency is conducting an aggregate (single chemical, multi-route) risk assessment of amitraz.

8.1. Scientific considerations

The Agency's WOE document and DERs for amitraz describe the study design and results of the amitraz acute oral and dermal toxicity human studies and the human metabolism study. The WOE document also discusses the Agency's conclusions regarding the usefulness of the human studies in the single chemical risk assessment for acute and chronic oral exposures in addition to dermal and inhalation exposures of various durations. For oral exposure, the Agency has concluded that the combined results from the single oral dose study and human metabolism study establishes a dose response relationship in human subjects and that the single oral dose study is appropriate for developing a point of departure for acute and chronic dietary risk, short-

term oral exposure, and inhalation exposures of various durations. The Agency has further concluded that the human dermal study is appropriate for developing a point of departure for dermal exposures of various durations.

Please comment on the scientific evidence that supports these conclusions.

Board Response to the Charge

Overview

The HSRB was asked to comment on the scientific evidence that supports the following conclusions:

1. For oral exposure, the Agency concluded that the combined results from the single oral dose study and human metabolism study establish a dose-response relationship in human subjects, and that the single oral dose study is appropriate for developing a point of departure for acute and chronic dietary risk, short-term oral exposure, and inhalation exposures of various durations.

2. The Agency further concluded that the human dermal study is appropriate for developing a point of departure for dermal exposures of various durations.

Amitraz [N'-(2,4-dimethylphenyl)-N-[[(2,4-dimethylphenyl)imino]methyl]-N-methylmethanimidamide] is an insecticide/acaricide with wide range of registered uses in the United States. The toxicity profile for amitraz has not been characterized completely, especially developmental and reproductive effects. Neurotoxicity is considered the most sensitive effect resulting from exposure to amitraz. Neurotoxicity has been demonstrated in multiple species (baboon, cat, dog, mouse, rat and rabbit). Clinical signs include central nervous system depression, ataxia (loss of coordination), ptosis (droopy eyelids), emesis (vomiting), labored respiration, muscular weakness, tremors, hypothermia and bradycardia (slow heartbeat). Similar signs are seen in humans. The Agency's evaluation of amitraz included three human studies: a 1984 oral dose metabolism study Campbell 1984), a 1992 oral dose tolerance study (Cass 1992) and a 1997 dermal dose tolerance study (Langford 1997).

Critique of Oral Dose Metabolism Study (1984)

Radio-labeled amitraz was orally administered to rats, mice, baboons and humans. The two human volunteers received a single 0.25 mg/kg dose of amitraz by capsule. This dose caused dry mouth, drowsiness, disorientation, decreased temperature, bradycardia, and slightly pale appearance in both subjects, persisting up to 12 hours after dosing. One subject fell asleep for 6 hours following dosing. Psychomotor testing was not included in the protocol. Approximately 44% of the dose was excreted within 12 hours, with 63% excreted within 24 hours. Study deficiencies included the small number of subjects (N=2), testing of males only, and no control group. Sex differences in the repeat dose animal studies have shown that females are more sensitive than males. The study investigators concluded that humans were more sensitive to amitraz than were other species included in the metabolism study.

The occurrence of multiple signs and symptoms in both subjects and the continued bradycardia up to 12 hours post-dosing suggest that the 0.25 mg/kg dose was not the lowest adverse effect level for amitraz in humans. Because there was only one dose level, it was not possible to glean any information regarding dose-response from this study. Incomplete excretion of the amitraz metabolites at 24 hours post-dosing suggests that some portion of the dose was present on the day following dosing, albeit not at levels sufficient to produce the frank signs and symptoms observed on the day of dosing.

Critique of Single Oral Dose Study (1992)

The design was a single oral dose double-blind tolerance study. The primary objective of this study was to determine the tolerance of male volunteers to a single dose of amitraz at two different dose levels (0.0625 and 0.125 mg/kg), with a placebo control. Six subjects completed the study. Each subject received each of the doses and the placebo. Dosing events were separated by at least 14 days for each subject. The dosing regimen was thoughtfully constructed, beginning with the lower dose, and moving to the higher dose when it was observed that the lower dose was well tolerated. The subjects were admitted to a clinical pharmacology unit the evening before each dosing and remained there for 36 hours. Complete physical examinations were conducted before and after the study. The subjects were followed for at least three weeks following the study. The most sensitive neurologic endpoints measured in this study were two psychomotor performance tests: choice reaction time and critical flicker fusion threshold. The psychomotor performance tests were administered pre-dose, 2.5 hours, and 8 hours following dosing. The report provides tabular and graphic representation of the results of these tests, but no statistical analyses were conducted. Study deficiencies included the small number of subjects (N=6), and the testing of males only. The study investigators concluded that no effects were observed from the single oral dose in any of the subjects.

