SAP Minutes No. 2003-06

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

Physiologically-based Pharmacokinetic/Pharmacodynamic Modeling: Preliminary Evaluation and Case Study for the N-Methyl Carbamate Pesticides: A Consultation

December 11 and 12, 2003
FIFRA Scientific Advisory Panel Meeting,
held at the Sheraton Crystal City Hotel,
Arlington, Virginia

NOTICE

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The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and was established under the provisions of FIFRA, as amended by the Food Quality Protection Act FQPA of 1996. The FIFRA SAP provides 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 persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

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

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Myrta R. Christian, M.S.
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: March 1, 2004

Christopher Portier, Ph.D.
FIFRA SAP, Session Chair
FIFRA Scientific Advisory Panel
Date: March 1, 2004

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
December 11 and 12, 2003

Physiologically-based Pharmacokinetic/Pharmacodynamic Modeling: Preliminary Evaluation and Case Study for the N-Methyl Carbamate Pesticides: A Consultation

PARTICIPANTS

FIFRA SAP, Session Chair

Christopher J. Portier, Ph.D., Director, Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Designated Federal Official
Myrta R. Christian, M.S., FIFRA Scientific Advisory Panel Staff, Office of Science Coordination and Policy, EPA

FIFRA Scientific Advisory Panel Members
Stephen M. Roberts, Ph.D. (FIFRA SAP Chair), Professor & Program Director, University of Florida, Center for Environmental & Human Toxicology, Gainesville, FL

Steven G. Heeringa, Ph.D., Research Scientist & Director for Statistical Design, University of Michigan, Institute for Social Research, Ann Arbor, MI

Gary E. Isom, Ph.D., Professor of Toxicology, School of Pharmacy and Pharmacological Sciences, Purdue University, West Lafayette, IN

FQPA Science Review Board Members
William Stephen Brimijoin, Ph.D., Chair, Department of Pharmacology, Mayo Clinic and Medical School, Rochester, MN

Lutz Edler, Ph.D., German Cancer Research Center, Heidelberg, Germany

Hisham A. El-Masri, Ph. D., Technical Director of the Computational Toxicology Laboratory, Agency for Toxic Substances and Disease Registry, Computational Toxicology Laboratory, Atlanta, Georgia

Jeffrey W. Fisher, Ph.D., Professor and Department Head, Environmental Health Science, The University of Georgia, Athens, GA 30602-2102

Bettina M. Francis, Ph.D., Associate Professor, Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Peter D.M. Macdonald, D. Phil., Professor of Mathematics & Statistics, McMaster University, Hamilton, Ontario, Canada L8S 4K1

James N. McDougal, Ph.D., Professor and Director of Toxicology Research, Wright State University, School of Medicine, Dayton, OH 45435-0001

Nu-may Ruby Reed, Ph.D., D.A.B.T., California Environmental Protection Agency, Department of Pesticide Regulation, Sacramento, CA 95812

Raymond S. H. Yang, Ph.D., Professor of Toxicology, Director, Center for Environmental Toxicology & Technology, Department of Environmental and

Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523-1690

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to physiologically-based pharmacokinetic/ pharmacodynamic modeling: preliminary evaluation and case study for the N-methyl carbamate pesticides.

Advance notice of the meeting was published in the *Federal Register* on October 22, 2003. The review was conducted in an open Panel meeting held in Arlington, Virginia, on December 11 and 12, 2003. Dr. Christopher J. Portier chaired the meeting. Mrs. Myrta R. Christian served as the Designated Federal Official.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters. These meeting minutes address the information provided and presented at the meeting, especially the response to the charge by the Agency.

CHARGE

The Agency is in the early stages of developing a methodology that incorporates PBPK modeling to assess the cumulative risk for the N-methyl carbamate pesticides. The intent of this methodology is to provide a basis for extrapolation of cumulative risk of multiple common mechanism chemicals between species, from high to low doses, and across temporal dosing patterns and routes of exposure. As a starting point, the Agency will present its PBPK approach for one N-methyl carbamate. The purpose of the meeting will be to review a pilot analysis of this PBPK model. The Agency will request comment from the panel on various technical aspects of the pilot approach (e.g., model structure, pharmacokinetic and dynamic parameters).

SUGGESTED QUESTIONS FOR THE SAP:

1. Development of the Preliminary PBPK/PD Model Structure

Conceptually, PBPK/PD models offer great promise in cumulative risk assessment, such as the ability to incorporate species, sex, or age-specific information on biological processes and the explicit consideration of pharmacokinetic and mechanistic data. At present time, the appropriate pharmacokinetic data are not available for the majority of N-methyl carbamate pesticides. The Agency has developed preliminary model structure in two computer languages (See Section III.D, Figures 2 and 3) for this common mechanism group based on information available at the present time. Specifically, the structure of the preliminary model is based on: limited available pharmacokinetic data from the literature; AChE inhibition data and rat metabolic profiles from the scientific literature and/or from studies submitted for pesticide registrations; and previous PBPK/PD models developed for organophosphorus chemicals.

Question 1.1 Please comment on the proposed PBPK/PD model structure for the N-methyl carbamate pesticides as described in the document, with specific consideration of the biological and mechanistic basis for this structure.

2. Data Needs for the N-Methyl Carbamate PBPK/PD Model

The document under review describes an iterative process for model development

where the model developer and laboratory scientist work collaboratively, first to identify and then to fill in areas where data or information are missing for a particular chemical(s). At the present time, the Agency has developed a preliminary model and has identified areas where pharmacokinetic and/or pharmacodynamic data are not available. These data needs, along with the purpose of the experiment in the modeling effort, are described in the document.

Question 2.1 Please comment on the adequacy and appropriateness of the data needs identified for the purpose of developing PBPK/PD models for individual N-methyl carbamates and also for developing the PBPK/PD model for the common assessment group as a whole.

Question 2.2 Typically, parameter estimation is performed using a set of available physiological, pharmacodynamic, and pharmacokinetic data. Data used for model development are not used for evaluating model reliability. Instead, separate data sets are used. Given the considerable resources needed to conduct in vivo pharmacokinetic studies, particularly mixture pharmacokinetic studies, identification of a minimum amount of data needed to achieve an acceptable level of residual uncertainty in the PBPK/PD model for the common assessment group is preferred. Please comment on the types of data needed to evaluate model reliability.

