UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

January 26, 2005

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held December 2, 2004 on the Use of Pharmacokinetic Data to Refine Carbaryl Risk Estimates from Oral and Dermal Exposure

TO: James J. Jones, Director
Office of Pesticide Programs

FROM: Joseph E. Bailey, Designated Federal Official /s/
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Larry C. Dorsey, Executive Secretary /s/
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Joseph J. Merenda, Jr., Director /s/
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on December 2, 2004. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to the use of pharmacokinetic data to refine carbaryl risk estimates from oral and dermal exposure.

Attachment

cc:

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OPP Docket

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SAP Meeting Minutes - 2004-02

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Use of Pharmacokinetic Data to Refine Carbaryl Risk Estimates from Oral and Dermal Exposure

December 2, 2004
FIFRA Scientific Advisory Panel Meeting
held at the Holiday Inn Rosslyn at Key Bridge
Arlington, VA

NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation of use.

The FIFRA SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested parties are invited to contact Joseph E. Bailey, Designated Federal Official, via e-mail at bailey.joseph@epa.gov.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency as well as information presented in public comments. This document addresses the information provided and presented within the structure of the charge by the Agency.

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held at the Holiday Inn Rosslyn at Key Bridge
Arlington, VA

Mr. Joseph E. Bailey
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: 1/26/2005

Steven G. Heeringa, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date: 1/26/2005

Federal Insecticide, Fungicide and Rodenticide Act
Scientific Advisory Panel Meeting

December 2, 2004

Use of Pharmacokinetic Data to Refine Carbaryl Risk Estimates from Oral and Dermal Exposure

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific and technical issues being considered by the Agency pertaining to its review of the Use of Pharmacokinetic Data to Refine Carbaryl Risk Estimates from Oral and Dermal Exposure. Advance notice of the meeting was published in the *Federal Register* on October 19, 2004. The review was conducted in an open panel meeting held in Arlington, Virginia, December 2, 2004. The meeting was chaired by Steven G. Heeringa, Ph.D. Mr. Joseph E. Bailey served as the Designated Federal Official.

The Agency received a proposal from Bayer Crop Sciences to use pharmacokinetic data about carbaryl to refine risk estimates from oral and dermal exposure to carbaryl from use on residential turf. The proposal offered a refined approach to calculating a margin of exposure based on target tissue concentrations. The purpose of this SAP meeting was to evaluate whether comparison of internal doses in target tissue is a useful way to refine carbaryl risk estimates, and to evaluate the approach used to estimate brain concentrations from intermittent exposure using pharmacokinetic data. Kitt Farwell, D.V.M. (Health Effects Division, Office of Pesticide Programs) provided an introduction and highlighted the goals and objectives of the meeting. Michael E. Krolski, Ph.D. and Mr. Curt Lunchick (Bayer Crop Sciences) provided clarifying comments on a number of issues and questions the Panel had regarding Bayer's proposal to refine carbaryl risk estimates. Mr. Joseph J. Merenda, Jr. (Director, Office of Science Coordination and Policy) and Randolph Perfetti, Ph.D. (Associate Director, Health Effects Division, Office of Pesticide Programs) offered opening remarks at the meeting.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency, as well as information presented by public commenters. This document, especially the response to the Agency's charge, addresses the information provided before and presented during the meeting.

PUBLIC COMMENTERS

Oral statements were made by:

Jennifer Sass, Ph.D., Natural Resources Defense Council

No written statements were provided.

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

The Panel supported the pharmacokinetic studies conducted by Bayer Crop Sciences in an effort to investigate the premise that pharmacokinetic data can be used to refine estimates of risk from oral and dermal exposure to carbaryl. Further, it was agreed that the margin of exposure (MOE) can be more appropriately estimated based on an internal dose metric in a toxicologically relevant target tissue. However, there was disagreement with regard to what the dose metric should be. Some Panel members strongly indicated that the internal dose metric should be based on cholinesterase (ChE) inhibition rather than the concentration of any single anticholinesterase agent.

The studies presented were designed to provide data pertaining to peak brain carbaryl from acute exposures for the proposed refinement of toddler exposure to carbaryl from treated lawns. However, due to the focused nature of the design of the studies, limited pharmacokinetic data were generated regarding the actual peak concentrations of carbaryl in the brain, significantly impacting the reliability of the proposed alternative MOE calculations.

Some Panel members raised the question of whether peak carbaryl level in the brain is the sole reasonable measure for carbaryl acute toxicity. Therefore, evidence should be given to support the presumption that peak level in the brain is a sensitive and most appropriate metric for representing the acute toxicity of carbaryl. Some Panel members expressed concerns about the lack of information on the toxicity and pharmacokinetics of the metabolites. However, others believed the short-term toxicity of such metabolites was very unlikely to impact the acute toxicity risk assessment being considered. The Panel was also concerned that the understanding of the metabolism and distribution of carbaryl itself may be incomplete. Some data or discussion to clarify these apparent discrepancies could lend support for the proposed metabolic pathways.

The Panel commented on the dermal absorption of carbaryl with regard to the surface area of application, delivery method and vehicle, and the choice of experimental model for comparison to humans. However, the conclusion reached from these studies was that the peak level of carbaryl obtained from the oral dose would be much larger than that from the dermal absorption contribution. Thus, the prolonged and delayed peak dermal absorption measurements in the mixed-dose study were not expected to significantly contribute to the peak level that was used in the revised MOE calculation.

The Panel considered it important to implement the concept of using pharmacokinetic data to establish risk limits. Peak carbaryl levels in the brain should only be used as a comparison with the total absorbed dose approach. It was agreed that it is necessary to obtain a valid half-life for carbaryl in the brain and plasma if the results from modeling are to be considered acceptable. The proposal was weakened by the lack of complete understanding of the pharmacokinetics of carbaryl in the brain and the lack of identification of the true peak level. The Panel discussed using area under the curve (AUC) for carbaryl in the brain as an alternative to peak brain levels of carbaryl.

