July 7, 2006

EPA-HSRB-06-02

George Gray, Ph.D.
Science Advisor
Office of the Science Advisor
United States Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: May 2-3, 2006 EPA Human Studies Review Board Meeting Report

Dear Dr. Gray:

The United States Environmental Protection Agency (EPA or Agency) requested the Human Studies Review Board (HSRB) to review scientific and ethics reviews of chromium, carbofuran and methyl isothiocyanate. The enclosed HSRB report addresses the Board's response to EPA charge questions for the Board's consideration at its May 2-3, 2006 meeting.

A summary of the Board's conclusions on the scientific and ethical considerations of the human toxicity studies for the three pesticides are provided below.

Chromium

Scientific Considerations

- The Board concluded that the 1994 Nethercott et al. dermal sensitization study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of dermal exposure to hexavalent chromium.

- The study was properly designed, well-conducted, and employed appropriate scientific and clinical methods to determine a minimum elicitation threshold for dermal sensitization due to hexavalent chromium. The MET_{10} reported in the study provided a reasonable point of departure for risk assessment.

Ethical Considerations

- The HSRB concluded that this study appeared to have not deviated significantly from the ethical standards prevalent at the time the research was conducted, noting that this conclusion was hampered by a lack of supporting documentation concerning independent ethical review.

- 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 the deficiencies did not result in serious harm, nor seriously impair the informed consent of the research subjects and;

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

Carbofuran

Scientific Considerations

- The HSRB concluded that there were numerous technical issues regarding the conduct of the oral and dermal studies with carbofuran and that overall, the weakness of the studies far outweigh the strengths. Accordingly, the HSRB did not recommend any of the oral or dermal studies conducted with carbofuran in human subjects for the single chemical assessment or for in informing the interspecies uncertainty factor for the cumulative assessment.

Ethical Considerations

- For the oral human toxicity study, there was no evidence that the study failed to fully meet specific ethical standards prevalent at the time the research was conducted.

- For the oral human toxicity study, 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.

- For the oral human toxicity study, there was no clear and convincing evidence that the research was fundamentally unethical (e.g., intended to seriously harm participants or that informed consent was not obtained).

- The HSRB found deficiencies in both dermal human toxicity studies relative to specific ethical standards prevalent at the time the study was conducted.

- For both dermal human toxicity studies, there was clear and convincing evidence of significant deficiencies in the ethical procedures for minimizing risk that could have resulted in serious harm (based on the knowledge available at the time the study was conducted). The first dermal toxicity study was significantly deficient given the delay in

the administration of atropine to more than one subject experiencing the signs and symptoms of carbamate toxicity. The second dermal toxicity study was considered significantly deficient in that the lack of information provided about the results from the initial dermal toxicity study seriously impaired their informed consent.

- However, for both dermal human toxicity studies, there was no clear and convincing evidence that the research was fundamentally unethical (e.g., intended to seriously harm participants or that informed consent was not obtained).

Methyl Isothiocyanate

Scientific Considerations

- The Board concluded that air concentrations of methyl isothiocyanate sufficient to produce eye irritation would lead to a conservative and prudent point of departure for inhalation risk (i.e., eyes were a sensitive endpoint in relation to the respiratory system). The Board reached its decision based on the observation that eye irritation LOAELs are often lower than respiratory irritation LOAELs for irritant gases. While the use of eye irritation data as a surrogate for respiratory data is reasonable in this situation, one must be cautious as only appropriate controlled human studies of the respiratory system can provide a final and definitive respiratory point of departure, if ever determined.

Ethical Considerations

- The HSRB determined there were minor deficiencies in the ethical procedures relative to those prevalent at the time, however;

- There was no clear and convincing evidence that the conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent) and;

- There was no clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board also provided commentary of its scientific criteria for review of human dosing studies. The Board's criteria encompassed the following: (1) justification; (2) dose selection; (3) endpoint selection; (4) participants; (5) method; and (6) statistical analyses. In addition, the Board established criteria for evaluating the utility of single dose level studies.

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

Sincerely,

Celia B. Fisher, Ph.D.
Chair
United States Environmental Protection Agency
Human Studies Review Board

NOTICE

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

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

United States Environmental Protection Agency Human Studies Review Board

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* Recused from carbofuran discussion and deliberation
**Not in attendance at May 2-3, 2006 Public Meeting

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INTRODUCTION

On May 2-3, 2006, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB) met to review scientific and ethical issues concerning human toxicity studies involving two pesticide active ingredients, carbofuran and methyl isothiocyanate (MITC), and chromium, a constituent of wood preservative products (wood preservatives are regulated as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act).

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

Both MITC and carbofuran are undergoing reevaluation in the reregistration process. EPA is considering the human health risks of chromium both in its reregistration program and as part of its review of an application for registration pending under FIFRA and PRIA.

For each of the human studies under consideration, the Agency provided the Board with the complete study report and any supplements available to the Agency. Each of these studies was assigned a unique identifier, the Master Record Identifier or MRID, which the EPA, Office of Pesticide Programs (OPP) uses to manage documents in its archive. When a company submits multiple documents pertaining to a single study, each document is assigned a unique MRID as it is received and catalogued. Thus a study with several supplements, such as the MITC study discussed at the meeting, may be associated with several MRIDs.

For each study, the Agency had provided 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. EPA has intentionally deferred making a final determination of whether an individual study satisfies 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; the Board was provided with one or more DERs for carbofuran and MITC. DERs contain summaries of the study design, methods and results, describe potential deficiencies, and provides conclusions about the usefulness of the study in risk assessment.

In addition to the DERs, the Agency had prepared a Weight of Evidence (WOE) memorandum for carbofuran and MITC discussing the differences and similarities between the

human and animal responses to each chemical and characterizing the usefulness of the human toxicity studies for human health risk assessment. The WOE memos expressed the Agency's current scientific conclusions on which the Agency was soliciting the Board's comments. To maintain the historical record of review, the Agency may, in some cases, include a DER for a study that expressed scientific conclusions differing from those in the WOE document.

For chromium, the Agency provided a set of documents which contained similar information to DERs and WOEs, but which had a slightly different format and presentation, due to the procedural history of the EPA's review of this chemical. As noted above, chromium is a constituent in wood preservative products. The Agency has concerns about the potential for chromium to elicit an allergic response in sensitized individuals who come in contact with residues remaining in products made from wood that had been treated with chromium-containing wood preservatives. To assess the risk of potential dermal exposure, the Agency reviewed, among other information, a study involving intentional exposure of sensitized subjects to different levels of chromium (Nethercott et al. 1994). This assessment was one of the first assessments of this kind performed by the Agency, and it raised significant scientific issues. Accordingly, the Agency prepared a background document for its independent, peer review advisory committee, the FIFRA Scientific Advisory Panel (SAP). The SAP is a federally chartered advisory committee of scientific experts who provide advice to the Agency on pesticides and pesticide-related issues as to their impact on human health and the environment of regulatory actions. The Agency provided a copy of the materials given to the SAP for its review, as well as a copy of the SAP's final report. After receiving the SAP's recommendations, the Agency sought review and comment from other Agency scientists through the steering committee of the Agency's internal Science Policy Council (SPC) to ensure consistency across programs in the approach to regulating substances that are skin sensitizers. Using the advice of the SAP and the steering committee of the SPC, the Agency developed a memorandum describing how it intended to use the results of the Nethercott study to derive a sensitization Reference Dose.

The HSRB has reviewed 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 scientific reasons or because they do not meet the standards in EPA's final human studies rule, 40 CFR Part 26. The Agency asked the HSRB to advise the Agency on a range of scientific and ethics issues and on how the studies should be assessed against the provisions in 40 CFR sections 26.1701 – 26.1704 of EPA's final human studies rule**.** This report transmits the HSRB's comments and recommendations from its May 2-3, 2006 meeting.