3. Model Evaluation and Quality Control

This document outlines a five-step approach to evaluating a PBPK/PD model for use in cumulative risk assessment. These steps include: 1) determining and stating model purpose, 2) development of model structure based on characterization of the biological and toxicological profiles of the individual members and the common assessment group as a whole; 3) description of the mathematics of the model; 4) implementation in a computer language; and 5) estimation of parameters and evaluation of model fit.

Question 3.1 Please comment on this five-step approach to evaluating PBPK/PD models, with particular consideration of their use in regulatory settings. Does this approach encompass the main issues related to model evaluation and quality control? If not, what additional issues need to be considered?

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

The FIFRA SAP reviewed the Agency November 10, 2003 Document and made suggestions for the development of the proposed PBPK/PD model. Additional related issues are also noted.

Below is a summary of findings and recommendations.

1. The Panel commended the Agency for the initiative of developing a much needed methodology and tool for refining health risk assessment of N-methyl carbamate pesticides in general, and for their cumulative risk assessment.

2. The documentation for the PBPK/PD modeling needs greater coverage on the background of its development (e.g., historical perspectives, anticipated and general goals, its use in N-methyl carbamate risk assessment, and referencing the source of data used). The agency should explicitly address how a PBPK/PD model impacts the use of uncertainty factors in risk assessments (e.g. will the model reduce the need for uncertainty factors?).

3. The model construct should ensure the accommodation and proper accounting for all pathways that are major contributors to the pharmacokinetics and toxicity of the parent and active metabolites. Relevant pathways of exposure, including dermal, should be incorporated into the model. Transport across the blood brain barrier (BBB) should be considered. In addition to brain and red blood cell (RBC) AChE, the binding to plasma ChE and to AChE at key target peripheral sites should be modeled. For comparison between model output and experimental data, the Panel provided general guidance for the proper assay of carbamylated ChE and AChE.

4. The characteristics of the four components of the model (PBPK, cumulative exposure, PBPD, population PK/PD) can be made more distinct in their development, coding, and evaluation. The complexity of the model should be balanced with its need to adequately address the critical pathways. The model should accommodate stochastic analysis and the conducting of uncertainty and sensitivity analysis. The model code should be appended to the report and published. The choice of programming language should include consideration of accessibility of the language for the scientific community.

5. Sources of input parameters to the model, including rate constants, should be referenced and cross checked with a historical database. Data are needed for all key metabolic pathways and enzymes kinetics over a pertinent dose range for a single carbamate and for cumulative risk assessment. The Panel provided comments and suggestions regarding the needed data, namely, the usefulness of *in vivo, in vitro, and in silico* data, and the need for data in human tissues and pertinent to developmental age. For cumulative risk assessment, chemical interactions (e.g., additivity or not) should be characterized in terms of time- and dose-range.

6. Regarding the use of PBPK/PD model, the Panel generally agreed that model validation is less crucial than providing an accurate model algorithm and good quality data. The Panel offered suggestions on the

goal and approaches to model evaluation and validation. Articulation of needs for model evaluation may spur the generation of data to fill the existing data gaps.

7. Instead of the 5 steps of model development presented in the Agency Document, the Panel suggested approaches to quality control and an eight-step flow chart for model development that connect the model input-output to the process of risk assessment specifically practical to cumulative risk assessment.

8. Overall, the following tasks associated with the development of PBPK/PD were suggested:

- The Agency is encouraged to establish data quality criteria for input data and investigate the availability of pertinent data for pharmaceutical products of similar or comparable mechanisms of action.

- The Agency is encouraged to open a parallel track of dialogue for defining the toxicity criteria in light of the obtainable parameters now available through modeling (e.g., benchmark peak AChE inhibition, length of AChE inhibition above a benchmark).

- A linkage between the model output and the risk assessment processes is needed, including the discussion of whether safety factors will be included in PBPK/PD models (as conservative estimates of parameters) or applied to the final result as traditional uncertainty factors.

- Define the goal for cumulative risk assessment endpoint - carbamylation of AChE by carbamates or the inhibition of AChE. The latter goal would require the consideration of concomitant OP exposure.

- A Good Modeling Practice guidance document would be useful for the evaluation of PBPK models in risk assessment.

PANEL DELIBERATIONS AND RESPONSE TO CHARGE

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

Response to Charge

1. Development of the Preliminary PBPK/PD Model Structure

Conceptually, PBPK/PD models offer great promise in cumulative risk assessment, such as the ability to incorporate species, sex, or age-specific information on biological processes and the explicit consideration of pharmacokinetic and mechanistic data. At present time, the appropriate pharmacokinetic data are not available for the majority of N-methyl carbamate pesticides. The Agency has developed preliminary model structure in two computer languages (See Section III.D, Figures 2 and 3) for this common mechanism group based on information available at the present time. Specifically, the structure of the preliminary model is based on: limited available pharmacokinetic data from the literature; AChE inhibition data and rat metabolic profiles from the scientific literature and/or from studies submitted for pesticide registrations; and previous PBPK/PD models developed for organophosphorus chemicals.

Question 1.1 Please comment on the proposed PBPK/PD model structure for the N-methyl carbamate pesticides as described in the document, with specific consideration of the biological and mechanistic basis for this structure.

Response

The Scientific Advisory Panel (SAP, hereafter also referred to as the Panel) acknowledged the work on the PBPK/PD modeling by the U.S.EPA (hereafter referred to as the Agency) as an urgently needed start in developing the methodology for its use in the risk assessment <u>and</u> as a very useful tool for the risk assessment of multiple chemical exposures. The Panel welcomed both the November 10, 2003 written report *Physiologically-based pharmacokinetic/pharmacodynamic modeling: Preliminary evaluation and case study for the N-methyl carbamate pesticides* (hereafter referred to as the Document) as well as the oral presentations given at the December 11, 2003 SAP meeting. These reports show that the Agency is on the right track to find a workable, manageable, and acceptable solution to a pressing issue in risk assessment.

