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

OFFICE OF
PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

November 13, 2007

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held August 16 - 17, 2007 on Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides

TO: Debbie Edwards, Director
Office of Pesticide Programs

FROM: Steven M. Knott, Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Elizabeth Resek, Acting Director
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 August 16 - 17, 2007. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides.

Attachment

cc:

James B. Gulliford
James J. Jones
Anne Lindsay
William Jordan
Margie Fehrenbach
Janet Andersen
Steven Bradbury
William Diamond
Tina Levine
Lois Rossi
Frank Sanders
Betty Shackleford
Richard Keigwin
Jack Housenger
Anna Lowit
Michael Devito
Michael Hughes
Edward Scollon
Rogelio Tornero-Velez
R. Woodrow Setzer
Douglas Parsons
Dale Kemery
Vanessa Vu (SAB)
OPP Docket

FIFRA Scientific Advisory Panel Members

Steven G. Heeringa, PH.D. (FIFRA SAP Chair)
Janice E. Chambers, Ph.D., D.A.B.T. (FIFRA SAP Session Chair for August 16-17, 2007 Meeting)
John R. Bucher, Ph.D., D.A.B.T.
Stuart Handwerger, M.D.
Gary Isom, Ph.D.
Kenneth M. Portier, Ph.D.
Daniel Schlenk, Ph.D.

FQPA Science Review Board Members

Lutz Edler, Ph.D.
Derek W. Gammon, Ph.D., D.A.B.T.
Richard Greenwood, Ph.D.
Dale Hattis, Ph.D.
William L. Hayton, Ph.D.
Wendy J. Heiger-Bernays, Ph.D.
Sastry S. Isukapalli, Ph.D.
Nu-may Ruby Reed, Ph.D., D.A.B.T.
Matthew K. Ross, Ph.D.

SAP Minutes No. 2007-07

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

Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides

August 16 - 17, 2007
FIFRA Scientific Advisory Panel Meeting
held at the
Environmental Protection Agency Conference Center
Arlington, Virginia

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). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes 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 for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and 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 Environmental Protection Agency, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. FQPA 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 Steven Knott, SAP Designated Federal Official, via e-mail at knott.steven@epa.gov.

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

TABLE OF CONTENTS

PARTICIPANTS.....5
INTRODUCTION.....7
PUBLIC COMMENTERS.....9
SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS.....10
PANEL DELIBERATIONS AND RESPONSE TO CHARGE.....12
REFERENCES.....29

SAP Minutes No. 2007-07

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

Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides

August 16 - 17, 2007
FIFRA Scientific Advisory Panel Meeting
held at the
Environmental Protection Agency Conference Center
Arlington, Virginia

Janice E. Chambers

Janice E. Chambers, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date: NOV 13 2007

Steven M. Knott

Steven M. Knott, M.S.
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: NOV 13 2007

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
August 16-17, 2007

Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides

PARTICIPANTS

FIFRA SAP Session Chair

Janice E. Chambers, Ph.D., D.A.B.T., William L. Giles Distinguished Professor and Director, Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Designated Federal Official

Steven M. Knott, M.S., FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, EPA

FIFRA Scientific Advisory Panel Members

Stuart Handwerger, M.D., Professor of Pediatrics, University of Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Kenneth M. Portier, Ph.D., Program Director, Statistics, American Cancer Society, Statistics and Evaluation Center, Atlanta, GA

Daniel Schlenk, Ph.D., Professor, Aquatic Toxicology, Department of Environmental Sciences, University of California, Riverside, CA

FQPA Science Review Board Members

Lutz Edler, Ph.D., Head of the Department of Biostatistics, German Cancer Research Center, Heidelberg, Germany

Derek W. Gammon, Ph.D., D.A.B.T., Staff Toxicologist, Medical Toxicology Branch, California Environmental Protection Agency, Sacramento, CA

Richard Greenwood, Ph.D., Head of School, School of Biological Sciences, University of Portsmouth, Portsmouth, United Kingdom

Dale Hattis, Ph.D., Research Professor, Center for Technology, Environment and Development (CENTED), George Perkins Marsh Institute, Clark University, Worcester, MA

William L. Hayton, Ph.D., Professor, Division of Pharmaceutics, Associate Dean for Graduate Studies and Research, The Ohio State University, Columbus, OH

Wendy J. Heiger-Bernays, Ph.D., Associate Professor, Department of Environmental Health, Boston University School of Public Health, Boston, MA

Sastry S. Isukapalli, Ph.D., Assistant Professor, Department of Environmental and Occupational Medicine, University of Medicine and Dentistry of New Jersey - Robert Wood Johnson Medical School, Piscataway, NJ

Nu-may Ruby Reed, Ph.D., D.A.B.T., Staff Toxicologist, California Environmental Protection Agency, Sacramento, CA

Matthew K. Ross, Ph.D., Assistant Professor, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

INTRODUCTION

The FIFRA Scientific Advisory Panel (SAP) has completed its review of Assessing Approaches for the Development of Physiologically Based Pharmacokinetic (PBPK) Models of Pyrethroid Pesticides. Advance notice of the SAP meeting was published in the *Federal Register* on May 16, 2007. The review was conducted in an open panel meeting August 16 – 17, 2007 held in Arlington, Virginia. Dr. Janice Chambers chaired the meeting. Steven Knott served as the Designated Federal Official.