REVIEW PROCESS

On May 2-3, 2006 the Board had a public face-to-face meeting in Arlington, Virginia. Advance notice of the meeting was published in the Federal Register "Human Studies Review Board: Notice of Public Meeting (71 Federal Register 19725). At the public meeting, following welcoming remarks from Agency officials, Celia B. Fisher, HRSB Chair, proposed a set of scientific and ethics criteria consistent with the language of 71 Federal Register 6137 to guide Board evaluation of each completed study. The Chair's scientific criteria asked the Board to consider the following two questions: (1) did the research design and implementation meet

scientific standards and (2) did the data generated by the study have implications for the Agency's Weight of the Evidence (WOE) review and when applicable aspects of the risk assessment? The Chair'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 deficiencies identified could have resulted in serious harm based on knowledge available at the time the study was conducted *or* the information provided to participants could seriously impair informed consent). The Board then heard presentations from the Agency on the following topics: science and ethics of chromium human studies, science and ethics of carbofuran human studies and science and ethics of methyl isothiocyanate human studies. The Board heard oral public comments from the following individuals:

Chromium
Jennifer Sass, Ph.D. representing the Natural Resources Defense Council

Carbofuran
Donald Carson, Ph.D. and Ms. Jane McCarty on behalf of FMC Corporation
Jennifer Sass, Ph.D. representing the Natural Resources Defense Council

In addition, the Board received written public comments from CRLA Foundation, FMC Corporation, and the Natural Resources Defense Council. 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. Chromium

Charge to the Board

Hexavalent chromium is a component of a pesticide product intended to be used as a wood preservative. Members of the general public may experience dermal exposure to residues of hexavalent chromium remaining on wood treated with a wood preservative. Because chromium has caused allergic contact dermatitis (ACD) in occupational settings, EPA has determined that it should assess the potential for ACD in the general public resulting from the use of wood preservatives containing chromium.

In a meeting of the FIFRA Scientific Advisory Panel (SAP) in May 2004, EPA obtained independent peer review of scientific issues related to the assessment of the potential dermal risk resulting from exposure to chromium. See www.epa.gov/scipoly/sap/2004/final.doc The Agency

had carefully considered the report of the SAP, as well as the advice of EPA scientists through the steering committee of the Agency's Science Policy Council. Taking all of this into account, EPA had derived a "sensitization reference dose" (RfD) based on the 10% Minimum Elicitation Threshold (MET 10) and use of a 10-fold uncertainty factor for potential variability within the human population and other uncertainties. See ADTC Memorandum, "Hexavalent Chromium - Finalization of Issues related to Quantitation of Dermal Risk from exposure to treated wood containing hexavalent chromium," August 31, 2004.

Scientific considerations

EPA had identified a study performed with subjects who had documented sensitivity to chromium (Nethercott, et al., 1994). The study was conducted to identify a level of exposure to chromium below which dermal exposure did not appear to elicit an ACD response. Regarding the Nethercott human study, the Agency had concluded that the study contains information sufficient for assessing human risk resulting from potential dermal exposure.

Please comment on whether the Nethercott study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of dermal exposure to hexavalent chromium.

Board Response to the Charge

Introduction

Hexavalent chromium (CrVI) is known to cause allergic contact dermatitis (ACD). CrVI is a component of a pesticide product used as a wood preservative, and members of the general public may be exposed through contact with treated wood. ACD is a delayed, immunologically mediated, inflammatory skin disease consisting of various degrees of erythema, edema, and vesiculation. ACD is typically characterized by two phases, termed induction and elicitation. Induction occurs when there was an exposure of sufficient magnitude and/or duration to activate specific immune mechanisms resulting in the acquisition of sensitization, while elicitation occurs from a subsequent exposure to the same chemical allergen. In general, the amount of allergen exposure needed to produce induction is greater than that needed to produce elicitation in previously sensitized individuals. Thus, the study of elicitation can provide an appropriate critical endpoint for risk assessments. One approach to estimate an acceptable area dose for protection against elicitation is the determination of a minimum elicitation threshold, or MET. The concept behind the MET is that there was an elicitation threshold below which no sensitization reaction is expected.

The EPA FIFRA Scientific Advisory Panel met in May 2004 to review human and animal studies related to CrVI (SAP, 2004). In August, 2004 the Agency's Antimicrobials Division Toxicity Endpoint Selection Committee issued a memorandum that summarized its assessment of dermal risk from CrVI (ADTC, 2004). The Agency identified a study performed with human subjects who had documented sensitivity to chromium (Nethercott et al., 1994). The study was conducted to identify a level of exposure to chromium below which dermal exposure did not appear to elicit an ACD response. Regarding the Nethercott et al. study, the Agency had concluded that the study contained information sufficient for assessing human risk resulting from

potential dermal exposure. The Agency had asked the HSRB to comment on whether this study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of dermal exposure to hexavalent chromium.

Critique of the 1994 Nethercott et al. Study

The purpose of the study was to determine the MET as mass of allergen per skin surface area for CrVI by a patch testing technique. The study also included response to CRIII, but these data were not discussed here. Five concentration levels of CrVI (4.4, 0.88, 0.18, 0.088, 0.018 $\mu g/cm^2$) were used in the patch test, and "TRUE-Test" patches were manufactured specifically for use in the study to reduce the variability inherent in earlier patch preparation methods. The highest concentration (4.4 $\mu g/cm^2$) were used as a screening concentration to identify those who were sensitized to CrVI. This first round of testing involved 102 volunteers (78 men and 24 women) previously shown to be sensitive to developing allergic contact dermatitis (ACD) in response to an allergen. CrVI elicited ACD in 54 (39 men and 15 women) of these 102 subjects. Two lower concentrations (0.018 and 0.088 $\mu g/cm^2$) were tested in these 54 volunteers in round two. Those who had no ACD response during round two were tested with the next two higher concentrations (0.18 and 0.88 $\mu g/cm^2$) in round three. These concentrations were chosen to provide a maximal ACD response. The study was double blind as to concentration, and each concentration was matched with a control (placebo) concentration within each volunteer. Patch concentrations were validated analytically and found to be within Contract Laboratory Procedure criteria for acceptability. The serial escalation of patch concentration level permitted the authors to determine a dose-response relationship and to calculate MET values. The authors calculated a 10% minimum elicitation threshold (MET_{10}) of 0.089 $\mu g/cm^2$.

This study had a number of strengths. It involved both sexes, the study concentrations were selected carefully based on previous studies, and the investigators determined *a priori* what sample size and dosing group size were needed to establish statistical accuracy for the MET_{10}. Many elements of the experimental protocol (e.g., employment of the control patch, serial increase of the concentration until manifestation of ACD, double blinding of patch concentration levels) were thoughtfully developed. The study was designed in accordance with current scientific standards to address a clearly defined research question, included representative study populations for the endpoint in question, and met requirements for adequate statistical power. It appears to have been conducted in accordance with recognized good clinical practices, including appropriate monitoring for safety. Finally, the study authors reported the design, conduct and analysis very comprehensively.

There are several questions that can be raised regarding the scientific validity of this study. First, the authors developed a cumulative response curve that included subjects who did not respond to any of the doses presented in rounds two and three. These subjects were assigned a minimum elicitation threshold value of 4.4 $\mu g/cm^2$, although none were tested at doses between 0.88 and 4.4 $\mu g/cm^2$. The assignment of this MET value appeared arbitrary, and potentially distorts the shape of the cumulative response curve. However, this use of the high MET value does not affect the calculation of the MET_{10}, and so it was of no consequence to the study's primary conclusion. Second, a recent study by Hansen et al. (2003) reported a MET_{10} of 0.03 ug/cm^2 for 18 subjects, a value substantially lower than that reported by Nethercott et al.