The need for a more realistic risk assessment has been expressed in the literature. The following citations merely serve to illustrate the articulation of these needs. Regarding the role of PBPK modeling, Andersen (2003) stated that, *"Pharmacokinetic (PK) models have the potential to estimate time-course concentrations of parent compounds and metabolites for different exposure conditions"* and *"The stage is set for wider penetration of these approaches in the risk assessment process by a wide group of modeling practitioners throughout the world.*" This publication stresses the need to accelerate acceptance of these modeling tools to both improve mechanistic studies in toxicology and integrate diverse data in current risk assessment practice. Regarding cumulative risk assessment, it has been widely recognised that humans are not exposed to one chemical but to mixtures. Many authors have therefore called for an integrated approach to the analysis of toxicological interactions of chemical mixtures both from a scientific as well as regulatory point of view (e.g., Mumtaz *et al.*, 1993, El-Masri *et al.*, 1997, Verhaar *et*

al., 1997). In response to the challenge of addressing the cumulative risk, the Agency has taken the lead and recently completed the *Guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity* (USEPA, 2002). The current effort on PBPK/PD modeling is a reasonable follow up for providing an adequate modeling framework and the computational tools for cumulative risk assessment.

The Agency is commended for taking a major step toward providing applicable methodology for refining the risk assessment. Although some published case studies show encouraging results in PBPK modeling, no systematic approach has been formulated with the scope as presented now by the Agency. When applied, PBPK/PD modeling can reduce uncertainties in cumulative risk assessment for N-methyl carbamate pesticides. The Panel affirms the importance of PBPK/PD modeling in risk assessment and its use in providing a refined methodology for assessing cumulative risk. It encourages the Agency team of policy makers, toxicologists, statisticians, computer modelers/engineers from many different units as well as external expertise, including CIIT Centers for Health Research, to proceed with this work.

At this early stage, the Panel has many suggestions for the model development. These are presented for the following areas.

Model Presentation

The model as presented by the Agency consists of two components; the PBPK model in general and the model for mixture exposure. Incorporated into these are two additional structural elements, the substructure of pharmacodynamics (PD) and the substructure of population kinetics. Although at the present stage the PD component constitutes only a minor portion of the model, it complicates both the discussion and the validation of the model. The population kinetic modeling is to be implemented using the MCSim programming language suitable to judge the uncertainty as well as the application of the model to populations. This module adds a statistical dimension to an otherwise completely deterministic model.

The Panel has the following comments on model presentation: 1) Several Panel members commented that there was an apparent difference between the Document and the oral presentations at the SAP meeting regarding the breadth and depth of information given about the model. More information should be presented in the written Document. 2) The written Document could be improved by accurate presentation of equations (e.g., Equation 1, 3; Figure 2); consistent use of abbreviation (e.g., "AChE" instead of "Ace" in Figure 1); and explicit statement of all assumptions. 3) Source references should be given for all model parameters and the parameter values should be cross checked against the historical database. 4) The presented model appeared to be structurally similar to other earlier models by Gearhart *et al.* (1990) and Knaak *et al.* (1993). Reference to these models would provide the perspective on the scientific foundation of the current model, as well as the logical introduction of new features, especially if more recent *in vitro* and *in viv*o data have been used to update those earlier modeling approaches. 5)

Instead of only giving computational equations, a diagram describing the AChE inhibition, such as presented in Gearhart *et al.* (1990), can be a more transparent way to illustrate the model. 6) The confidence bounds should be given for all the predictions and their appropriate statistics should be developed. 7) The Panel unanimously agreed with the Agency regarding the stated advantages of a PBPK/PD model. However, some issues could be further elaborated, e.g., any additional promise for its use in cumulative risk assessment, and the unique application to N-methyl-carbamates.

Model Construct

In general, the Panel considered the model structure for the N-methyl carbamate pesticides (actually for anticholinesterases in general) as thoughtful and sound. The Panel recommends structuring the model and its presentation to account for each of these four model components (PBPK, cumulative exposure, PBPD, population PK/PD) separately. The distinctions between the four components can be made in the initial model building process, in computer coding, and in addressing questions of model evaluation and model validation.

Neither the Documents nor the presentations described clearly the metabolic and clearance pathways of the parent chemicals and their metabolites, or the difference between parent and metabolite bindings to AChE. In order not to expand the model complexity beyond what is necessary, the current model proposes to combine in a group all the biotransformation steps that do not result in active metabolites. However, care should be taken to account for any steps within this group that may have rate-limiting effects on the key biotransformation pathways (e.g., depleting enzyme pools, such as carboxylesterase, during developmental stage).

The model should include the dermal route of exposure. As a key potential route of entry, skin should probably be a stand alone compartment in Table 1, including penetration rates, skin volume, blood flow, and vehicle/skin & skin/blood partition coefficients for parents and active metabolites. It was noted that skin surface water is an input compartment to the ERDEM model (Page 18 – Figure 2). This is probably only valid during bathing. Input should be based on concentration and skin/vehicle partition coefficient for each chemical. Chemicals may be provided as granules or wettable powder, and cutaneous penetration of granules and aqueous solutions could differ by orders of magnitude.

The two modeling approaches using either ACSL or MCSim software tend to diverge at present. The ERDEM-model has focused on exposure and various transitions of pesticides, whereas the second model, solved with MCSim, has been built in a simpler structure. It focuses on the metabolites and the similarity of the fate of the parent compound and its metabolites. In principle, both languages should be applicable to both models. Consideration should be given to the transportability and acceptability of the computer implementations in choosing programming languages. One Panel member encouraged EPA to follow up the option of providing computer code in the R-language,

because of its free availability and its broad usage for scientific data analysis and statistical computing.

There will be serious issues to consider regarding model uncertainty, stochastic variability, parameter estimation and evaluation of the fit. Although the entire project is well thought out and well presented, some Panel members look forward to a stochastic version of the model which would allow statistical estimation of risk assessment targets and the analysis of uncertainty. As the current model is deterministic, it can most easily accommodate stochastic analysis by allowing for distributions of the parameters. The process being modeled, however, may in many cases have to be stochastically run on fixed rate constants. Whether a fully stochastic model with fixed deterministic parameters or a partially stochastic model with deterministic structure and stochastic parameters is more appropriate should be decided depending on adequacy for risk assessment purposes.

The code and all mathematical formulas should be available for review during the development of the model. This should include the objective function of the model fit for statistical estimation purposes as well as an analysis of uncertainty. It is noted that there can not really be two independent models if they are based on the same conceptual framework. To decide if one or both models are correct, consider that they are over-parameterized so it should always be possible to fit the data, and the models will agree with each other only if they both make the same errors. Ultimately, a model must make sense scientifically if it is to be used. The question is whether the two models are based on the same conceptual framework. Since the flow chart descriptions (Figs 2 & 3) appear to be quite different for the two models, they can be expected to give different results even if both are coded correctly.