However, experimental data revealed that the AUC for carbaryl was not equivalent to total dose. The Panel also discussed an alternative use of measurement or model-based estimation of peak brain ChE inhibition rather than carbaryl level and establishment of the correlation between both endpoints. Despite some limitations in the current data set, the currently available data provide a base from which a more refined pharmacokinetic modeling process and results that support the validity of the approach can emerge. For an improved carbaryl risk assessment it was recommended to clearly separate the three modeling components, namely exposure, pharmacokinetics, and pharmacodynamics, and to use the most appropriate model for each component.

PANEL DELIBERATIONS AND RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background documents, references and Agency's charge questions.

Charge Question 1 - Design of Pharmacokinetic Studies

A series of pharmacokinetic and metabolism studies were completed that serve as the basis for the proposed approach associated with children's exposure to carbaryl after lawn treatments. These studies included dosing rats via several routes (i.e., oral, dermal, and intravenous). In a subsequent study, carbaryl was administered to rats via the oral and dermal routes simultaneously at exposure levels similar to those calculated in the Agency's deterministic exposure assessment for toddlers playing on treated lawns.

(1A) Please comment on the design of these experiments with respect to the usefulness of results to estimate peak tissue levels for risk assessment purposes.

The majority of the Panel supported the design of these studies; however, they were concerned about the lack of identification of the actual peak level of exposure. Basic pharmacokinetic (PK) studies should provide sufficient data to derive a set of PK parameters for any compound, including peak concentration, area under the curve (AUC), and estimation of various values of half-life (e.g., half-life at target sites and half-life of systemic elimination).

The Panel commented on the peak carbaryl levels that were presented. They noted that the actual peak level in the oral study was likely achieved before the time at which the first measurement was made, i.e., 15 minutes after dosing. While it is unlikely that the actual peak level occurred prior to 5-10 minutes after exposure, with a half-life on the order of 15 minutes, the observed peak carbaryl levels in the brain reported in this study may be significantly lower than the actual peak levels. This is an important issue since the peak carbaryl level was the starting point for the proposed margin of exposure (MOE) calculations.

The Panel expressed concern that the available evidence was not sufficient to support the presumption of peak carbaryl level in the brain as the most appropriate metric representing acute toxicity of carbaryl. Partial AUC (e.g., AUC to the Cmax or peak level) for carbaryl could also be considered. While dermal absorption appeared to affect AUC for carbaryl, the delayed and low peak levels from dermal absorption suggested no significant contribution from dermal absorption to the peak brain levels used in the revised MOE calculation.

Other general comments pertain to the general quality of the data and the lack of complete data analyses. The set of data on the level of carbaryl in the brain after oral dosing was incomplete. This deficiency hindered an accurate determination of the

elimination rate and half-life of carbaryl. Considering the decline in carbaryl levels after oral dosing, it appeared that the plasma half-life of this agent might be as long as one hour. However, assuming first-order kinetics of elimination, the brain accumulation of the chemical suggested that the half-life might be as short as 15 minutes. For modeling exercises, a valid half-life value for carbaryl in both brain and plasma are required. The Panel raised concern with the apparent inability to measure brain carbaryl levels due to the level of sensitivity of the mode of detection. To assist in any conclusions drawn from a data set, a more accurate representation of the population data, including measures of variance, should always be accounted for and reported.

The Panel members had differing opinions on whether peak carbaryl level in the brain is a reasonable measure for acute carbaryl toxicity. The brain, as opposed to the blood, is considered a relevant target tissue and offers the possibility of comparing chemical levels and ChE inhibition. The peripheral nervous system can also be considered a potential target tissue; however, ChE activity in this tissue is technically difficult to assay. Peripheral nervous system tissue was collected for ChE determination, and the Panel encouraged the Agency to consider evaluation of correlations with the central nervous system between the peak carbaryl levels and ChE activity. In general, peak carbaryl level is a less biologically relevant parameter than the measurement of peak ChE inhibition. However, such measurements may be technically difficult and highly variable with a rapidly reversible carbamate such as carbaryl. Data on ChE inhibition, ChE activity, and the time course of tissue concentration might help clarify the question of how peak carbaryl level would correspond to ChE inhibition. A correlation analysis of ChE inhibition and toxicity endpoints (i.e., cholinergic signs and symptoms) to the tissue level of the active form of the chemical, in this case, parent compound, would be very valuable.

Some Panel members expressed concerns about the lack of information on the toxicity, pharmacokinetics, and peak levels of the carbaryl metabolites such as the hydrolysis product. The Panel thought it was important to consider the toxicity of metabolites, although it is highly likely that, for the ChE inhibition endpoint, none of the metabolites are nearly as potent as the parent chemical, carbaryl. Some members of the Panel raised questions regarding possible contribution to the overall toxicity by minor metabolites, including epoxides and quinone intermediates that may inhibit/modify the activity of critical molecular targets. Although >90% of the total radioactive residue (TRR) was recovered, less than 50% of the components were identified.

Other concerns were that the understanding of the metabolism and distribution may be incomplete. Data suggested that metabolism (or at least, elimination rates) might vary with respect to dose (page 4 of Bayer report). Lower doses led to a shorter half-life and faster loss of detectable ChE inhibition. Given the possibility of underestimation of peak or plateau tissue levels, the differences with respect to dose should be considered. It was also of concern that, according to the report, hydroxymethyl carbaryl was found in the brain after oral and i.v. exposure but not in the mixed-dose study, in which only 1-naphthol sulfate was detected. Some data or discussion to clarify these apparent discrepancies could lend better support for the proposed metabolic pathways.