However, these two studies differed with respect to the reading scale employed. The reading of the tests in the Nethercott et al. study followed rules adopted for the diagnostic patch test; that is to say, the definition of a positive reaction was the appearance of erythema infiltration and papules. This approach was consistent with current international clinical standards. For the Hansen et al. study, the investigators used the same reading scale for diagnostic patch testing, but for definition of thresholds they used any degree of reaction, including erythematous and follicular reactions. The logic for this approach was that at very low concentrations irritation was not an issue, so that the question of threshold was not a diagnostic decision. This more sensitive reading approach, which at present was considered experimental, accounts for the difference in MET_{10} values reported in these two studies. Third, Nethercott et al. (1994) used patches that covered a very small area of skin (0.81 cm^2). Workers, and presumably members of the public, would typically be exposed over a much larger skin surface area than that used in this study. In their article Nethercott et al. discussed the potential importance of patch surface area, and described an additional experiment with four of the study subjects who had exhibited MET values at 0.88 $\mu g/cm^2$. In this experiment five patches were used for each subject, and the exposure level of CrVI was reduced to 0.18 $\mu g/cm^2$ for each patch. The data that resulted from this experiment were not presented, but the authors stated that "sub-MET concentrations of CrVI applied over a larger skin surface area did not elicit the positive responses seen when the MET concentration was applied in the standard patch." Current evidence indicated that the dose per unit area was the most important parameter for studies of this kind. But there was no doubt that if an extended area was exposed, such as the full arm, there may be an effect from absorption of an ACD-producing compound. This type of exposure could lead to a *systemic* contact dermatitis reaction with spreading of the dermatitis to a vesicular palmar eczema, and eventually flexural eczema. Such systemic spreads are well known in relation to major contact dermatitis reactions, as occur in occupational exposures. The Nethercott et al. study, where relatively small skin surface areas were exposed, does not exclude that such effects could happen if larger areas were exposed.

HSRB Consensus and Rationale

The Board concluded that the 1994 Nethercott et al. dermal sensitization study was sufficiently sound, from a scientific perspective, to be used to estimate a safe level of dermal exposure to hexavalent chromium.

The 1994 Nethercott et al. study was properly designed, well-conducted, and employed appropriate scientific and clinical methods to determine a minimum elicitation threshold for dermal sensitization due to hexavalent chromium. The MET_{10} reported in the study provided a reasonable point of departure for risk assessment.

Charge to the Board

Ethical considerations

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

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

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

Board Response to the Charge

Brief Overview of the Study

A previously-published study involving dermal exposure of 102 healthy volunteers to increasing doses of CrVI was evaluated, hereinafter referred to as Nethercott et al.1994. The study sponsor was unknown, but is likely to be either the Chem Risk Division of McLaren/Hart Environmental Engineering, Alameda CA, or a client of McLaren/Hart. The study was conducted in 1992 at five U.S. and one Canadian academic institution: the Cleveland Clinic Foundation (Cleveland, OH), Johns Hopkins University (Baltimore, MD), Pennsylvania State University (Hershey, PA), Stanford University (Palo Alto, CA), the University of British Columbia (Vancouver, BC), and the University of Louisville (Louisville, KY). The study was conducted after the promulgation of federal protections for the protection of human participants in research (i.e. Common Rule) (§45CFR46; adopted by the EPA in 1991 and published at §40CFR26), so the regulatory requirements of the Common Rule were applicable. Furthermore, all five US academic institutions participating had a valid Multiple Project Assurance of Compliance with U.S. Department of Health and Human Services (DHHS) Regulations for Protection of Human Research Subjects at the time the study was performed. The University of British Columbia, in contrast, held a Cooperative Project Assurance at that time, allowing its participation in DHHS-recognized research programs and documenting the University of British Columbia's commitment to the protection of human research subjects in accordance with §45CFR46.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in USEPA (2006a). However, further comments were raised regarding: 1) whether the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent and 2) whether the three step protocols used were designed to minimize risks to study participants.

1) Voluntary Informed Consent

The Common Rule provides a comprehensive framework for initial and continuing review of research involving human subjects. In order to ensure that a study like Nethercott et al. was performed ethically, the Common Rule requires that: 1) people who participate as subjects in research are selected equitably and give informed and voluntary written consent; and 2) research involving human subjects be reviewed and approved by an independent oversight group such as an Institutional Review Board (IRB). As published, however, the Nethercott et al. study did not contain sufficient information for the Board to adequately determine whether or not the

informed consent process used to enrolled study participants met the standards outlined in §45CFR46. All that is known about the informed consent process is that "all volunteers provided their doctors with written consent to participate in the study" (Nethercott et al. 1994).

Given the paucity of documentation, the Board concluded there was no evidence that the voluntary informed consent process used failed to meet the regulatory and ethical standards applicable to research conducted in the United States and Canada in 1992. All six academic institutions participating in this study had an assurance of compliance with DHHS Regulations for Protection of Human Research Subjects at the time, requiring independent review of the research protocol and consent documents by IRBs. These review boards were expected to approve a study involving human subjects only if: 1) the risks to subjects were minimized by using procedures which were consistent with sound research design and which do not unnecessarily expose subjects to risk; and 2) the risks to subjects were reasonable in relation to anticipated benefits to subjects, if any, and the importance of the knowledge that may reasonably be expected to result (see, e.g., §45CFR46.111). The HSRB believed that it was unlikely that all six of these IRBs would overlook deficiencies in the consent process that would seriously impair the voluntary informed consent of the research subjects.

2) Minimization of Risks to Study Participants

The Nethercott et al. study employed a three-step exposure protocol. Initially, 102 volunteers were screened for hexavalent chromium sensitivity by dermal exposure using a chromium concentration equivalent to the standard dose used in patch testing for skin allergies (4.4 μg Cr(VI)/cm^2). Pregnant women, individuals receiving immunosuppressive or steroid medications, and patients with recent or concurrent dermatological conditions were excluded from study participation. 54 chromium-sensitive subjects were identified by Nethercott et al. These chromium-sensitive subjects then participated in up to two rounds of additional testing. In the first round, subjects were exposed to 0.018 and 0.088 μg CrVI/cm^2 using a skin patch approach. Five subjects developed allergic contact dermatitis to one or both of these lower doses; these subjects were excluded from further testing. Subjects who failed to respond to either the 0.018 or 0.088 μg dose, however, were subsequently exposed to ten-fold higher doses (0.18 or 0.88 μg Cr(VI)/cm^2. 27 subjects developed allergic contact dermatitis to one or both of these higher doses.

In sensitized individuals, chromium exposure elicits an allergic contact dermatitis similar to a poison oak or poison ivy rash. The result typically is an itching, red rash with bumps or blisters; these transient symptoms usually are mild and can be treated with calamine lotion and hydrocortisone cream. The use of patch testing, even when it knowingly results in allergic contact dermatitis, thus meets the generally accepted definition of minimal risk. Furthermore, Dr. Torkil Menne, a consultant to the HSRB, commented that most studies designed to determine the minimum elicitation threshold to a dermal sensitizing agent like chromium have used a single-step protocol in which study subjects were exposed to the entire range of dermal concentrations in a single round of testing. The study exclusion criteria and the use of a three-step exposure protocol, involving initial screening of subjects for chromium sensitivity followed by additional rounds of testing, using doses significantly smaller than those routinely employed for allergy testing and excluding reactive study participants from further exposure, seems designed

specifically to minimize the risk of serious harm to research participants. Thus, the Board believed that there was not clear and convincing evidence that these studies could have resulted in serious harm based on the knowledge available to the investigators at the time.

HSRB Consensus and Rationale

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 the deficiencies did not result in serious harm, nor seriously impair the informed consent of the research subjects.

The Board determined that there was no clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board based these two determinations on its conclusion that this study appeared to have not deviated significantly from the ethical standards prevailing when the study was conducted. However, this conclusion was based, in part, on a process that was hampered by a lack of supporting documentation concerning independent ethical review by the study investigators' home institutions. The Board strongly recommended that for all studies submitted to the HSRB, the Agency make a good faith effort to obtain such documentation in the future.

2. Carbofuran

Charge to the Board

Carbofuran is an *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. Carbofuran can, at sufficiently high doses, lead to a variety of clinical signs. The Agency is conducting acute, aggregate (single chemical, multi-route) and worker risk assessments of carbofuran. In addition, carbofuran 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.

Scientific considerations:

The Agency's WOE document and DERs for carbofuran described the study design and results of a carbofuran human oral study and two human dermal toxicity studies. The WOE document also discusses the Agency's conclusions that these studies were useful in establishing points of departure, both oral and dermal, for the single chemical assessment and in informing the interspecies uncertainty factor for the cumulative assessment.

Please comment on the scientific evidence that supports these conclusions.