Multiple Chemical Interactions

The interactions of N-methyl carbamates were inadequately addressed for the wealth of information on various endpoints now obtainable through PBPK modeling. Addressing the combined effects of multiple N-methyl carbamates is of high priority and should be done. One key question to be addressed for the cumulative risk assessment is, additivity versus non-additivity at a given exposure to several compounds. In the "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity" (USEPA, 2002a), it was assumed that at lower levels of exposure typically encountered environmentally no chemical interactions are expected (i.e., simple additivity). For additivity to hold true, a further assumption must be that all the common mechanism chemicals behave the same pharmacokinetically and pharmacodynamically [i.e., have the same pharmacokinetics (PK) and pharmacodynamics (PD)] (USEPA, 2002a). In reality though, a case study of cumulative risk assessment of 33 organophosphorus pesticides provided BMD (benchmark dose) and BMDL (lower bound of BMD at ED_{10}) with a range of 3,977- to 7,848-fold difference between the highest BMDL for malathion to the lowest BMDL for dicrotophos (USEPA, 2002b). These 3-4 order of magnitude differences among "common mechanism chemicals" suggest strongly

that the PK and PD are not the same among these chemicals. Thus, the probability of toxicological interactions at the level of PK and PD exists.

The specific areas commented by individual members of the Panel are: 1) Lack of frank description of the interaction mechanism (the type of enzymatic inhibition mechanism). 2) Lack of consideration for the need and approach to experimentally validate the mechanism of competitive enzymatic inhibition *in vivo*. 3) The need to specify interaction corresponding to the AChE level. Would the model be structured such that there will always be AChE available for binding with the carbamates? 4) Interactions at the metabolic processes of the parent chemicals that could dictate the reactions. 5) Lack of adequate description on how the model can accommodate the potential variation of effects due to the different patterns of exposure, including the exposure dose, duration, and frequency (e.g., bolus, short- and long-term of exposure). A discussion is also needed on the value of using the PBPK model versus the simple additive models which might provide similar answers to the risk estimates, especially at low environmental levels and whether the same uncertainty factors used in a simple additivity model will still be needed for the PBPK/PD model.

Input Data

The two presented models are suggestive of generic models for a class of compound (i.e., N-methyl carbamates or, more broadly, anticholinesterases) and yet, throughout the discussion, it was quite clear that the authors of this Document think along the line of data availability of the particular carbamate(s) to be included and the possibility of building models for those individual compounds. In other words, if there are five chemicals in the cumulative assessment group, the expectation is that each of them would have sufficient data for building five PBPK models. This expectation could severely limit the number of chemicals to be considered in the cumulative risk assessment process. Alternatively, a generic PBPK model for a class of compound is theoretically possible. However, in such a case, the PBPK model must be coupled with other modeling techniques including QSAR, molecular modeling, and reaction network modeling as discussed in more detail in item 4, "Additional Comments from the SAP" at the end of this report.

Several Panel members discussed the model structure and the mass balance equations for the adequacy of zero order and first order synthesis rates for AChE and whether the present choice of zero order synthesis is really significant for the carbamate inhibition. If the zero order AChE synthesis rate was derived from the work of Abbas and Hayton (1997) from Rainbow trout, this rate constant was actually the result of curve-fitting in their study. Considering further the AChE turnover work of Hu *et al.* (2003) in neuroblastoma where the elevation of AChE activity reflected slowed AChE degradation rather than accelerated synthesis, further experimental characterization of this important

rate constant (i.e., AChE synthesis rate), preferably at the mRNA level in human tissues under a variety of normal and treatment conditions, would be prudent. It may be that the intrinsic AChE synthesis rate is different (e.g., first order process and a slower rate) from when anticholinesterases are present. In the latter case, under external stress, maximal AChE synthesis rate (i.e., zero order) kicks in to compensate for the loss of AChE activities.

One Panel member argued that carbamate inhibition is a fast and early event such that the use of a complicated kinetic model must be weighed against a simpler method. In particular, it is unclear at this stage that terms for rates of enzyme synthesis and degradation are really needed since even in rodents the turnover time for AChE is measured in days while the regeneration rate is measured in seconds and the metabolic clearance of toxicant is measured in hours. The Agency team should address this option under the premise of building the most parsimonious model. If synthesis and degradation are to be considered, however, it may be necessary to introduce even further complexity. In that case, for example, the model building should also address the possibility of an up-regulation of AChE at chronic exposure conditions. Such up-regulation has previously been demonstrated in cell culture systems and at least in one case in a rodent model of chronic intoxication with chlorpyrifos (Chiappa *et al.*, 1995). The impact of these effects is probably not large, but it would be appropriate to take them under consideration.

Endpoint and Toxicity

Since the purpose of the model is for risk assessment, proper linkage between the defined AChE endpoint (e.g., 10% inhibition) and the relevance in toxicity is essential. The PBPK/PD approach opens up various options for defining the endpoints for risk assessment that are not previously possible (e.g., peak AChE inhibition, length of time above pre-defined level of AChE inhibition).

In addition to brain and RBC AChE inhibition, the PBPK/PD model should also have the capability for modeling plasma ChE and other AChE at key target sites. More relevant could be the target organs starting from which the AChE inhibition causes toxicity. The modeling should explicitly address processes happening at those sites where the toxic action takes place. These sites would include e.g., peripheral tissue, heart, gut, lung, skeletal muscle or the autonomic ganglia. Measurement at those sites is not a routine matter and will call for special attention to procedures for tissue sampling, homogenization and enzyme assay. On the other hand, such data would be feasible to obtain and it would be appropriate to elicit a call for more data for the purposes of the modeling endeavour. To some extent, the PBPK/PD model could still be useful in the absence of complete data.