Dermal Exposure Study

The Panel made several comments on the experimental designs and observations related to dermal exposure. The absorbed dose from a dermal exposure depends on the application area of the administered dose. Often, the percentage of the administered dose that is absorbed increases as the application area increases. This effect was observed in the dermal absorption studies of carbaryl, in which, during a 24-hour exposure period, 3% was absorbed from an administered dose of 3.46 mg cm^{-2} compared with 25% absorbed from an administered dose of 0.0356 mg cm^{-2}. Similar results were observed at shorter exposure times. As a result, extrapolating the measured percent absorption values to smaller administered doses (in terms of mass per area) could significantly underestimate the actual absorption and the consequent potential risk.

To translate the dermal internal dose measurement from the mixed-dose study in rats to children, some Panel members suggested that the administered doses should be comparable on both a weight and an area basis. The administered dose in the rat mixed-dose study was 0.225 mg, which corresponded to 0.871 mg kg^{-1} on a weight basis and 0.0174 mg cm^{-2} on an area basis (i.e., the dosing area was 1.0 in x 2.0 in = 12.9 cm^2). The equivalent administered dose in a 15-kg child would be 13 mg and would cover an area of 750 cm^2. If the same 13 mg of carbaryl were distributed on an area larger than 750 cm^2, then the internal dose arising from dermal absorption could be larger than estimated from the rat mixed-dose study. If this occurred, the relative importance of dermal and oral absorption could be greater than was measured in this study. Given the delay associated with dermal absorption, however, the peak concentration from the oral dose would still probably be larger than from the dermal absorption contribution.

Panel members also commented on the dermal application method and suggested that dermal application should take into consideration the absorption of carbaryl or components from the vehicle into the application material. A few Panel members raised the concern that the application method used in these studies may have reduced the effective administered dose below the intended administered dose. Overall, some level of confirmation of the availability and contamination of dose should be provided. Specifically, carbaryl was applied to a water-resistant adhesive bandage in 0.4 mL of a mixture of acetone and water and the acetone was allowed to evaporate in open air for two minutes. Carbaryl absorption from acetone has been shown in other studies to be less than that seen from aqueous vehicles (Baynes and Riviere, 1998). Confirmation of the loss of acetone and the availability of the entire administered dose (not bound to bandage material) was not conducted. The potential problems as raised by a few Panel members are as follows:

(1) Any carbaryl absorbed into the bandage coating may be released more slowly. This could possibly contain acetone that partitioned along with carbaryl into the polymeric bandage coating prior to evaporation.

(2) Carbaryl may interact with the components of the bandage, including the gauze and polymeric coating, and this may reduce the bioavailability of the administered dose and the rate of dermal absorption.

Consideration of dermal contact to carbaryl by children was raised as an issue in the methodology used in the experimental animal model. In most exposures, children would be exposed to carbaryl residue rather than in an aqueous medium. One Panel member raised the concern that the absorption of such compounds applied directly to skin can sometimes be larger than from an aqueous solution. In addition, the use of water as a vehicle for the lipophilic compound, carbaryl, as delivered with occlusion, would maintain an elevated hydration level of the exposed skin and this may alter absorption.

Some Panel members suggested that whenever compounds that undergo significant dermal metabolism are under investigation, a simple in vitro human skin diffusion study using fresh viable tissue (Bronough methods) is good for a validation comparison to animal skin. Therefore, any animal model for skin permeation should be compared to human skin since it is relatively easy to do and, in most cases, provides reliable results.

(1B) The design of the multi-route study was intended to mimic the concurrent oral and dermal exposures of toddlers playing on treated lawns. Please comment on this approach.

The concurrent administration of carbaryl through two oral doses at a one-hour interval and one dermal dose over two hours was carried out to mimic the plasma and brain levels of carbaryl possible in human toddlers from exposure to treated lawns. The protocol is appropriate for examining possible pharmacokinetic interactions between carbaryl given by two different routes of administration. In comparison to oral and i.v. dosing, the dermal exposure experiments provide data to suggest that this additional route of exposure, while offering slow absorption and possibly not significantly contributing to the peak level, may contribute to the longer term internal exposure levels. Since the peak is less defined and pronounced from dermal exposure, the contribution to brain levels of carbaryl from dermal absorption is then considered negligible compared to the contribution provided by oral exposure in the mixed-dose approach. However, because the half-life of the ChE inhibition is considerably longer than that of carbaryl in the brain, this conclusion of a negligible contribution from the dermal exposure needs to be reevaluated in light of the much slower reactivation rate of the carbaryl-inhibited ChE. Considering the presently available facts, there is some likelihood that ChE inhibition from the dermal exposure may make some non-trivial contribution to peak ChE inhibition, depending on the relative mass transfer of carbaryl by the oral versus dermal routes.

In the experimental rat model, oral exposure was accomplished by gavage of 2 oral doses. As would be expected, in the rat, the peak level is both higher and achieved more rapidly after the two bolus doses, as compared to smaller doses given more frequently. Evaluation of this data with regard to the mixed-dose study should take into

consideration the half-life of this compound when evaluating the contribution from each exposure route. The mixed-dose study confirmed what could be predicted based on the single route studies. It also provided a third data point for the correlation between TRR and carbaryl brain level. The Panel suggested that the Agency consider further data comparison between the single route and mixed-dose study regarding data consistency and validation. This can be done by using the information from the single route study to model the results of the mixed-dose study and compare them to the actual results measured from the mixed-dose study. This exercise also could reveal any dose-related differences in pharmacokinetic patterns.