The major concern with this study was whether "no effect" was truly observed. The study report was deficient in its description of the psychomotor performance testing, and in the rationale for selecting these tests. There were no descriptions of the specific procedures followed for the psychomotor tests, no standard operating procedures, and no quality assurance documentation. Also, there was no evidence provided in the report to indicate that these tests were the most sensitive available. A review of the current scientific literature, and discussions with leading scientists in this area of expertise would permit the Agency to determine if these psychomotor test results are adequate to characterize a "no effect" level in regard to neurotoxicity for amitraz.

Critique of Dermal Toxicity Study (1997)

This study was a double-blind sequential dosing study with a randomized crossover and a placebo group. The study aims were to 1) establish a no effect level for acute effects following repeated dermal doses of amitraz, 2) produce data that would permit calculation of margins of safety for agricultural workers. Eight male volunteers were given a total dose of either 0, 8, 16 or 24 mg/kg amitraz, applied dermally as four equal doses of 0, 2, 4 or 6 mg/kg in an aqueous 1:1 (w/w) slurry every 2.5 hours over 10 hours. Starch, 1:1 (w/w) in water, was used as a placebo.

Each of these amitraz experiments involved a timed series of dosing events (0, 2.5, 5.0, and 7.5 hours), followed by a washing event at 10 hours. Each dose within an experiment was applied to a different 20 cm^2 skin surface area, so the total skin surface treated in each experiment was 80 cm^2. Thus, skin loadings for the three experiments were 7,000, 14,000, and 21,000 $\mu g/cm^2$, corresponding to the three dose levels (8 mg/kg, 16 mg/kg, 24 mg/kg), assuming a body weight of 70 kg. No differences were observed between treatment and placebo for any of the measured endpoints. No urinary metabolite monitoring was conducted to confirm that amitraz had been absorbed. The study report is deficient in its description of the psychomotor performance testing, and in the rationale for selecting these tests. There is no description of the specific procedures followed, no standard operating procedures, and no quality assurance documentation. Also, there is no evidence provided in the report to indicate that these tests were the most sensitive available. A review of the current scientific literature, and discussions with leading scientists in this area of expertise would permit the Agency to determine if these psychomotor test results are adequate to characterize a "no effect" level in regard to neurotoxicity for amitraz.

The primary deficiency of this study is that it does not provide a realistic worker exposure scenario; that is, the exposures of the subjects in these experiments did not correspond to exposures likely to be seen among workers. Large amounts of amitraz (140-420 mg) were applied to a relatively small skin surface area (80 cm^2) in the experiments, whereas we typically see much larger skin surface areas exposed to smaller amounts among workers. For example, the hands, a skin surface commonly exposed to pesticides, have a total surface area of 990 cm^2, or 12 times greater than the exposed skin surface area in this experiment (USEPA 1997). If both the hands and forearms were exposed, the total exposed surface area would be 3,900 cm^2, or 49 times the surface area exposed in this study. This discrepancy is important, as it has a major impact on the amount of compound that can be absorbed within a defined time period. Dermal dosing studies require careful consideration of three factors: mass applied to the skin, surface area treated, and the duration of exposure. These three factors are typically used to determine the flux of a chemical through the skin (e.g., $mg/cm^2/hr$). Percent absorbed is highly dependent on the skin loading, or mass applied per unit area (e.g., mg/cm^2). The relationship between skin loading and percent absorbed was made clear in a review of studies in the rat by Zendzian (2000). In the case of azinphos-methyl, for example, a loading of 3 nanomole/cm^2 for 10 hours resulted in 23% absorption, a loading 10-fold higher (29 nmolesM/cm^2) resulted in 15% absorption, and a further 10-fold increase in loading (293 nmoles/cm^2) resulted in only 2.9% absorption. These azinphos-methyl loadings, expressed as mass per unit area, were 0.95, 9.2, and 93 $\mu g/cm^2$, respectively. In earlier dermal dosing studies involving humans, radio-labelled pesticides were applied at loadings of 4 and 40 $\mu g/cm^2$ (Feldmann and Maibach 1974). It is evident that the skin loadings in the amitraz study were several orders of magnitude higher than those used in the above cited studies. The key point is that chemical loadings on small areas of skin result in relatively small amounts of chemical absorbed. In this dermal toxicity study, the loadings were so high that the three "dose" levels were essentially equivalent in terms of dermal absorption potential.