Other Comments

One Panel member pointed out that modeling of N-methyl carbamates can strongly benefit from already existing knowledge on organophosphates (OPs). Given that binding

and inhibiting AChE is one source of toxicity for both organophosphates (OPs) and N-methyl carbamates (carbamates), the question is raised whether the carbamates alone constitute an appropriate common mechanism group (CMG), or should OPs and carbamates be placed in one CMG. The answer clearly depends on the definition of mechanism. Is the mechanism of interest the carbamylation of AChE by carbamates, or the inhibition of AChE? Toxicologically, it is the inhibition of AChE that matters, regardless of whether it results from carbamylation or phosphorylation. Concurrent exposure to carbamates and OPs is plausible, even probable. Moreover, since OPs, for practical purposes, irreversibly bind AChE, even an earlier exposure to OPs could alter the amount of AChE available in carbamate exposure. Therefore the OPs cannot simply be ignored in modeling exposure to mixtures of AChE-inhibiting pesticides.

2. Data Needs for the N-Methyl Carbamate PBPK/PD Model

The document under review describes an iterative process for model development where the model developer and laboratory scientist work collaboratively, first to identify and then to fill in areas where data or information are missing for a particular chemical(s). At the present time, the Agency has developed a preliminary model and has identified areas where pharmacokinetic and/or pharmacodynamic data are not available. These data needs, along with the purpose of the experiment in the modeling effort, are described in the document.

Question 2.1 Please comment on the adequacy and appropriateness of the data needs identified for the purpose of developing PBPK/PD models for individual N-methyl carbamates and also for developing the PBPK/PD model for the common assessment group as a whole.

Response

In general, the data needs identified in the Document for developing the model are appropriate. A list of chemicals of interest would be helpful for a more specific evaluation of data needs because data needs are defined by the chemical of choice and data structure. Specific recommendations and comments from the Panel are provided for the following areas.

In vivo, In vitro, and In silico

The Panel agrees that both oral and intravenous (iv) *in vivo* pharmacokinetic data are needed. Data on several modes of oral administration is valuable, since the vehicle used can significantly alter rates of absorption. *In vivo* studies are very valuable in the development of models and should be given emphasis when possible. For the present initiative, the incorporation of *in vitro* human data is essential. Credible human enzyme kinetic studies using human tissues with the quality of organ transplant conditions should be carried out (Lipscomb and Garrett, 1998; Lipscomb *et al.*, 2003). Alternatively, c-

DNA expressed human AChE from recombinant organisms commercially available could be used to generate human enzyme kinetic parameters. Structure-activity studies for developing partition coefficients need validation in tissues that are unusual. One Panel member suggested that, because of the large number of potential combinations and interactions, *in silico* toxicology (e.g., Molecular Modeling and Molecular docking based on QSAR and the known 3D structures of AChE, and reaction network modeling) could be used to assist the evaluation of mixtures (more detail in item 4, "Additional Comments from the SAP" at the end of this report).

Pharmacokinetic Data

Data on metabolism are especially important in the development of the models. It is useful to establish criteria for data quality, such as the sample size (e.g., number of animals or measurements) in a study that would be considered as sufficient for use in the development of the model. All the key enzymes involved in carbamate toxicity should be studied so that a more robust model can be developed not only for cholinesterase carbamylation but also for toxicity description. Data on biotransformation enzymes should include those that can potentially become rate-limiting for a single chemical as well as multiple chemical exposures. Studies on clearance of the chemical are also important. Complete development of a model for one chemical with quality data will make development of the other chemical models easier.

Kinetic and metabolic data should be collected over a range that is relevant for identifying the change in the shape of dose-response relationship (i.e., change from linearity to non-linearity), especially because the calculated benchmark dose levels for 33 organophosphorus pesticides in the USEPA case study of cumulative risk assessment (USEPA, 2002b) have been reported to differ by 3 to 4 orders of magnitude (see earlier discussion and citations under Question 1.1). Data for a sufficient range of dose-response relationship is also useful for identifying a possible pattern of interactions (e.g., additivity) for a cumulative chemical exposure. Moreover, coupled with proper analytical determination of AChE (see next paragraph for more detail), data over a range of exposure would also allow a meaningful comparison between the model output and the exposure estimates, and between the exposure and toxicity. The Agency may consider a parallel track of dialogue to define the model endpoint of risk assessment (e.g., peak AChE inhibition).

Pharmacodynamic Data

It is important that AChE activity is appropriately measured. Determination of AChE or BChE inhibition by quasi-reversible agents such as carbamates is problematic because, when enzyme regeneration half-times are measured in seconds, a new equilibrium is typically established before a standard assay can be completed. Therefore, assessment of reversible AChE inhibition should follow best scientific practice, which means using a method that is rapid and that involves the smallest practical dilution of the sample. The standard practice of preparing tissue homogenates at high dilution should be discouraged.

Optimally, the effect of dilution should be explicitly factored into the data analysis so that it is possible to extrapolate back to the undiluted case, in other words to determine what level of enzyme inhibition existed in the intact animal (or human subject). In choosing among specific biochemical procedures for assay, it will sometimes be advantageous to use the radiometric assay of Johnson and Russell (1975), which is based on liberation of tritiated acetate from radiolabeled acetylcholine. This assay is extremely sensitive. Compared to spectrophotometric methods, radiometric assay is less subject to interference from hemoglobin or myoglobin in tissue extracts. However, in many cases the conventional Ellman assay (or as modified for plate readers) is appropriate and even preferable because of its wide dynamic range and its potential to yield real time kinetic data.

In addition to brain and blood AChE, AChE in peripheral tissues, especially those that are target sites for acute toxicity, should be modeled. A previous SAP (September 21 – 24, 1999, Section IV) highlighted the need for more information about AChE inhibition in peripheral tissues, particularly the heart, lung (and respiratory tract in general), gut, skeletal muscle, and autonomic ganglia. Although there are technical difficulties in measuring AChE activity accurately in such tissues (principally because they are difficult to homogenize), it should be feasible to obtain data for one or more carbamate anticholinesterases. The PBPK/PD modeling could then be utilized to predict the behavior of other agents, or of the same agent under altered conditions of exposure or timing of toxicant.

Even though inhibition of serum esterases (also known as aliesterases or nonspecific esterases) by carbamates has not been directly linked to toxic effects, serum esterases cannot be ignored in modeling the inhibition of AChE by carbamates. Binding of carbamates to these nonspecific esterases results in significant degradation of the toxicants by limiting the amount reaching the nervous system. In effect, the nonspecific esterases can play a significant role in the disposition of carbamates.