Regarding information from the mixed-dose study, it was noted that dermal absorption might significantly contribute to plasma levels at later time points. Similar concerns were raised of the mixed-dose study as were addressed in Question 1A with regard to dermal exposure. It was noted by a few Panel members that dermal absorption rates are usually normalized by body surface areas. A critical variable is the percent of body surface area dosed in rats compared to a typical exposure in humans. In this and any experimental animal model the exposure area is a fixed component that needs to be considered in any extrapolation to human exposure. The dermal exposure experiments compared to oral and i.v. dosing experiments are important to demonstrate the slow absorption from dermal exposure. The results indicated that an additive effect took place to produce the composite brain level curve showing a rapid peak and slower decline because of the slower absorption of the dermal dose. Given the results observed in the mixed-dose study, it seems appropriate to use data from single oral and dermal doses given separately to animals and then to model the brain levels over time of a combined oral and dermal dose regimen.

The Panel noted several aspects of differences between the mixed-dose study and toddler exposure to a lawn treated with carbaryl. It was recognized that, for the single oral dosing studies, fasting before dosing would give a conservative estimate of peak target dose, given a slower absorption due to contents in the digestive tract. However, the slower absorption could also mean that peak carbaryl level from oral (hand-to-mouth action) and dermal exposure could possibly overlap in time, especially when, in the child, the exposure may be the result of multiple hand-to-mouth deliveries occurring over a period of time. It was noted by one Panel member, however, that not all hand-to-mouth activities would result in exposure. A member of the Panel raised the alternative approach of chemical delivery via intragastric cannulation that would allow for repeated chemical delivery over a specific period of time. However, this would limit the delivery to the stomach and would bypass other sites of chemical absorption involved in oral exposure, such as buccal absorption.

The Panel noted that these studies are not designed to form the basis for derivation of a factor to translate biomonitoring data to peak concentrations. They also do not account for human genetic diversity (polymorphisms, etc.), address chronic exposures, nor account for indirect transfer (from toys to mouth). Moreover, the pharmacokinetic data obtained in 7-week rats (young adults) have limited usefulness for modeling the exposure of human toddlers due to the current inability to adequately

describe the pharmacokinetics (absorption, distribution, metabolism, elimination) of young children.

A few Panel members raised the issue that no information is available to support the assumption that the rat responds to an acute exposure from carbaryl in a way that is similar to human toddlers. The value of the present data on the pharmacokinetics and pharmacodynamics of carbaryl in rats for purposes of risk assessment is limited by design flaws in the experiments from which the data were obtained. It was suggested that to utilize such data in the manner outlined by EPA might actually hinder the progress being made by this and other studies toward a more scientific approach to risk assessment.

Charge Question 2 - Pharmacokinetic Approach

Historically, risk assessments completed by the Agency have been based on comparison of endpoints associated with total administered dose levels from toxicology studies with daily human exposure. The proposed pharmacokinetic approach presented in this paper instead relies on the use of peak internal dose at the target tissue. Because of the rapid pharmacokinetics and pharmacodynamics of carbaryl, a more appropriate dose metric may be the use of peak target tissue levels for calculating exposure estimates instead of total daily absorbed dose values.

(2A) Please comment on the appropriateness of using peak levels for estimating exposure.

While the Panel agreed that having some internal target tissue measurements of a compound aids in the estimation of exposure, additional dose metrics can be considered in addition to or as alternatives to peak levels. For example, the peak carbaryl level in the brain depends upon several experimental factors including timing of sampling. The Panel expressed concerns about this lack of accuracy in the determination of the peak level, compounding its further concerns on the appropriateness of this dose metric in isolation.

Measurements of carbaryl concentrations were available from the animal bioassays of the two Bayer studies (Metabolism Study and Mixed-Dose Study) for two oral doses (1 mg/kg/d and 10 mg/kg/d), two i.v. doses (1 mg/kg/d and 10 mg/kg/d), two dermal doses (20 mg/kg and 100 mg/kg) and one combination experiment using two oral doses (0.084 mg/kg) separated by one hour in combination with one dermal dosing (0.871 mg/kg) over two hours. The concentrations in the brain, red blood cells, whole blood and plasma were measured at 15 and 30 minutes, and at 1, 4, 6, 12, and 24 hours after start of treatment. Except for the experiments with dermal application, the observed carbaryl concentrations decreased from the first measurement taken after 15 minutes. Based on the data derived from the oral and i.v. studies, it was postulated that carbaryl concentration was maximal at the 15-minute time point, although no measurements were recorded during the preceding interval. Therefore, the actual peak levels in these studies may not have been identified because they could have occurred earlier than the 15-minute

time point and would have been at least as high or higher than the level reported at 15 minutes.

Peak Level as Exposure Metric

The Panel agreed that properly identified peak levels should not be excluded as a potential dose metric. It was also pointed out that in the recent International Agency for Research on Cancer risk assessment for formaldehyde (Cogliano et al., 2004), using data of the National Cancer Institute cohort of occupationally exposed persons (Hauptmann et al., 2003 and 2004), peak levels were used as a critical exposure measure to evaluate the concentration-response relationship concurrently with cumulative concentration, average dose intensity and duration of exposure. In this occupational cohort study, the most pronounced concentration-effect relationship was obtained between leukemia specific mortality and the estimated peak level of formaldehyde exposure. The Panel also noted that in its discussion of the cumulative risk analysis of the carbamate family in December 2003, the FIFRA SAP had considered the length of time above a pre-defined level of ChE inhibition as a toxicity endpoint. In that case, however, it was the response level (ChE inhibition) for which the maximum level was investigated and not the dose measure (level of cholinesterase inhibitor itself). Thus, these are qualitatively different situations.