Board Response to the Charge

Study Overview

Three separate studies (one oral, two dermal) were carried out with carbofuran in human subjects. The study details are described separately below.

Overview of Oral Study

The oral study conducted with carbofuran was carried out in nine healthy male volunteers using an ascending dose schedule and single doses of 0.05, 0.1 and 0.25 mg/kg (1976). The goal of this study was to determine the threshold for toxicity following a single oral dose. Initially, the study was conducted in an open design (subject and investigator knew that carbofuran was ingested) until a dose level produced symptoms determined to be intolerable (described below). Once the intolerable dose was achieved (0.25 mg/kg), the study was completed in a randomized, double blind manner. Carbofuran was administered as a single dose in a capsule immediately following breakfast, after which subjects remained under observation for 24 hours. Blood samples were collected for analysis of plasma and RBC cholinesterase activity at 0.5, 1, 2, 3, 6 and 24 hours after dosing. The baseline level of RBC cholinesterase activity was established from a predose sample collected immediately prior to dosing. For each subject, additional physiological parameters including ECG, blood pressure, pupil size and accommodation and the Fukuda step test were collected, and subjects were monitored continuously for additional symptoms of toxicity, including sweating, salivation, headaches and nausea and vomiting throughout the 24-hour post-dosing period. A complete clinical chemistry profile was performed predose and at 24 hours. The next highest dose was not initiated until data from the 24-hour post-treatment period were evaluated. Plasma and RBC cholinesterase levels were determined using a modification of the Ellman colorimetric method with propionylthiocholine as substrate. Subjects were allowed to smoke during the 24-hour sample collection period.

After administration of the 0.05 mg/kg dose (2 subjects), no symptoms were noted and RBC cholinesterase activity was decreased by 11 or 22% from baseline (plasma cholinesterase was decreased by 32 and 36%, respectively). Accordingly, the dose was escalated to 0.1 mg/kg (2 subjects). In this leg of the study, one subject exhibited an abnormal vestibular mechanism prior to dosing and showed further deterioration after exposure to carbofuran. This subject also showed changes in cardiovascular parameters including sinus bradycardia and sinus arrhythmia. Two subjects presented with mild symptoms including headache (1 subject) or lightheadedness (the other subject). RBC cholinesterase activity decreased 33 and 31%, respectively, whereas plasma cholinesterase activity was more variable (decreased 56 and 35%, respectively). Based on these results, the dose was escalated to 0.25 mg/kg (2 subjects) where marked symptoms, including drowsiness, nausea, vomiting, headache, salivation, and sinus bradycardia were noted. Accordingly, this dose level was considered to have achieved the level of intolerable symptoms, and an additional 2 subjects were exposed to this level along with one control subject in a double blinded manner. At this dose level, RBC cholinesterase inhibition ranged from 46-63% and plasma cholinesterase inhibition ranged from 33-100%.

Overview of Dermal Studies

The dermal studies conducted with carbofuran (1977 and 1978) involved application of the compound to the backs of subjects for 4 hours. The two studies were similar in design, but differed with respect to the commercial formulations tested and the mass applied per unit area of skin.

The 1977 dermal study (i.e., first dermal study) was carried out as a single, ascending dose study and was designed to determine the threshold for toxicity under conditions of normal and elevated temperatures. Carbofuran was provided in labeled capsules containing 75.4% carbamate powder or placebo. This powder was applied to the backs of each subject over an area described by a paper template and was then mixed with either water, an artificial sweat medium, or normal saline to insure adhesion. Under normal temperature conditions (approximately 70°F and 35% humidity), the doses evaluated were 2, 4, 8 and 32 mg/kg (2 subjects per dose level), whereas under elevated temperature conditions (approximately 90°F and 68-89% humidity), the doses evaluated were 0.5, 1 and 2 mg/kg (2 subjects per dose level). A control group (2 subjects) was included in the high temperature leg of this study. For the high temperature conditions, subjects were also made to exercise by riding a stationary bicycle (5 minutes of exercise followed by 15 minutes of rest) throughout the entire 4-hour exposure period. The parameters outlined above under the overview of the oral study were performed on all subjects in this study.

Under normal temperature conditions, no symptoms were noted at any dose level, and changes in RBC and plasma cholinesterase were variable. RBC cholinesterase inhibition did not exceed 24% (observed at 32 mg/kg). Plasma cholinesterase activity was highly variable, with a maximal inhibition of 33% noted at the 4 mg/kg dose, whereas only 0 or 2 % inhibition was reported in the 2 subjects dosed with 32 mg/kg.

Under conditions of high temperature and humidity, symptoms were observed in the two subjects dosed at 2 mg carbofuran/kg. One subject at this level exhibited severe symptoms (including hazy vision, vomiting, defecation with muscle cramps and chills) and required atropine (at 3 separate times) to ameliorate symptoms. Maximal inhibition of RBC cholinesterase activity at this dose level was 46 and 65% in the 2 subjects (4 hours), whereas plasma cholinesterase inhibition was maximal at 24 hours (12 and 16 %, respectively).

The 1978 dermal study (i.e., second dermal study) was carried out as a single, ascending dose study and was conducted under conditions of elevated temperature and humidity as described above. The carbofuran used in this study was a formulation containing 44% active ingredient and was applied at a concentration of approximately 0.5 mg/cm^2 using a 50% dilution of the formulation. The doses evaluated were 0.5, 1, 2 and 4 mg/kg (2 subjects per dose level). There was no control group. The same parameters outlined above under the overview of the oral study were performed on all subjects in this study.

One subject dosed at 0.5 mg/kg reported nausea after treatment and the other subject noted burning at the application site. In contrast, neither subject dosed with 1 or 2 mg/kg experienced any symptoms. A dose of 4 mg/kg resulted in symptoms of nausea, dizziness and weakness in both subjects, and atropine was administered to these subjects. Inhibition of RBC

cholinesterase activity showed some evidence of dose-dependence but was variable, ranging from 22 and 7% to 61 and 49% in the 2 subjects treated with 0.5 and 4 mg/kg, respectively. Plasma cholinesterase levels were highly variable, with 33 and 46% inhibition observed at 0.5 mg/kg and 6 and 9% at 4 mg/kg, respectively.

Critique of the Oral and Dermal Studies Conducted with Carbofuran

In the three studies described above, the major strength of the work was that the experimental design included the evaluation of at least three dose levels from which dose response relationships could be evaluated. Furthermore, the study outcomes were generally consistent with fundamental principles of xenobiotic disposition including observations that exposure from the oral route likely exceeded that from the dermal route (reflected by the observation of toxicity at much lower oral doses) and that dermal exposure was increased in an environment of increased temperature and humidity. However, in evaluating all of the studies, numerous weaknesses were noted by the HSRB. These weaknesses included:

1) There was no justification or rationale for the selection of doses used in any of the three studies.

2) The sample size was very small (typically two subjects per dose or condition) with few or no controls (no more than two control subjects in any study). Such a design prevented evaluation of statistical significance for any parameter measured in the studies.

3) The values obtained for RBC and plasma cholinesterase levels were highly variable. Factors that contributed to this variability included the small sample size, the inclusion of only a single baseline sample collected immediately prior to dosing used to compare all post-dosing samples, the small number of control subjects, and an uncommon method for analytical determination of cholinesterase activities. The contribution of potential laboratory error cannot be ruled out.

4) Plasma cholinesterase levels were highly variable in all studies so as to preclude any useful interpretation. In general, plasma cholinesterase levels were not consistent with changes in RBC cholinesterase activities.

5) One subject who presented with abnormal vestibular mechanisms in the pre-dose evaluation was used in the oral study and showed serious symptoms after treatment.

6) Subjects were allowed to smoke during the study period.