Data Pertinent to Humans

It is unlikely that animal data will suffice to model inter-individual variability in response to carbamates, because the commonly used laboratory strains of animals are too homogeneous to represent human variability. Even the so-called outbred rats exhibit a considerable degree of inbreeding, probably because of inadvertent selection of traits associated with high fecundity. Developmental data are also needed for the model development for assessing the age-related toxicity response of N-methyl carbamates. Using data from laboratory animals (e.g., rats) as surrogates for humans would require proper matching of developmental stages between animals and humans. The Agency is encouraged to explore the availability of comparative data between laboratory animals and humans and any data that are pertinent to humans. One possible source of data (e.g., physiological, PK, and PD data) might be research on pharmaceutical products that are similar to carbamates or organophosphates.

Question 2.2 Typically, parameter estimation is performed using a set of available physiological, pharmacodynamic, and pharmacokinetic data. Data used for model development are not used for evaluating model reliability. Instead, separate data sets are used. Given the considerable resources needed to conduct *in vivo* pharmacokinetic studies, particularly mixture pharmacokinetic studies, identification of a minimum amount of data needed to achieve an acceptable level of residual uncertainty in the PBPK/PD model for the common assessment group is preferred. Please comment on the types of data needed to evaluate model reliability.

Response

Model validation is a difficult concept. Panel members discussed extensively the meaning of validation and its utility. In general, model validation appears to be less important than other issues in the use of PBPK/PD models in risk assessment, such as appropriate model algorithms and good data quality for model development. When a dataset used in model validation or evaluation disagrees with the model output, it only indicates that further investigation is necessary. Where a model with 50 or more parameters is used to describe a curve that simply peaks and returns to zero, it will be over-fitted. Agreement of the fitted and the observed peaks does not necessarily validate the model.

One Panel member expressed concern that the classical concept of validation, as discussed in the Document, should not be used for PBPK/PD models in the context of risk assessment. In general, he noted that there would never be sufficient statistical power in validation data sets to reject a model that adequately fits the estimation data sets. Instead, guidance for quantifying hazards will be better provided by giving closer attention to objective methods of parameter estimation; use of formal statistical hypothesis testing of critical hypotheses included in the model (such as additivity versus synergy) in the context of the PBPK/PD model; focused design for new data to go into the model estimation; use of all data in model characterization; and presentation of all predictions as means and confidence bounds. This Panel member suggested that this classical statistical approach applied to data analysis, in this context, is a form of validation that matches EPA's historical approach to quantifying health risks and is sufficient for the proposed usage of PBPK/PD models.

The following collective comments and suggestions were made by the Panel for model evaluation. There is no implication that any of these suggestions alone will meet the needs to have a 'validated or reliable model'. The Panel agrees that validation increases confidence. A Good Modeling Practice guidance document would be useful for the use of PBPK models in risk assessment.

Several techniques help inform whether PBPK models are robust, and describe the kinetics and dynamics of a biological system in a fashion that is plausible and useful.

Data sets collected for model development can be used for validation, as described in Keys *et al.* (2003). One Panel member noted that cross validation gives protection from over-fitting. If the model fits the fitting data better than the validation data, it shows that parameters have been used to fit non-significant features of the fitting data.

Panel members discussed several informal approaches that can assist in model validation. There are statistical methods to assist with validation of large data sets. When there are thousands of observations (as in data mining, e.g.), the data are divided randomly into 3 sets, one for choosing the model, one for fitting the model and one for validating the fit. Note that this is one data set split randomly which is different from fitting the model to a series of laboratory studies and then testing the fit on a completely new study.

Another approach is to use part of the existing data set for model development and the remaining data for model reliability. However, this may not be beneficial if little data is available and there are fewer resources to build the model. If sparse data exist in the literature, one can simulate the exposures and try to predict the outcome, e.g., levels of chemical in the urine or blood, AChE inhibition. Experiments can be designed with limited kinetic data up to full time course studies for validation. If a sensitivity analysis is completed, validation could be attempted only on important model parameters. Running experiments *in silico* (i.e. running simulation programs) to identify key parameters is useful for mixture studies. A fractional factorial design should be used to get as much information as possible with a limited number of simulations.

It is important to consider how the models will be used for validation, e.g., "How well does the model behave in the low dose and response region for relevance to environmental exposures?" Species extrapolation of PD responses should be considered. The variability in humans is expected to be greater than in laboratory animals. To help with validation, inhibition of red blood cell AChE could be evaluated between humans and animals. Frank toxicity is another important aspect in the validation process. Cross check the simulated parameters against endpoints from toxicity studies across dose- and time-range, and for different routes of exposure, including dermal. Validation should include other tissues such as muscle as a target organ for the PD aspect of the model. The Document states that the results of simulations are time curves. It will be easier to discuss and compare results if these are summarized by characteristics, as was done in Tables 2 to 6. Are these characteristics (Peak inhibition, AUC at 1 hr, etc.) the most stable and useful descriptors of a curve? The Document provides six model simulations based on different exposures or potential physiological changes which are predictions from the PBPK/PD model. Thus, the Agency has a unique opportunity to design sets of experiments based on these simulation scenarios to validate these predictions. From such exercises, more and more experience will be obtained for the "minimal data sets" needed for model validation.

Model failure and model parsimony are important. Models test our thinking about how a system works and help us discover something new. New studies could be conducted to test hypotheses concerning model failure.

3. Model Evaluation and Quality Control

This document outlines a five-step approach to evaluating a PBPK/PD model for use in cumulative risk assessment. These steps include: 1) determining and stating model purpose, 2) development of model structure based on characterization of the biological and toxicological profiles of the individual members and the common assessment group as a whole; 3) description of the

mathematics of the model; 4) implementation in a computer language; and 5) estimation of parameters and evaluation of model fit.

Question 3.1 Please comment on this five-step approach to evaluating PBPK/PD models, with particular consideration of their use in regulatory settings. Does this approach encompass the main issues related to model evaluation and quality control? If not, what additional issues need to be considered?

Response

The five-steps given above (item 3 of the **Charge Questions, FIFRA SCIENTIFIC ADVISORY PANEL)** should be better coordinated with the Document. Panel members had difficulty discerning the beginning and end of each step. The steps should be incorporated into the case study in the revised document.