The Panel agreed that the use of the peak carbaryl level could potentially be appropriate, but it was not yet of proven value for estimating exposure. Using peak levels of carbaryl to estimate exposure is a useful first step; however, issues were raised as to how this would estimate toxic effects. In the current model, without additional data about the predictive validity of peak level of carbaryl and subsequent ChE inhibition, use of the peak level of carbaryl itself may be misleading given its short half-life in the brain (< 15 minutes) relative to the longer half-life of ChE inhibition (>1.7 hours). If the peak level should be used as one component in further exposure assessments, it will be important to measure this level accurately for the target tissue(s) of interest, as well as fully understanding the dose metrics as a function of route of exposure. This does not appear to have been accomplished with carbaryl in the Bayer study, although extrapolation from half-life or a full PK modeling approach with the data obtained might give a reasonable estimate of the true peak level. The lack of an actual peak level and other kinetic information limits the Panel's enthusiasm for this approach, and its use in this case would not identify the critical exposure metric.

A major issue raised by the Panel was whether or not carbamate levels are adequate to address the biological effects of these inhibitors. Such effects will depend on many other factors, including the difference between the half-life of carbaryl and the half-life of ChE inhibition. Modeling of ChE inhibition was suggested as an important component to be included in this risk assessment. One needs the data to extrapolate from measures of exposure to estimate the peak potential for ChE inhibition in the target tissue. For that purpose, one must allow for the possibility that inhibition will cumulate for a longer period and reach a peak much later than the compound itself, given that the half-life for inhibition is about 8 times longer than the compound's elimination and redistribution half-life.

Peak carbaryl concentrations in the brain should only be used as a basis for comparison with approaches using the total absorbed dose. Using peak carbaryl levels for risk assessment and, in particular for exposure assessment, may seem justified. However, Bayer's proposal to do so is seriously weakened by remaining uncertainty regarding the pharmacokinetics of carbaryl in the brain, by the dubious means used to calculate MOE from peak levels in the brain, and by questionable conversions of urinary 1-naphthol concentrations.

A discussion was initiated by a few Panel members regarding the use of AUC for carbaryl in the brain as an alternative to peak level. This alternative dose metric would, however, also need further exploration. One would expect the AUC for carbaryl to be equivalent to the total dose; however, the experimental data provided showed some discrepancy.

Further Pharmacokinetic Considerations on the Appropriateness of Peak Levels for Estimating Exposure

The Panel agreed that it is important to implement the concept of using pharmacokinetic data to establish risk limits. Despite some limitations in the current data set, these data on carbaryl do establish the general validity of the pharmacokinetic approach. With better data on peak level and a consideration of the kinetic effects of carbaryl on its enzymatic target, cholinesterase, this model would improve.

From a pharmacokinetic perspective of predicting carbaryl concentrations for use in extrapolating across studies, the use of peak internal dose at the target tissue is an appropriate metric in a risk assessment for a compound such as carbaryl with a short half-life, low mammalian toxicity, and rapidly developing ChE inhibition as the primary endpoint. This may not hold for other compounds or other endpoints, for example, more chronic effects, where the area under the curve at the target tissue is an acceptable exposure metric. Additional constraints are placed on the design of experiments due to the need to determine size and timing of a peak if the time course is broadened in the human exposure scenario. Sampling times have to be clustered around the anticipated peak concentrations for an accurate estimate of this metric. In the pharmaceutical arena, peak levels or fractional AUCs are often used for rapidly acting drugs, and a number of studies have also determined the difficulty in reliably estimating when this peak occurs. If total carbaryl residues are the metric, these may not reflect active carbaryl delivered to the tissues.

Because of the rapid PK/PD of carbaryl, it is plausible to use a peak level of carbaryl at one of the target sites. However, when using peak level in a target site such as the brain, which has limited accessibility, it is unlikely that peak carbaryl levels would have practical use in exposure assessment. Therefore, an MOE based on peak carbaryl levels will need to be modified before it can be used for comparisons with biomonitoring data.

Fundamentally, it makes more sense to focus on the biological event of brain ChE inhibition rather than peak levels of carbaryl. For carbaryl this is particularly true due to the chemical's very short half-life relative to the longer half-life (2-3 hrs) of the ChE inhibition. Thus, the longer half-life for ChE inhibition would mean the possibility of a greater cumulative effect on this physiological endpoint than is seen in the level of chemical in the brain. Such effects, as calculated by a simple model, are illustrated in Figures 1 and 2. These figures show the effect of a 1.7-hour half-life on the accumulation of an "index of cholinesterase inhibition" over 2- and 8-hour periods of repeated dosing. The index of cholinesterase inhibition is simply constructed as the accumulation of carbaryl level present at any time, reduced by degradation using whatever reversal rate is deemed appropriate.

Various Panel members raised a number of issues for the Agency's consideration, such as additional methods to approximate peak levels of carbaryl in tissues, identifying correlation between peak level of carbaryl in the brain, and subsequent level of ChE inhibition as a measure of biological effect. Given the attempts to model exposure of children on treated lawns, the use of dermal and oral routes of exposure was a logical first step. In order to evaluate these data, it would be of interest to know how additional exposures from other sources would influence the level and location of the peak for carbaryl in the brain.

(2B) This pharmacokinetic approach assumes that toddlers put their hands in their mouths at a rate of 20 times an hour for 2 hours. A laboratory-dosing regimen that exactly mimics this toddler behavior is impractical. As such, oral doses were administered in the multi-route rat study once per hour for 2 hours. The proposed approach uses an algorithm to adjust the results for 2 hourly bolus doses to that of a toddler, which occurs 20 times per hour. Given the rapid metabolism of carbaryl, please comment on whether this algorithm can be reasonably used to predict the expected pharmacokinetic behavior of carbaryl.

(2C) To convert the four 24-hour time periods in the biomonitoring study to a shorter time period and to account for plateau tissue concentrations, Bayer proposed extrapolating results from the rat mixed-dose study to the biomonitoring study in this manner. Because the margin-of-exposure calculated using estimated plateau brain concentrations was approximately 20-fold greater than the margin-of-exposure calculated using EPA's SOPs for Residential Exposure Assessment, Bayer proposed multiplying results from the biomonitoring study by an adjustment factor of 20. Please comment on whether this approach is appropriate for extrapolating from results in the rat pharmacokinetic study to the biomonitoring study.