If it is assumed that the flux of amitraz in these experiments was approximately 2 $\mu g/cm^2/hr$, as has been estimated from a study of chlorpyrifos, a lipophilic insecticide with a similar molecular weight (Nolan et al. 1984), then the total mass absorbed in the amitraz experiments over 10 hours would have been approximately 1,000 µg (1 mg), or an absorbed dose

of 0.014 mg/kg. If this same flux were applied to an exposure to the hands and forearms of a worker, the total mass absorbed would be approximately 50,000 µg (50 mg), or an absorbed dose of 0.71 mg/kg, well above both the 0.125 mg/kg single oral dose "no effect" level, and the 0.25 mg/kg single oral dose that elicited multiple signs and symptoms of neurotoxicity. It is for this reason that the amitraz dermal toxicity study cannot be considered a "no effect" study for risk assessment purposes.

HSRB Consensus and Rationale

a. The Board concluded that the results from the single oral dose study are informed by the human metabolism study such that the single oral dose study is appropriate for developing a point of departure for acute and chronic dietary risk and short-term oral exposure.

The appearance of overt signs and symptom of toxicity in the 1984 oral metabolism study at a dose of 0.25 mg/kg provides some confidence that the highest dose (0.125 mg/kg) in the 1992 single oral dose study represent a no observable adverse effect level (NOAEL) for an acute dietary exposure to amitraz No effects of neurologic toxicity were observed in any of the study subjects at 0.125 mg/kg. This conclusion rests on the assumption that the psychomotor tests applied to the subjects are the most sensitive endpoints for neurotoxicity, and that they were conducted properly. There was considerable uncertainty regarding these psychomotor tests, as the procedures used were not described in the study, and on quality assurance information was provided. Thus, the findings from this study should be used with caution for risk assessment purposes.

b. The Board concluded that the combined results from the single oral dose study and the human metabolism study were not appropriate for developing a point of departure for chronic dietary risk, short-term oral exposure, or inhalation risk.

A single oral dose of 0.25 mg/kg elicited frank symptoms of toxicity in both subjects in the 1984 metabolism study. Multiple signs and symptoms were observed in the subjects for several hours post-dosing, and at least one sign (bradycardia) was still present at 12 hours post-dose. Excretion of radio-labeled metabolites was incomplete (62%) at 24 hours. The proposed NOAEL is only one-half the dose used in the 1984 study. Thus, subjects exposed to repeated daily doses, as would occur in short-term or chronic oral dose studies, would achieve a cumulative dose of 0.25 mg/kg within 48 hours. It seems quite possible that repeated short-term oral exposures or chronic oral exposures would elicit at least some effects in humans within a very few days. Thus, the available data do not demonstrate a no-effect level for multi-day oral exposures. In regard to inhalation exposures, absorption through the respiratory tract is generally more efficient than absorption through the gastro-intestinal tract. It is quite possible that adverse effects would have been observed in an inhalation exposure study that delivered 0.125 mg/kg as a single dose. Also, an oral dose study would not capture adverse effects unique to exposure by the inhalation route. Thus, the available data do not demonstrate that the oral NOAEL and the inhalation NOAEL are equivalent.

c. The majority of the Board concluded that the human dermal study was not appropriate for developing a point of departure for dermal exposures of various durations.

The HSRB members did not consider the dermal toxicity study to be a valid scientific study for risk assessment because: 1) the study involved extremely high loadings (mass per unit surface area) of amitraz on the skin, making the three dose levels used in the study very nearly equivalent in regard to dermal absorption potential, and not equivalent to worker exposure scenarios; 2) the study did not demonstrate an effect (i.e., the study was equivalent to a "NOAEL-only" study); and 3) there was no corroborating evidence to demonstrate that an internal dose had been delivered to the subjects by the dermal route. One member of the HSRB, while agreeing with these scientific criticisms, considered that the study might still be of use to the Agency.

8.2. Ethical considerations

Charge to the Board

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

With respect to the Campbell (1984) research, whether the lack of medical surveillance of subjects, following the termination of dosing, to establish that subjects' signs of adverse effects had returned to normal should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

With respect to the Cass (1992) and the Langford (1998) studies, whether references to the test material as a drug and other statements that could indicate the study constituted medical research, that appear in the materials used to obtain informed should be considered significantly deficient relative to the ethical standards prevailing when the study was conducted; and

b. The Agency asks that the Board provide comment on the following, taking into account all that is known about the ethical conduct of each study:

OPP's conclusion that there is not clear and convincing evidence that the conduct of the research was fundamentally unethical.