In general, the five-step approach to evaluating PBPK/PD models of carbamate anticholinesterases is reasonable but overly simplified. The steps are probably borrowed from a publication that tried to generalize the approach to developing models. They should not be used for specific issues that have unique questions and problems to be solved by modeling in this specific case. For example, the steps describe the process of building the model, starting with its purpose and ending with estimation of parameters. Ending with the parameter estimation step indicates that this is the goal for the modeling. However, parameter estimations and model fits are an outcome of the model but not necessarily the reason the model is developed. Therefore, for the model at hand, the steps need to be more specific and detailed.

The steps in the Document show only how to build the model, not how to evaluate it. The Agency presenters seemed well aware of this limitation, however, in referring to the necessarily iterative nature of the development process. Some of the steps are interrelated and should not just follow each other successively. The steps need to relate to each other, especially when validation of the model leads to model modifications to refine the different hypotheses therein.

The five-step approach does not cover evaluation or use in a regulatory environment. It

would be more useful to expand the approach to cover the context of risk assessment [NRC/NAS, 1983 ("The Redbook")]. Specifically, putting this work into a regulatory setting implies an extra step, ensuring that the PBPK/PD model fits into a larger exposure and risk model. The PBPK/PD model will have to accept inputs from the exposure model and return outputs to the risk model. The choice of implementation may depend on which one performs satisfactorily. Unless the PBPK/PD model can be used in a larger context of exposure and risk model, it will be little more than a scientific curiosity.

Quality control obtained by using two programming languages will work only when the model building and the programming are performed by two independent teams for each model. One could check the output for identity of the results from the application of the two approaches to the same data and the same question. 'Similarity' of the outcome from two programs created by the same team would be insufficient for assuring correctness to the extent the agency may want. On the other hand, one may at this stage concentrate on the development of one model that meets the risk assessment requirements for the N-methyl carbamates.

One Panel member suggested the simulation study could be better organized and, through this organization, be better designed to address specific issues. For example, the following scheme could be used. The test of a set of chemicals of a common mechanism group (CMG) is primarily determined through the following choices of

the chemicals	$C_1, \ldots, C_M$,
the doses	$d_1, \ldots, d_K$,
and, the times of dosing (bolus dose)	$t_1, \ldots, t_I$.

Using, amongst others, these three design components, a simulation study can then be characterized. For example, using these three design components, the simulations used by the Agency PBPK/PD modeling can be described as follows:

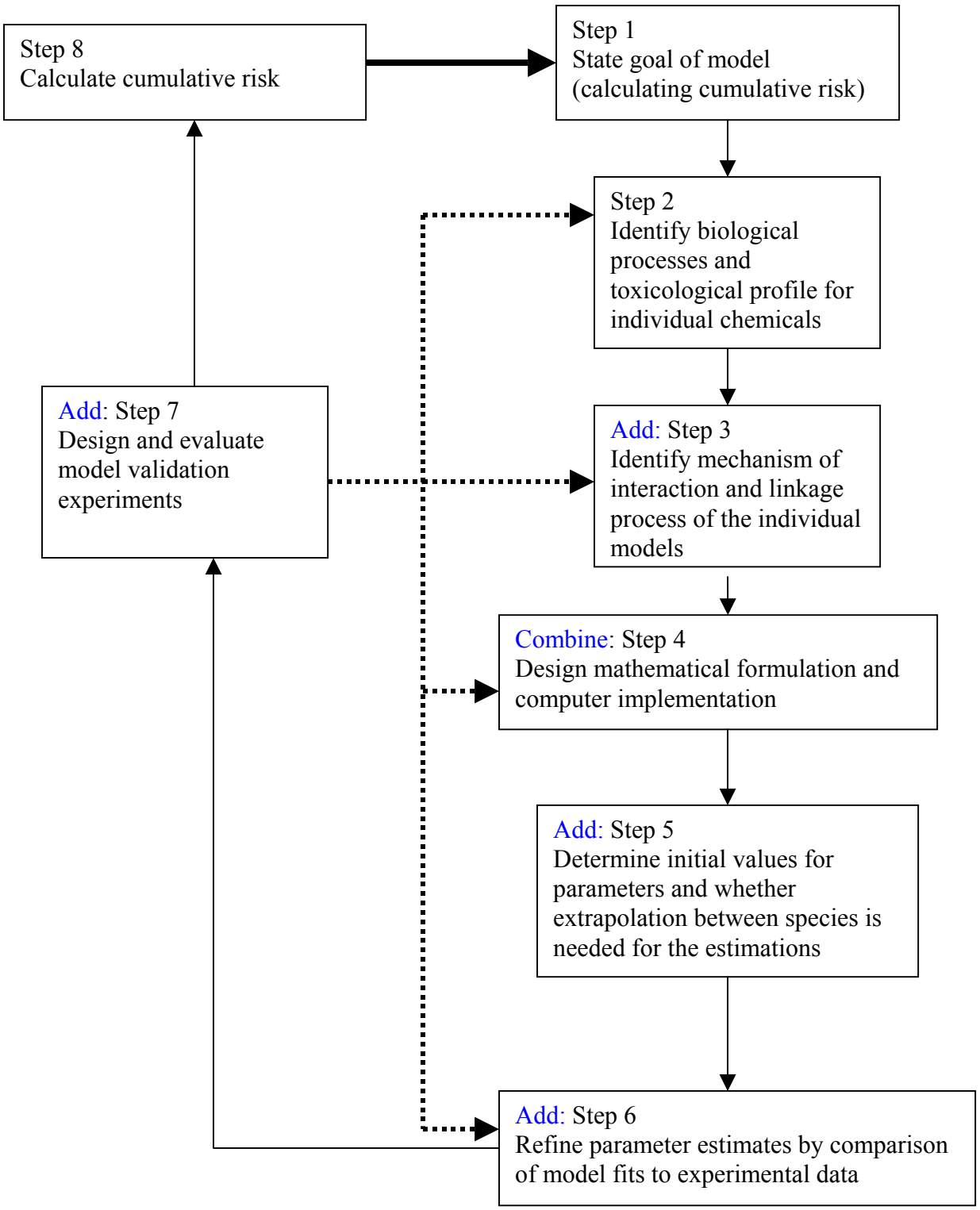
Simulation No	C_1				C_2		
	d_{11}			d_{12}	d_{21}		
	t_{111}	t_{112}	t_{113}	t_{121}	t_{211}	t_{212}	t_{213}
S1	x	-	-	-	-	-	-
S2	x	-	-	-	-	-	-
S3	x	-	-	-	-	-	-
S4	x	x	-	-	-	-	-
S5	x	-	x	-	-	-	-
S6	x	-	-	-	x	-	x

The simulations S2 and S3 are modifications of S1. "X" indicates a simulation design component; for example, S6 uses 2 chemicals with C1 at one exposure time and C2 at 2

exposure times. The simulation study can be further expanded and designed by filling and extending the scheme shown above. Using such schema, it would be easy to prospectively and efficiently design key simulation studies that will meet the requirements for model improvement and increase confidence levels of PBPK/PD models.