Question 2B addresses the mixed-dose study. Conducted on a single group of rats, this study was designed to mimic toddlers exposed orally to a total of 0.15 mg/kg/d over two hours and simultaneously exposed dermally to a total of 0.75 mg/kg per day. In the rat, the oral dose was administered as two oral bolus doses of 0.0841 mg/kg each and

the dermal dose was administered continuously over 2 hours with 0.871 mg/kg (corresponding to 0.0174 mg cm^{-2}) applied with the bandage.

Basically, the proposed pharmacokinetic analysis used first-order kinetics, resulting in the linear equations and calculations as presented in the documents. Although simple, this is a modeling approach that is subject to the normal standards of good pharmacokinetic modeling practice. It should be clearly stated at the outset that the approach to mixed-dose exposures and their extrapolation to toddlers used deterministic calculations throughout. In order to assess the validity of the model, one must clearly define the model and the underlying assumptions. Based upon the report and the presentation the following assumptions appear to have been made:

1) Under real life conditions with human toddlers, hand-to-mouth events occur every three minutes, resulting in 40 doses over a 2-hour exposure period. Each dose, therefore, would be equivalent to 0.15 mg/kg/ 40 = 0.00375mg/kg. This dose is below the detection limit of the assays used to measure carbaryl in the brain, but its contribution to brain concentration was estimated from the data of the mixed-dose study combined with the oral low-dose and high-dose studies. From these data, using a three-point interpolation and an assumed log-log relationship: log carbaryl = -1.0516 + 1.23 log oral dose, the incremental step in the brain carbaryl level was calculated to be 0.000091 ppm.

2) The half-life of the carbaryl in the brain was estimated to be 15 minutes (from the alpha portion of the decay curve), and the half-life of TRR was estimated to be 19 minutes using information from the literature.

3) Using the addition-subtraction rule (every three minutes 0.000091 ppm can be added, while every minute 0.025 of the total is subtracted), the plateau value of carbaryl was estimated to be 0.011 ppm. This value was then used for MOE calculation.

Quality of the Modeling Approach

The Panel judged this modeling approach as one that is at once both oversimplified and too detailed, applied to a different species (rat) in a very different context (specific oral-dermal dosing over time). The large amount of guesswork in this approach diminished confidence that the results can offer anything to the risk assessment of carbaryl. A more realistic model is needed to simulate how exposure occurs, accounting for hand-to-mouth behavior; and contact with grass, toys and pets outside as well as inside the house. However, it does seem appropriate to model the toddler's exposure at 20 occurrences per hour from data on exposure once per hour for two hours. It is even appropriate to model it from a single exposure, provided that the basic pharmacokinetics is understood. In particular, one needs to know precisely what is the half-life and whether the half-life is stable over a wide dose range, i.e., that it follows true first-order kinetics. Secondly, a validated means of extrapolating from such information is required to calculate the cumulative and peak levels of ChE inhibition, based on the half-life of the carbamylated enzyme itself.

The lack of pharmacokinetic data for carbaryl throughout the report, the proposal and other documents provided to the Panel was believed to be a substantial problem. Also, only two pharmacokinetic endpoints were reported: the half-lives and the peak levels of carbaryl in different specimen samples as obtained by either a simple mathematical calculation or observation. Such derived half-lives vary with administered doses for the same route of carbaryl administration. Unless the pharmacokinetics of carbaryl are dose-dependent, this variation raises the question of how robust are the animal studies. A full pharmacokinetic analysis would provide a set of results with a greater level of confidence.

The Panel drew the inference that the present study was conducted in the attempt to add an exposure scenario onto the pharmacokinetic model. This, however, resulted in opening the whole model to the criticism of being oversimplified. A better approach, as referenced by one Panel member during this meeting, would be the approach planned for discussion by the SAP during the subsequent meeting on December 3, 2004 on cumulative risk assessment. This approach separates the exposure scenario modeling from the applied dose-to-effect component which, in and of itself, needs a full PBPK/PD model.

If one looks at the mixed-dose study as a multi-route study, its usefulness comes in understanding whether interactions exist in the pharmacokinetics of oral versus dermal exposure; not in trying to mimic an exposure scenario. For an improved carbaryl risk assessment, the Panel recommends that the three components of exposure, pharmacokinetics and pharmacodynamics be separated and that the most appropriate model be used for each component.

The log-log dose-response projection to low doses used in the empirical modeling approach is not justified mechanistically. One Panel member proposed to assume that the detoxification process, probably taking place in the liver, follows saturation, Michaelis-Menten type kinetics. That would more appropriately model any non-linearity in the carbaryl brain level versus administered dose. EPA has, in fact, used this type of analysis for a cumulative dose/exposure study of organophosphates (US EPA. 2001). A similar analysis should be done with carbaryl, and estimates of uncertainty should be derived using the confidence limits on the underlying raw data of the study. It should be noted that plots of the data with simple log versus simple linear models do not show that a strict linear interpretation can clearly be rejected (see Figures 3 and 4 below), and some low dose linearity is strongly implied by pharmacokinetic theory. Both the apparent non-linearity at the high dose and the low dose linearity can be modeled using the Michaelis-Menten framework, allowing integration of the information on brain levels of carbaryl and observations of apparently shorter half-lives for carbaryl as a function of dose.