While the oral and dermal studies shared these common weaknesses, there were also serious limitations regarding the application of carbofuran in the conduct of the dermal studies. In particular, it is known that dermal absorption is influenced by the concentration of compound applied per unit surface area of skin, and it was clear that the studies were extremely different in this regard. For example, as shown in the Table 1 below in the first dermal study (high temperature/humidity), the mass loading range was 6,000 to 12,000 µg carbofuran/cm^2. These extremely high loading levels were not appropriate for evaluating potential dermal absorption

from occupational or environmental exposure to carbofuran. In the first dermal study, the greatest skin surface area treated in the normal temperature leg of this study was 40 cm^2; a mass of 3,264 mg was applied to this area, equivalent to a loading of 81,600 µg carbofuran/cm^2. In contrast, mass loading was controlled to achieve approximately 500 µg carbofuran/cm^2 at all dose levels in the second dermal study.

Table 1. Calculation Of Loading Levels For Carbofuran For Subjects In The First Dermal Study (High temperature/humidity conditions)

Subject	Dose (mg/kg)	Body Wt (kg)	Mass (mg)	Template (cm^2)	Loading (ug/cm^2)
1	0	63	0		
2	0	65	0		
3	0.5	72	36	6	6,000
4	0.5	66	33	5.72	5,769
5	1	74	74	8.55	8,655
6	1	64	64	7.94	8,060
7	2	74	148	12.16	12,171
8	2	78	156	12.49	12,490

A primary deficiency of the first dermal study was that it did 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 carbofuran (up to 3,000 mg) were applied to a relatively small skin surface area (6-40 cm^2) in the experiments, whereas we typically see much larger skin surface areas exposed to smaller amounts among workers (e.g., 1-10 µg/cm^2). For example, the hands, a skin surface commonly exposed to pesticides, have a total surface area of 990 cm^2 (EPA Exposure Factors Handbook, 1997). Dermal dosing studies require careful consideration of three factors: mass applied to the skin, surface area treated, and the duration of exposure. Therefore, the skin loadings and skin surface areas exposed in both carbofuran dermal studies were not appropriate for determination of a NOAEL or a LOAEL for risk assessment purposes.

HSRB Consensus and Rationale

The EPA concluded that the oral and dermal studies conducted with carbofuran in human subjects were useful in establishing points of departure, both oral and dermal, for the single chemical assessment and in informing the interspecies uncertainty factor for the cumulative assessment.

However, while these studies were informative, the HSRB concluded that there were numerous technical issues regarding the conduct of the oral and dermal studies with carbofuran and that overall, the weakness of the studies far outweigh the strengths. The weaknesses included the small sample size, the lack of control subjects, the highly variable results for RBC cholinesterase activity and the improper dermal loading used in the dermal studies. Accordingly,

the HSRB did not recommend any of the oral or dermal studies conducted with carbofuran in human subjects for the single chemical assessment or in informing the interspecies uncertainty factor for the cumulative assessment.

Additional Considerations: Potential For The Carbofuran Human Studies Data

The Board provided additional analysis in response to the Agency's charge to the Board concerning the potential for the data in human subjects for carbofuran to be applied to: (1) the calculation of a benchmark dose (BMD_{10}) and identification of the BMD_{10L} (lower confidence limit); (2) the identification of a NOAEL or LOAEL for effects or (3) the comparison to other species for possible adjustments to uncertainty factor for the cumulative assessment.

The HSRB provided the following additional perspective relative to the Agency's question:

The utility of the human studies with carbofuran was limited by the very small sample size used in all of the studies. The Agency proposed to use the RBC cholinesterase data for determination of the $BMDL_{10}$. However, under conditions where the group size was only two, it would be imperative to have highly accurate, valid, reliable and consistent measures of RBC cholinesterase activity in both control and carbofuran-treated subjects. This rigor was simply not achieved in the human studies. Rather, RBC cholinesterase activities were compared to a single baseline value, were highly variable across subjects, including controls, and did not show any consistency with plasma cholinesterase levels. As such, although EPA scientists calculated a $BMDL_{10}$ from the time course of changes in RBC cholinesterase values in the nine subjects evaluated in the oral study, the HSRB concluded that the accuracy and reliability of this calculation was limited by the technical shortcomings noted for the study. Therefore, the HSRB reiterated its recommendation that the $BMDL_{10}$ calculated by the Agency from the human data should not be used.

In a similar manner, the small sample size, compounded by the lack of consistent changes in cholinesterase activities in all studies, the inappropriate methods used for dermal application of the compound in the dermal studies and the inclusion of at least one subject who presented with abnormal vestibular function in a pre-dose assessments limited the general utility of the data. Collectively, the weaknesses in the conduct and outcomes of the carbofuran human studies cast doubt on the utility of the data for identifying a NOAEL or LOAEL or for comparing across species in consideration of the interspecies uncertainty factor for the cumulative risk assessment. Thus the majority of HSRB members agreed the human oral data should not be used to identify a NOAEL or LOAEL, and there was unanimous agreement that the human dermal data should also not be used for these evaluations

Charge to the Board

Ethical Considerations

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

Oral Toxicity Study:

Is there clear and convincing evidence that the conduct of the human oral study conducted with carbofuran was fundamentally unethical?

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

Dermal Toxicity Studies:

Is there clear and convincing evidence that the conduct of either of the human dermal studies conducted with carbofuran was fundamentally unethical?

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

Board Response to the Charge

Study Overview

There were three studies involving either oral or dermal administration of carbofuran: an oral toxicity study performed in 1976 (IRB Review dated March 31, 1976; Final Report dated September 17, 1976); a dermal toxicity study performed in late 1976 and early 1977 (IRB Review dated August 25, 1976; Final Report dated March 18, 1977); and a second dermal toxicity study conducted in late 1977 (REC Review date unknown; Final Report dated February 15, 1978).

The location for the research was the Quincy Research Center in Kansas City, Missouri. All three studies were under the direction of a single principal investigator, John D. Arnold, MD. The research appeared to have been performed under contract to the Midwest Research Institute, also located in Kansas City. The responsible institutional review board was the Community Review Committee, Inc., again located in Kansas City. The research sponsor was FMC Corporation, located in Philadelphia, Pennsylvania with the manufacturing facility apparently located in Middleport, New York.

No ethical or regulatory standards were mentioned in any of the study documents. Given the dates of the research studies, Section 12 of FIFRA applied to the research. In addition, the 1975 version of the Declaration of Helsinki was available at the time.

Critique of Studies

The following comments apply to all three studies.

1) The fact that these studies have never been published should not be used as the sole criterion to determine whether the purpose of the research was to obtain generalizable knowledge. Publication is neither a necessary nor sufficient criterion of whether or not the research was designed to allow for either a descriptive or causal inference.

2) The risks were minimized by the study design (setting aside the actual conduct), assuming that there was a valid scientific purpose in escalating the dose until achieving a "lowest observable adverse effect level" (LOAEL). Examples of the procedures that were incorporated to minimize risk included the presence of a supervising physician who was readily available for 24 hours after dosing, confinement of the subjects for 24 hours, abstinence from alcohol during the study, the exclusion of other drugs within two weeks of performing the study, the availability and administration of atropine (discussed further below), and a delay in dose escalation (at least in the oral toxicity study) until the 24 hour clinical data was available. In addition, subjects only received the active compound once during each research study.

3) Measurements of RBC cholinesterase inhibition should serve as an adequate surrogate measure of toxicity, obviating the need to induce clinical signs and symptoms of cholinergic toxicity. The question however in judging these three studies was whether this standard was either appreciated or applicable in 1976 and 1977. The fact that the research was designed to cause clinical signs and symptoms of cholinergic toxicity as the study endpoint does not, in and of itself, establish that the interests of the subjects did not prevail over other interests. The Common Rule allows for the balancing of the risks of research against the knowledge that may reasonably be obtained. The central question then was whether the risks were reasonable, not whether the research was designed to elicit clinical toxicity.

4) With respect to informed consent, the list of signs and symptoms of cholinergic toxicity found in the consent documents was fairly complete. The consent documents were fairly straightforward about the fact that the testing involved a pesticide and that the research would be of no benefit to the subject. The freedom to withdraw was emphasized, along with the fact that additional testing to ensure the safety of subjects would be requested by the supervising physician. In spite of these strengths, the consent documents failed to provide a description of the study design (i.e., dose escalation) and the anticipated endpoint of clinical toxicity. The phrase "we do not expect any serious complications" is clearly open to interpretation. Some would and some would not consider the clinical signs and symptoms of cholinergic stimulation "serious." Regardless, the phrase does introduce a framing of these stated risks as "non-serious." Given the research design, the consent documents would have been improved if they had been explicit about the dose escalation, the place of the specific subject within that dose escalation, and the fact that someone would eventually have a 100% chance of experiencing clinical toxicity. Although these changes are an admirable standard going forward, the consent documents used for the oral and first dermal toxicity study met (and some might argue exceeded) the standards prevalent in 1976 and 1977. However, as discussed below, the consent document for the second dermal toxicity study was seriously deficient.