Another suggestion was that the Agency develops a means for determining the quality of data sets to be used in the model process. More discussion should be included in the steps for the methodology of selecting appropriate data sets for optimization and model calibration, and the steps should include explicit explanation of the criteria for data quality and data inclusion.

The Panel suggested the following scheme for the evaluation of the PBPK model that incorporated some of the steps proposed in the Document:


Step 8
Calculate cumulative risk
Step 1
State goal of model
(calculating cumulative risk)
Step 2
Identify biological processes and toxicological profile for individual chemicals
Add: Step 7
Design and evaluate model validation experiments
Add: Step 3
Identify mechanism of interaction and linkage process of the individual models
Combine: Step 4
Design mathematical formulation and computer implementation
Add: Step 5
Determine initial values for parameters and whether extrapolation between species is needed for the estimations
Add: Step 6
Refine parameter estimates by comparison of model fits to experimental data

4. Additional General Comments from the SAP

In addition to providing comments to the above questions 1 through 3, as requested by the Agency, the Panel made the following general comments on the Agency's approach to the PBPK/PD modeling as presented to the Panel for review.

One Panel member commented that it is not clear where the proposed PBPK/PD modeling approach fits into the 10-step cumulative risk assessment process as outlined by the Agency (USEPA, 2002a). Specifically, he questioned whether it will start at the initial screening stage or at the later comprehensive cumulative risk assessment stage. The advantage of integrating PBPK/PD modeling into cumulative risk assessment should be more clearly articulated. From the perspective of chemical mixture toxicology or toxicological interactions, which are necessary considerations in the cumulative risk assessment process, the incorporation of PBPK/PD modeling in cumulative risk assessment is not a matter of the Agency favoring one method vs. another. Scientifically, it is a matter of necessity to integrate PBPK modeling into the cumulative risk assessment if the cumulative risk assessment is to be successful.

One Panel member encouraged the Agency to consider linking PBPK/PD modeling with reaction network modeling, or something similar, to look at the overall dynamics of most key reactions, if not all, involved leading to the toxic endpoint (i.e., a systems biology approach to cumulative risk assessment). This is important because cumulative risk assessment must consider multiple chemical interactions. When we consider chemical mixtures, we soon realize that animal, or even cell culture, experimental work becomes impossibly complex. Thus, it is necessary to use computational technology beyond PBPK/PD modeling when dealing with chemical mixtures. Significant developments in such technologies are applicable to the study of chemical mixtures. David F. V. Lewis in England uses a COMPACT (Computer-Optimized Molecular Parametric Analysis of Chemical Toxicity) method which integrates QSAR and molecular modeling to predict Cytochrome P450 catalyzed drug metabolism and toxicity (Lewis *et al.*, 1998; Lewis, 2002a,b). Similarly, molecular docking, molecular dynamics, and density functional theoretical calculations have been applied to the predictions of metabolism of CYP2E1 catalyzed reactions (Park and Harris, 2003). These excellent tools could be used in understanding multiple chemical interactions. Such tools have been used in a reaction network modeling approach to consider large complex chemical mixtures (Klein *et al.*, 2002; Liao *et al.*, 2002; Yang *et al.*, 2003; Reisfeld and Yang, 2004; Yang *et al.*, 2004). Anticholinesterases as a class of pesticides are ideally suited for molecular modeling or molecular docking because crystalline structures are known for acetylcholinesterase in the human, mouse, electric eel, and Drosophila. This class of compounds is also ideally suited to the exploration of linking PBPK modeling with other modeling technologies (e.g., molecular modeling/docking, and reaction network modeling) for considering chemical mixtures in the cumulative risk assessment process. The advantage of this approach is that, because of the probable built in capabilities of computer-assisted modeling (i.e., automation), it may be possible to evaluate large candidate cumulative

assessment groups (CAG; Step 5 of Guidance on cumulative risk assessment, USEPA, 2002a) of say, 50 carbamates or more.

The Panel recommended that the Agency consider the role of the blood brain barrier (BBB) in their formulation of PBPK models for N-methyl carbamates. The uptake of carbamates and possible active metabolites into the brain may be affected by pharmacokinetic interactions. For example, Lawrence Livermore scientists recently reported that the anticholinesterase, DFP, crosses the BBB to differing extents in the presence and absence of other pesticides, such as multiple combinations of parathion and permethrin (Vogel *et al.*, 2002).

One Panel member expanded on the issue of chemical interaction relative to dose levels. The issue of possible toxicological interaction at the environmental exposure levels, usually at very low levels, should be addressed. The "common belief" is that at such low levels (say ppb of less), toxicological interactions do not occur. Thus, the additivity assumption may apply. In that event, the incorporation of PBPK/PD modeling into the cumulative risk assessment process might appear to be overkill. However, we really do not have sufficient scientific data to prove such "common belief." In fact Bae *et al.*, (2001) reported dose-dependent toxicological interactions in cell cultures where at very low doses (i.e., environmental exposure relevant concentrations such as in the low ppb range) hormesis effects were observed. At higher concentrations, additivity, and then synergistic toxicity prevail. Antagonistic toxicity was observed at the highest concentrations. Furthermore, in cases of occupational exposure involving workers such as farmers and pesticide operators, the exposure concentration to N-methyl carbamates or other pesticides is high. In those instances, toxicological interactions are probable. Thus, PBPK/PD modeling application in cumulative risk assessment is not only relevant but also necessary.

One Panel member suggested that the Agency should immediately develop a format for presentation of PBPK/PD models, data and parameter estimates in a manner that is clear to the diverse scientific audience, and which will be needed for peer review of these analyses. It is important that the presentation include access to the data and computer code so that interested reviewers can fully replicate the findings of the Agency.

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