Statistical Variability and Uncertainty

All presentations of data should include information about measurement of variations, e.g., error bars representing some number of standard errors or geometric standard errors depending on the statistical form of the spread of the individual measurements from central values. Half-life is more variable than was assumed (see

figures in Table 4 of the EPA background document for this meeting dated December 2, 2004). The dependence of the outcomes on the linear kinetic is obvious and needs to be addressed in an uncertainty analysis. There exists intra-assay variability (between aliquots) as well as inter-animal variation, neither of which was considered in the extrapolation and the calculation of the refined MOE.

Some Panel members raised a concern about the use of specific half-life determinations in these calculations. It was pointed out that accumulation is usually conducted using the slowest terminal half-life and that the "alpha" half-life is calculated after the slower terminal elimination process has been subtracted. All of this assumes dose linearity under a first-order concept that was not evident in the non-linearity data presented from the Bayer study.

When addressing the mixed-dose design, it was noted that there are complexities in comparing a 20-times exposure per hour ingestion of carbaryl for two hours in humans (essentially a dose every three minutes) with a once-hourly dosing of carbaryl in rats. If one assumes repeated dose delivery every 3 minutes and a brain half-life of 15 minutes, then accumulation would occur with this dose regimen until a plateau is reached, as illustrated in the simulation presented by Bayer. This basic approach is sound, and is often used with parenteral pharmaceutics. However, when this dosing scenario is applied to an oral route of exposure, the rate of administration may not be rate limiting. In that case, the rate of gastric emptying may modulate the rate of absorption whether absorption occurs from the stomach or intestines. Such effects are highly species dependent. Data presented after a single dose indicate peak absorption at 15 minutes, which suggests gastric absorption. However, this early peak could represent an initial gastric dumping due to the formulation used. Repeated dosing may modify these kinetics. Also, it should be noted that gastric emptying is sensitive to food and fluid consumption. Use of such a finely graded dosing regimen may not be appropriate for a process as variable as oral absorption. A further concern about this extrapolation is how rat and human gastrointestinal absorption times compare, as this extrapolation is critical to the pharmacokinetic dose accumulation approach. In any case, the effect of fasting on metabolism and on the concentrations measured should be explored more fully.

Refined MOE

Overall, the Panel held that when EPA's standard MOE approach is abandoned, and much larger MOE values are obtained, there is a compelling need to know which elements of modeling are driving this change. As it now stands, there are very large uncertainties in the newly postulated factors of 70 or 20 derived from the biomonitoring study.

Caution should always be exercised when extrapolating from urinary metabolites to parent compound in blood and between very different compartments. The current MOE conversion, as presented, is oversimplified, and does not have enough supporting data. A revised MOE of 20 might be appropriate if one were merely estimating peak

tissue level of the active compound. However, if one is concerned with peak levels of ChE inhibition, then this value is probably inappropriate as, in general, one should not lose sight of ChE inhibition as a presumed common (shared) mechanism of toxicity. Therefore, an extrapolation factor of 20 may not be acceptable. Furthermore, an MOE of 20 does not match EPA's existing model using total exposure. It is important to look at factors within each model to determine the basis for the discrepancy in MOE for these models rather than using a simple "adjustment factor".

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Figure 1

Modeled Brain Carbaryl Concentration and a Modeled Index of Cholinesterase Inhibition for Small Doses Repeated Every Three Minutes--2 Hour Plot

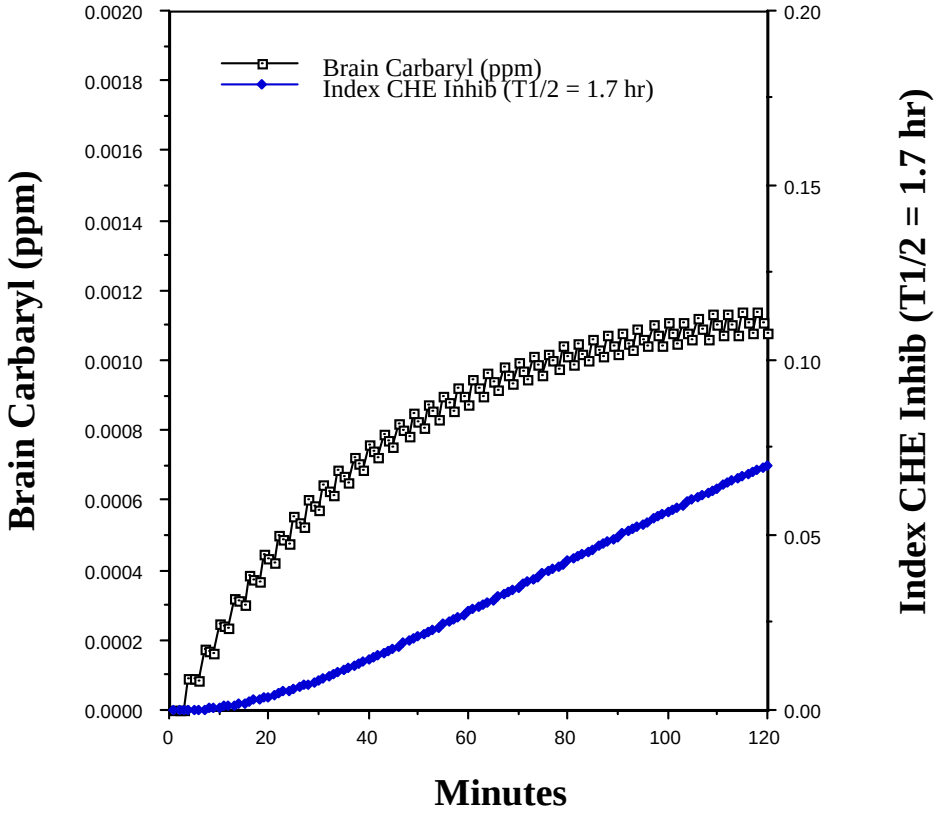

Figure 2

Modeled Brain Carbaryl Concentration and a Modeled Index of Cholinesterase Inhibition for Small Doses Repeated Every Three Minutes--8 Hour Plot