Whether there is clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

Board Response to the Charge

The compound amitraz is an alpha-two-adrenergic agonist that produces sympatholytic effects leading to sedation and other neurological signs and symptoms, as well as cardiovascular depression with a drop in blood pressure and heart rate. The studies under ethical review include two metabolic studies in 1984 using C^{14} radio-labeled isotope technology, a 1992 oral dosing study, and a 1998 dermal absorption study.

The two metabolic studies were performed in 1984 and involved two volunteer subjects. The oral dosing study was performed in 1992 and involved six research subjects (with an

additional (1) subject withdrawn for an unrelated rash). The oral dosing study was a double-blind, randomized, placebo-controlled, crossover study involving two different doses. The oral dosing study progressed in three phases, escalating from the lower dose to the higher dose. The protocol stated that the research was in compliance with the 1989 version of the Declaration of Helsinki, an early version of good clinical practice (GCP) guidelines promulgated by the EMEA (111-3976-88-EN), the ABPI Guidelines for Medical Experiments in Non-Patient Human Volunteers (1988, 1990), the 1986 RCP Guidelines on Research Using Healthy Volunteers, the FDA Compliance Program for Drugs and Biologics and the GCP guidelines from Japan that were current in 1992 (Notification No. 874 from the Ministry of Health and Welfare). The approval of the research ethics committee was given on November 20, 1991, but no information was provided about the substance and process of that review.

The dermal absorption study was performed in 1998 and involved eight research subjects. The study was a double-blind, randomized, placebo-controlled, crossover study involving three different doses (in a four phase dose escalation design). The protocol stated that the research was in compliance with the above standards, with the updated GCP guidelines promulgated in 1996. The approval of the research ethics committee was given on December 22, 1997, although no information is provided about the substance and process of that review.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the two 1984 metabolic studies, as detailed in Carley (2006g). Specifically, there is no justification for the dose selection, no record of independent committee review, and no record of informed consent (other than the description of the participants as volunteers. The dose selected did result in adverse events as might be expected from the administration of a product with this pharmacological profile. The period of recorded observations was eight hours, and stated observation time was 12 hours. Although the period of observation was insufficient to assure that the physiologic condition of the volunteers had returned to baseline, the known physiologic effects of the compound are such that the period of observation was sufficient to assure the lack of significant adverse effects.

The Board concurred with the factual observations of the strengths and weaknesses of the 1992 oral dosing study as documented in Carley (2006h). Of note was that the volunteer information included information about the risks of the administration of the study compound which were relatively complete in the scope of the risks described (yet absent a few details within each organ system).

The Board concurred with the factual observations of the strengths and weaknesses of the 1998 dermal absorption study, as documented in Carley (2006i). Although concern has been expressed about the choice of dose, the study design involved a dose escalation such that adverse events at lower doses could have been anticipated.

HSRB Consensus and Rationale

The Board concluded that:

a) The amitraz acute oral and dermal human toxicity studies failed to fully meet the specific ethical standards prevalent at the time the research was conducted.

b) There was no clear and convincing evidence that the research was fundamentally unethical--intended to seriously harm participants or that informed consent was not obtained.

c) There was no clear and convincing evidence of significant deficiencies in the ethical procedures that could have resulted in serious harm (based on the knowledge available at the time the study was conducted), nor that information provided to participants seriously impaired their informed consent.

Although the Board concurred in the noted deficiencies with the 1984 metabolic studies, the Board did not consider the lack of documentation of medical supervision following the termination of dosing in the 1984 metabolic studies to be significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board also acknowledged the observations that the 1992 oral dosing study and the 1998 dermal absorption study includes potentially misleading references in the informed consent documents. However, the Board does not believe that these deficiencies could have resulted in serious harm based on the knowledge available to the investigators at the time, nor seriously impaired the informed consent of the research subjects. As such, the Board did not consider these findings significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board concurred with the assessment of the Agency that there was not clear and convincing evidence that the conduct of the research was fundamentally unethical. The research as designed and conducted was not intended to seriously harm participants nor failed to obtain informed consent. Thus, the Board concluded that there was no ethical objection to the appropriate use of the data from these studies (as discussed in the scientific assessment) given the absence of clear and convincing evidence that the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time.

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