The Board had specific comments about the conduct of each of the studies that can be addressed under the general topic of the reasonableness of the risks (and the efforts to reduce those risks) that the subjects experienced in the conduct of this research.

1) Was it appropriate to expose additional subjects, in the oral toxicity study, to a dose which had already been shown to cause clinical toxicity if the scientific purpose was to establish a LOAEL? Given the criticism of attempting to determine a "no observable adverse effect level"

(NOAEL) using a small sample size, the design chosen in these three studies to elicit a LOAEL may be more reliable. However the small sample size, when combined with the variability and unreliability of the RBC cholinesterase measurements, undermine confidence that the study was designed to establish the real LOAEL. The repeat administration of the test substance absent dose escalation was used in other cholinesterase inhibitor studies, but the endpoint driving the decision to not escalate dosing was the more sensitive endpoint of the degree of RBC cholinesterase inhibition.

2) There was documentation (in a letter dated October 26, 1976) of the decision to start at the 2.0 mg/kg dose in the low-temperature and low-humidity phase of the first dermal toxicity study. Although the responsible IRB was not consulted (for which there were no procedural guidelines in 1976), was the decision to bypass the 16 mg/kg dose in favor of a 32 mg/kg dose in the low temperature and humidity phase of the dermal toxicity study reasonable? If the dose-response relationship based on the percent RBC acetyl cholinesterase inhibition was linear, yet the onset of clinical signs and symptoms reflects a threshold response, this decision could have placed the subjects given the higher dose at greater risk even though, in retrospect, the 32 mg/kg dose was well tolerated.

3) The administration of atropine as an antidote to cholinergic toxicity may have been delayed for one or more of the subjects in the high-temperature and high-humidity phase of both dermal toxicity studies. Although mention was made of written instructions for the administration of atropine, these instructions were not included in the submitted documentation. The question then was whether there could be any justification for the delay in the administration of atropine. Two possible justifications might be: (1) the signs and symptoms were from non-muscarinic cholinergic receptors and thus would not be responsive to atropine (which was not the case); or (2) the supervising physician was concerned that any resulting tachycardia or other side-effects from the administration of atropine would be of greater risk (highly unlikely). After considerable reflection, the Board could find no scientific or clinical reason to delay the administration of atropine.

4) Study participants were not fully informed of the risks of the study. It should have been clear to study investigators that the escalating dose design used was likely to result in serious harm to some research subjects. For example, several participants who received a 2.0 mg/kg dose of carbofuran during the high-temperature and high-humidity phase of the first dermal toxicity study exhibited clear clinical signs and symptoms of carbamate poisoning, requiring administration of atropine as an antidote. Plasma and red cell cholinesterase inhibition data also was obtained from these individuals, with participants demonstrating 46% and 65% peak red cell inhibition respectively. In the subsequent second dermal toxicity study, however, the data from the first dermal toxicity study were not used either to develop clear stopping criteria or to modify the dosing protocol, thus exposing study participants to an unacceptable level of risk. The two participants in the high-temperature and high-humidity second dermal toxicity study who received a 2.0 mg/kg dose of carbofuran did not exhibit any clinical symptoms of carbamate poisoning. These individuals did, however, exhibit peak red cell cholinesterase inhibition of 40% and 42% respectively, similar to the level of inhibition seen in one of the symptomatic participants in the first dermal toxicity study. These data suggest that the LOAEL for carbofuran was at or near 2.0 mg/kg. Nevertheless, the decision was made to expose

two subjects to a dose of 4.0 mg/kg carbofuran, once again resulting in severe clinical symptoms indicative of carbamate poisoning and requiring administration of atropine as an antidote. In light of the clinical and biomarker data obtained from the first dermal toxicity study, it should have been obvious to study investigators that exposure of additional research subjects to a dose of 4.0 mg/kg carbofuran was likely to have resulted in serious harm to these two study participants.

This conclusion, coupled with the observation that the consent documents from the second dermal toxicity study explicitly stated study investigators "[did] not expect a serious complications" raises serious questions about the informed consent process. At least some study participants were likely to experience clinical signs indicative of carbamate toxicity. To imply otherwise in the informed consent documents suggests that the consent process was severely flawed. Study participants were denied access to information that might have influenced their decision to voluntarily enroll in the second dermal toxicity study.

HSRB Consensus and Rationale

Oral Toxicity Study

For the oral study, there was no evidence that the study failed to fully meet specific ethical standards prevalent at the time the research was conducted.

There was no clear and convincing evidence that the research was fundamentally unethical (e.g., 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.

Dermal Toxicity Studies

The HSRB found deficiencies in both dermal human toxicity studies relative to specific ethical standards prevalent at the time the study was conducted.

The majority of the Board concluded there was no clear and convincing evidence that the research was fundamentally unethical (e.g., intended to seriously harm participants or that informed consent was not obtained). In light of the results obtained from the first dermal toxicity study, one Board member concluded that the second dermal toxicity study was fundamentally unethical in design. The Board member believed that this study was neither designed to minimize the risk of serious harm to participants nor to ensure an adequate informed consent.

For both dermal toxicity studies, there was clear and convincing evidence of significant deficiencies in the ethical procedures for minimizing risk that could have resulted in serious harm (based on the knowledge available at the time the study was conducted). The first dermal toxicity study was significantly deficient given the delay in the administration of atropine to

more than one subject experiencing the signs and symptoms of carbamate toxicity. The second dermal toxicity study was considered significantly deficient by Board members in that the lack of information provided about the results from the initial dermal toxicity study seriously impaired their informed consent.

3. Methyl Isothiocyanate (MITC)

Charge to the Board

MITC is an irritating compound that has a limited animal database for toxicity via inhalation, the key route of exposure. MITC can be used as a pesticide directly to treat wood poles, but the major pathway of exposure to MITC is from degradation of several fumigant pesticides (i.e., metam sodium, metam potassium, and dazomet). Due to its volatility, MITC has the potential to move off-site, which can result in exposure to bystanders near treated areas and, through ambient air, to people far away from treated areas. Use of the soil fumigants also results in exposure to those handling the pesticides or working in treated fields.

Scientific considerations

The Agency's WOE document and DER for MITC describe the study design and results of the MITC odor threshold and eye irritation human studies. The WOE document also discusses the Agency's conclusions that the eye irritation study is useful for the assessment of potential effects on bystanders and workers from exposures to MITC during acute (1-day) intervals. The Agency had concluded that the odor threshold study is less useful than the eye irritation study for assessing the human health effects of MITC, since the odor detection threshold for humans is higher than the level that causes eye irritation. The Agency had decided, however, to use the results of the eye irritation study for assessing the inhalation exposure of MITC.

Please comment on the scientific evidence that supports this conclusion.

Board Response to the Charge

Introduction

MITC is the primary and key degradate of these fumigant pesticides (i.e., metam sodium, metam potassium and dazomet). As a gas injected into soil, it can kill soil-borne pests, such as insects, microorganisms, weeds, and nematodes. The fumigant dissipates from the soil in a few days to a couple of weeks.

According to the EPA Weight of Evidence (WOE) document (USEPA 2006b) "The mode of toxic action for MITC is not known at this time. MITC is primarily an irritating compound that produces non-specific systemic effects in oral toxicity studies such as changes in body weight, food consumption, and hematological parameters. Following air exposures to MITC, consistent effects are observed in rats and humans. For example, clinical signs and pathological changes of the respiratory tract consistent with an irritant have been observed in

laboratory studies in rat. Humans exposed to MITC complain of symptoms such as itchy and burning eyes, rash and burning skin, nausea, scratchy throat, salivation, coughing, and shortness of breath. In acute toxicity testing with animals, MITC is considered Acute Toxicity Category I (corrosive) for skin and eye irritation." The animal studies were either long-term inhalation or oral studies and their use would be less protective of human health. Therefore, the Board recommended the eye irritation LOAEL as a point of departure.