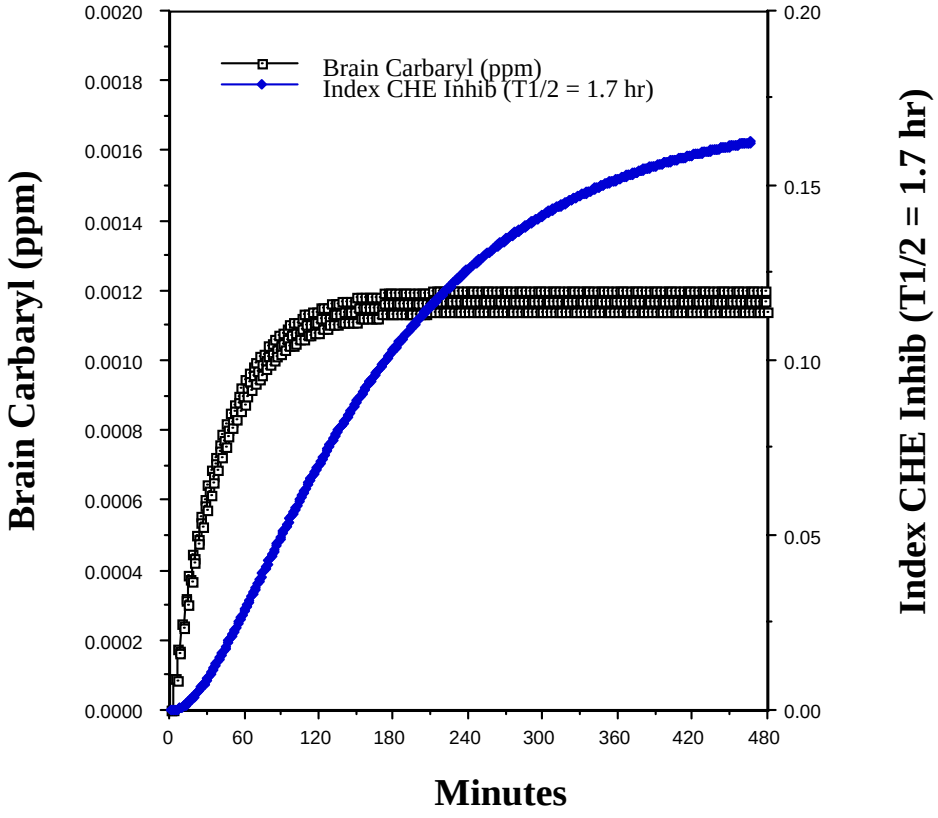

Figure 3

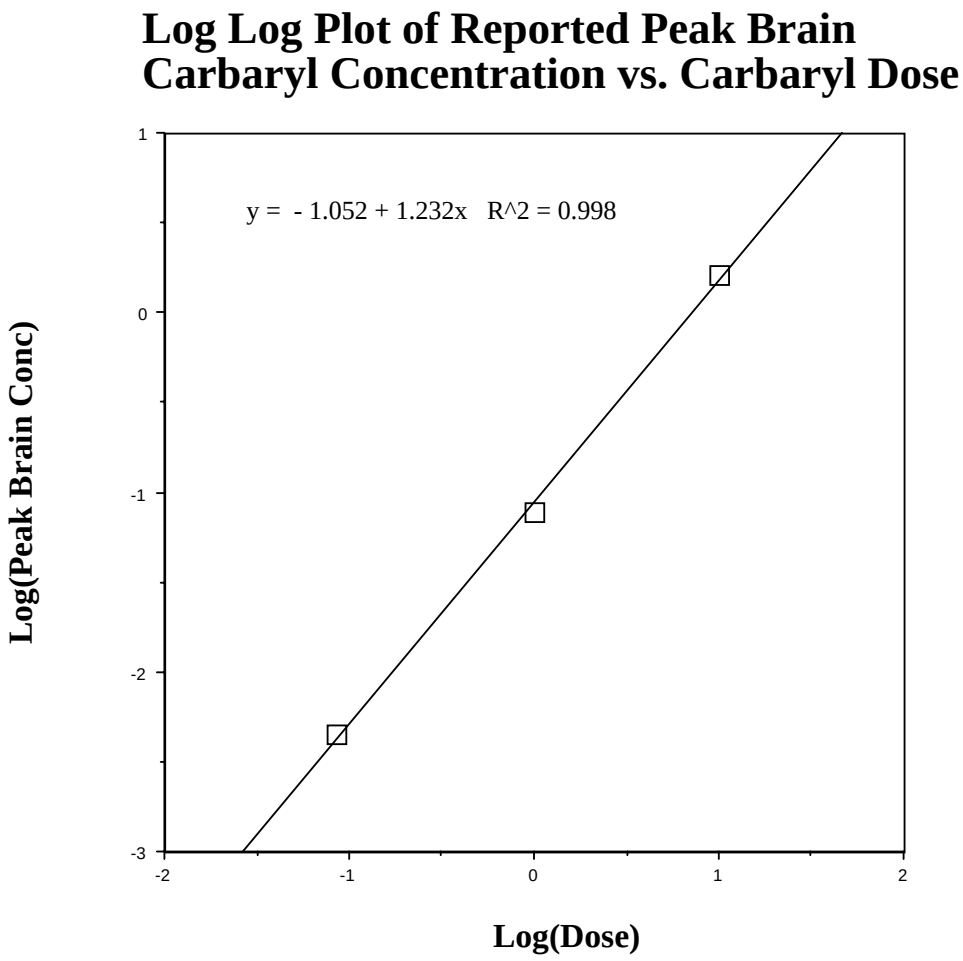

Figure 4