Brief Overview of Study

The EPA WOE extracted a description of the odor and eye irritation study (Russell and Rush 1996) directly from the Risk Characterization Document for MITC of the Department of Pesticide Regulation, Cal EPA (July 25, 2003, pp 53-59), which was considered accurate and quoted herein.

"In order to determine the NOEL for human eye irritation produced by MITC vapors, as well as its odor threshold, human volunteers were exposed to air concentrations of MITC in a laboratory setting (Russell and Rush, 1996). The study specifically focused on assessing these parameters at different times of exposure. An olfactometer was used which permitted the operator to dispense the test material through a manifold system. The test material could thus be diluted over a 100-fold concentration range. The material was dispensed by diffusion from a glass vessel which could be maintained at any temperature ±0.1°C over a range of 30 to 70°C. A Total Hydrocarbon Analyzer (THA) was used to monitor the flow of test material during the exposure period. In addition, carbon tube samples were drawn once the system was equilibrated prior to exposure, and at the end of the exposure. The test material was desorbed from the carbon and analyzed by gas chromatography. Every effort was undertaken to minimize the reaction of the test material with the tubing and other equipment used in the delivery system".

"In the olfactory threshold study, 33 individuals (16 males, 17 females) with a mean age of 25 years (range, 18 to 34 years) were tested. They were exposed to three positive control odorants, pyridine, acetic acid, and n-butyl alcohol as well as to MITC. The technician chose the odorant and concentration level. The odorant was dispensed in double blind fashion from one of three presentation ports. The subject was responsible for identifying from which of the presentation ports the odorant was dispersed. A 30-second rest period between exposures was permitted in order to allow the subject to recover prior to the next exposure. The operator tested each subject over the range of concentrations for each odorant until he was assured that the threshold had been adequately ascertained. A standard procedure was employed in order to make this determination."

"In the NOEL determination for eye irritation, the olfactometer was modified by attaching goggles to the presentation line. This permitted the test material to be directed only to the eyes. Five parameters were used to ascertain an irritation response: 1. the subjects' subjective estimation of irritation (using the "Likert" scale); 2. photographs of the subjects' eyes prior to and after exposure; 3. blink rate as measured by electromyography; 4. effect upon visual acuity; 5. tear production. Both a positive control (acetic acid) and a negative control (air) were employed. Baseline responses for each of the assessment parameters were determined under pre-exposure conditions ("zero-time controls") and upon exposure to the negative control ("air-only

controls") for the prescribed period. A positive irritation response was based on three criteria: 1. the average response must be quantitatively greater than the pre-exposure response; 2. the average response must be greater than pre-exposure and greater than could be expected statistically from individual to individual differences within the group; 3. the average treated response must be greater than the air-only group's response and greater than could be expected from individual differences observed within the group. Seventy individuals (38 males, 32 females) with a mean age of 32 years (range, 18-67 years; median age, 28 years) were exposed to air, MITC, and/or acetic acid. Between 9 and 16 subjects were examined under each dose/time period combination. Three exposure periods, 14 minutes, 4 hours and 8 hours were used. In the eight hour test, subjective responses, blink rates and tearing were assessed at 0, 1.5, 3, 3.5, 6 and 8 hours (tearing was not measured at 3.5 hours). Two 15-minute rest breaks and a 30-minute lunch break were permitted during the 8-hour period. In the four hour test, these same parameters were assessed at 0, 1, 2, 3 and 4 hours (tearing was not measured at 0, 2 and 3 hours). In the 14-minute exposure protocol, subjective responses and blink rates were measured at 0, 1, 4 and 14 minutes after the start of exposure. Tearing was measured at 14 minutes only. Visual acuity and ocular morphology were assessed at the beginning and end of each exposure period. All analyses were performed in a double-blind manner." T-tests were used to compare responses at each computed concentration level for each time period to both air control results and zero-time results. Both were significant and positive but responses to the control substance were not as dramatic.

Critique of the Study

Introduction

Table 2 shows what the investigators called the NOEL, which the Agency's DER and WOE call the NOAEL and LOEL respectively (EPA's RfC methodology document included eye, nasal, and throat irritation in its list of adverse effects).

Table 2. Summary Of MITC Eye Irritation Effects From Human Subjects

Exposure time	NOAEL (ppm)	LOAEL (ppm)	Source of observed Effect
1 minute	3.3	-	-
4 minutes	0.6	1.9	Subjective eye irritation
14 minutes	0.6	1.9	Subjective eye irritation
1 hour	0.23[a]	0.8	Subjective eye irritation
1.5 hours	0.22[a]	-	-
2 hours	0.23[a]	0.8	Subjective eye irritation and blink rate
3 hours	0.23[a]	0.8	Subjective eye irritation and blink rate
3.5 hours	0.22[a]	-	-
4 hours	0.23[a]	0.8	Subjective eye irritation
6 hours	0.22[a]	-	-
8 hours	0.22[a]	-	-

[a]The slightly different values obtained at the low dose NOAEL level (0.22 and 0.23 ppm) reflected the fact that they were derived from tests performed on different days.

As the WOE stated "Exposure to 0.8 ppm (800 ppb) MITC resulted in a statistically significant positive response based on averaging the subjective assessments by the subjects using the Likert scale methodology. As many as 8 out of 9 subjects showed a positive response at 1 and 2 hours, the first two time points examined [and also at 3 & 4 hours]. Shorter exposures to 0.6 ppm did not result in statistically significant Likert scale changes, though 1 of 9 individuals appeared to respond at 4 and 14 minutes. Exposure to 1.9 ppm or 3.3 ppm MITC for 4 or 14 minutes resulted in positive subjective responses at 4 and 14 minutes. At 1 minute of exposure, levels as high as 3.3 ppm did not evoke a statistically significant positive response."

"Mean blink rate determinations at 0.8 ppm were statistically significantly increased at the 2- and 3-hour time points compared both to air-only and zero-time controls. Statistical significance was not achieved at 1 and 4 hours, though a positive response was indicated in several individuals. The blink response to 0.6 ppm and 1.9 ppm at 1, 4 and 14 minutes did not show a positive response. At 3.3 ppm, statistical significance was achieved at 4 and 14 minutes. " The Board agreed with the Agency's conclusion that "A strong suggestion of a response was also present at 1 minute, though it was not statistically significant." In addition, the subjective (Likert scale) responses were the most sensitive and most variable. The eye blink rate was the next most sensitive. The other tests were not as sensitive and usually were not significant.

The Board agreed with the Agency conclusions as noted in their DER:

"• For a one-minute exposure, the NOAEL for eye irritation is 3.3 ppm due to a lack of response in any parameter tested."

"• For exposures 4-14 minutes, the NOAEL for eye irritation is 0.6 ppm based on responses on the Likert subjective scale at 1.9 ppm. "

"• For exposures of 1-8 hours, based on the statistically significant subjective (Likert scale) responses at 0.8 ppm MITC at 1-4 hours and the statistically significant eyeblink responses at 2 and 3 hours, 0.22 ppm was designated as the NOAEL for this study. The NOAEL for eye irritation was consistent for the 1-8 hour measurements. It is reasonable to assume that exposures up to 24 hours would likely yield a similar response."

Finally, in terms of the olfactory threshold study, the Board agreed with the Agency's conclusion that "The observed odor threshold for MITC ranged from 0.2 to 8 ppm with a geometric mean of 1.7 ppm."

Strengths of the study

The studies were well-designed, equipped, carefully controlled and performed by experienced investigators at a respected institute. The lowest concentration tested was the largest sample size. Exclusion criteria were appropriate: abnormal irritation, contacts, frequent headaches, recent asthma attacks, and pregnancy.

Weaknesses of the study

The eye irritation studies did not have a sufficient number of subjects in each of the experiments and phases. In addition there was no information on the susceptibility status of individuals tested nor information on within subject variation. Another shortcoming is that eye irritation does not predict dermal nor respiratory effects. Thus, there may be lower NOAELs for these latter effects.

Two-tailed t-tests were used to compare the responses of subjects receiving different doses of MITC despite the presence of substantial skew in the data of some groups, with some standard deviations exceeding the corresponding means. This was most common among the subjects receiving the lower doses, an issue of particular concern insofar as the goal of the study was to identify a NOAEL. A nonparametric test would have been a more appropriate choice. In addition, because responses were measured repeatedly on the same subjects over time, a statistical approach that took this into account would also have been more appropriate than the series of independent t-tests that were carried out.

The investigators were rather rigid in their approach to the interpretation of p-values. For instance, a group difference for which the p-value was 0.052 was not considered evidence of an effect. On the other hand, the investigators clearly stated their criteria for interpretation and applied these rules consistently. Moreover, inspection of the tables indicated that the conclusions reached would not have differed even if a somewhat more liberal criterion of statistical significance had been applied.

This issue does raise a more general concern relating to the size of the study sample. The investigators provided no rationale for the sample size that was used nor power calculations, despite the important influence that sample size has on whether a group difference reaches some level of statistical significance. The inclusion of a small number of additional subjects in the different groups could well have caused some of the borderline p-values to fall to a level that would have met the investigators' criteria for significance and, potentially, change the inferences drawn, as demonstrable by re-calculations of significance. Thus it is important that one could be confident that the sample size was adequate for the assessment of the study hypotheses. Ideally, the investigators should have begun by specifying the magnitude of the response that they consider meaningful and want to be able to detect, should it exist (e.g., a 50% increase in the response, a doubling of the response, etc). Then, after making additional assumptions, they could calculate the number of subjects that would be necessary. As stated, this was not done.

HSRB Consensus and Rationale

The Board concluded that air concentrations of methyl isothiocyanate sufficient to produce eye irritation would lead to a conservative and prudent point of departure for inhalation risk (i.e., eyes were a sensitive endpoint in relation to the respiratory system). The Board reached its decision based on the observation that eye irritation LOAELs are often lower than respiratory irritation LOAELs for irritant gases (WHO 1979ab, NRC 1986; WHO/EURO 1986). While the use of eye irritation data as a surrogate for respiratory data is reasonable in this situation, one must be cautious as only appropriate controlled human studies of the respiratory system can provide a final and definitive respiratory point of departure, if ever determined (NAS 1975).

Charge to the Board

Ethical considerations

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

a. Is there clear and convincing evidence that the conduct of the human eye irritation study with MITC was fundamentally unethical?

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

Board Response to the Charge

Brief Overview of the Study

The human eye irritation study was conducted in 1993 through 1995. The study was performed in Davis, California by researchers at the Sensory Testing Laboratory, School of Medicine, University of California, Davis, together with the Western Research Center of Zeneca Ag Products, Richmond, California. The study sponsor was the Metam Sodium Task Force

(representing chemical manufacturers), whose mailing address is in care of Zeneca Ag Products of Wilmington, Delaware. The documents provided by the sponsor specifically state that the research was conducted in compliance with the Declaration of Helsinki (presumably the 1989 version, though no date is specified) and the Human Subject's Bill of Rights (a provision of California law). The study was reviewed and approved by the Human Subjects Review Committee at the University of California, Davis, an institution which held a Multiple Project Assurance with the U.S. Department of Health and Human Services. The documentation provided by that Committee indicated that it reviewed this study pursuant to the standards of the Common Rule (45 C.F.R. Part 46, Subpart A) and determined it to be in compliance with that Rule.

The Board's comments only relate to the human eye irritation study and not to the human odor threshold study conducted by the same group of investigators. Consistent with the charge presented to the Board by the EPA, the Board made no comments with regard to the human odor threshold study.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the human eye irritation study, as detailed in USEPA (2006c). The Board concurred with the Agency's conclusion that although there were deficiencies with regard to the applicable ethical standards prevailing at the time this study was conducted, those deficiencies were relatively minor. In addition to the deficiencies specified in USEPA (2006c), the Board wanted to comment on two additional aspects of the study:

1. The Human Subjects Review Committee asked the investigators to add a provision to the protocol and the consent form indicating that "if significant irritation is experienced, no higher dose will be administered." The revised protocol never provided any specific criteria for determining how it would be determined whether a subject was experiencing significant irritation. It was appropriate that such stopping rules be relatively specific, if possible.

2. The original protocol for the eye irritation study involved exposing subjects to MITC for a series of two-minute periods, with twenty-minute breaks between each exposure. In the study investigator's memorandum to the IRB dated August 17, 1994, requesting renewal of the protocol, the investigator indicated that he had apparently finished conducting at least part of the study as initially described, and that it was "going well without any ill effects." He submitted a protocol amendment so that he might study the effects of longer exposure to MITC (up to eight hours at a time). In the document submitted to the EPA describing the results of this series of studies, however, no data were provided as to the results of the short-term study. On page 26 of the submitted documents, which outlines when subjects were exposed to this agent and for what periods of time, there was mention only of the 8-hour, 4-hour, and 14-minute exposure periods. The tables accompanying the report only gave details of the results of those longer exposure periods. Since the longer exposures were premised on the favorable results from the short-term exposures, it would have been appropriate for the report to have also included details relating to the results from the short-term (two-minute) trials. The absence of such details makes it difficult

to determine any ethical irregularities that might have been revealed by such additional information.

HSRB Consensus and Rationale

The Board concluded that:

There was no clear and convincing evidence that the conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent).

There was no clear and convincing evidence that the conduct of the study was significantly deficient relative to the ethical standards prevailing when the study was conducted.

The Board based these two determinations on its conclusion that this study, based on the evidence presented, appeared to have had only relatively minor deviations from the ethical standards prevailing when the study was conducted.

COMMENTARY ON SCIENTIFIC STANDARDS FOR HUMAN DOSING STUDIES

The Chair asked the Board to articulate the set of scientific standards that has and will continue to guide Board decision-making for human dosing studies. Following Board deliberation, scientific standards for human dosing studies in general and for single dose studies in particular were adopted.

Scientific Standards for Human Dosing Studies

1. Justification

- Is the scientific question worthwhile?
- Are human subjects necessary to answer the question?
- Is potential risk serious or irreversible?

2. Dose Selection

- Sufficient to test the question? (single dose in most cases is not sufficient to determine NOAEL and LOAEL)
- Based on appropriate data (e.g. preclinical; previous studies)

3. Endpoint Selection

- Consistent with the aim of the study?
- Appropriate to answer questions about human responses (e.g., sensitivity, accuracy, validity, replicability)?
- Measured accurately and reliably with good quality assurance?

- Participants
- Characteristics generalizable to question asked?
- Appropriate inclusion/exclusion criteria?
- Are measurements taken at appropriate times to answer the study question?

4. Method

- Is the sample size sufficient?
- Is selection of control and experimental groups appropriate?
- Is the staging of dose intervals, dose amounts, and type of exposure sufficient to answer the question?
- Is there quality assurance for observations, instruments and data?

5. Statistical Analyses

- Can data be statistically analyzed?
- Is the statistical method appropriate to answer the question?

<u>Scientific Standards for Single Dose Level Study</u>

Board definition of single dose level study - individual study that uses one dose level other than a control or placebo and irrespective of the number of subjects or frequency of dosing.

1. In general, single dose level studies have limited utility

- Such studies cannot be used in isolation to establish a NOAEL or LOAEL
- In rare instances they may have utility if interpreted within the context of one or more supplementary studies that provide information at other dose levels under analogous conditions.

2. Single dose level studies may be able to answer a very focused question

- However in such instances its utility will depend upon the robustness of study design, the rationale for the study and whether the study design was consistent with the rationale.
- Evaluation of robustness will include questions of: control, relevant endpoints, evidence that measures can identify an adverse effect or detect a change, use of a surrogate marker that is quantifiable and recognized as an established function of the compound and other criteria for scientific validity.

3. A single dose level study may have utility if it provides evidence of adverse effects observed at lower levels than other studies have indicated.

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