The development of PBPK models for pyrethroid pesticides offers many challenges, including:

a. As a class, pyrethroid pesticides have many structural similarities such that a "generic" model structure, with chemical specific adjustments as needed, can be developed. Chemical specific model parameters are anticipated to include partition coefficients, hepatic clearance rates and others.

b. It is anticipated that the PBPK models will be used for cross-species extrapolation of internal dose metrics for assessing the risk of pyrethroid neurotoxicity. Based on the results of *in vivo* experiments in rats, blood and brain concentrations of parent compound correlate with pyrethroid toxicity as measured by motor activity; either of these metrics could be a model output for use in a cumulative risk assessment.

c. Pyrethroids may have one or more chiral centers resulting in numerous stereoisomers. There is limited information on the toxicity and pharmacokinetics of the different stereoisomers. EPA proposes to evaluate three modeling assumptions in order to address the uncertainties resulting from the chiral chemistry of the pyrethroids.

d. Finally, there are limited human data to calibrate and evaluate these models for extrapolation to humans. EPA proposes to develop the human model through the use of computational and *in vitro* experimental approaches using human tissue. To evaluate this approach, EPA plans to develop equivalent rodent and human *in vitro* databases for metabolic and physiological parameters for use in the PBPK models. The utility of this approach will be assessed by comparing rodent model predictions with *in vivo* data. It is likely that scaling factors will be used in order to incorporate these *in vitro* parameters into the rodent model. When calibrating the human data, the scaling factors used in the rodent models will be used in the human models.

The purpose of the FIFRA SAP review was to request input on:

i. The appropriateness of a generic PBPK model,
ii. The proposed approach for developing these models with limited human dosimetry data.
iii. Potential dose metrics that are relevant for a cumulative risk assessment, and
iv. The proposed approach for the incorporation of chiral chemistry into model structure.
Planned methodologies for linking exposure models to PBPK models were also discussed.

Tina Levine, Ph.D., Director of the Health Effects Division (HED) in the Office of Pesticide Programs (OPP), provided opening remarks at the meeting. The agenda for this SAP

meeting included an introduction of the issues under consideration provided by Anna Lowit, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA. Presentations of technical background materials were provided by Michael DeVito, Ph.D., Michael F. Hughes, Ph.D., D.A.B.T., and Edward Scollon, Ph.D., National Health and Environmental Effects Research Laboratory, Office of Research and Development, EPA; Rogelio Tornero-Velez, Ph.D., National Exposure Research Laboratory, Office of Research and Development, EPA; and R. Woodrow Setzer, Ph.D., National Center for Computational Toxicology, Office of Research and Development, EPA.

PUBLIC COMMENTERS

Oral statements were presented by:

Myra L. Weiner, Ph.D., D.A.B.T., Research Fellow, Toxicology, Scientific & Regulatory Support, FMC Corporation. On behalf of the Pyrethroid Working Group

Written statements were provided by:

Myra L. Weiner, Ph.D., D.A.B.T., Research Fellow, Toxicology, Scientific & Regulatory Support, FMC Corporation. On behalf of the Pyrethroid Working Group

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

Although the Panel considered a generic pyrethroid PBPK model to be a good idea that should be implemented, the preliminary model derived by EPA may be too parsimonious. There may be subfamilies of pyrethroids, such as Type I and Type II, as well as very lipophilic non-ester pyrethroids, which may need to be considered separately before a family modeling approach can be developed to allow representation of all pyrethroids. In addition, as EPA realizes, absorption from the GI tract following oral dosing is critically dependent on the solvent system, both its nature and volume. Dosing should be standardized either to maximize absorption or to best model likely human exposure. Further, the administered dose(s) should be as low as possible in order to avoid complications arising from pharmacodynamic endpoints impacting the PBPK model(s); although the Panel recognized that a successful PBPK model should be largely dose-independent. Metabolism via esterase(s) and oxygenases can probably best be assessed using hepatocytes (rat and human). Also, the Panel encouraged efforts to study other tissues/organs, such as the GI tract. Furthermore, pharmacokinetic (PK) differences need to be studied from an age perspective, from fetus to adult. Such data could be critical for developmental neurotoxicity (DNT) studies. Current PBPK models are very adaptable and are able to handle many "special" situations. A variety of pharmacodynamic (PD) endpoints may need to be considered for a successful PBPK model. Only then can correlations be drawn between internal dose in a specific organ/tissue and neurotoxicity. The panel believed that limited information in the areas described above could complicate using a generic PBPK model for pyrethroids. However, this was not considered by the Panel to be a good reason to forgo the benefits of using such a model for the pyrethroid pesticides.

The Panel recommended that both the absorption from the GI tract, and into organs such as the brain, as well as the role of p-glycoprotein (Pgp) transporters for both pyrethroids and their metabolites needs to be better understood. If Pgp transporters are involved, stereoselective uptake and efflux may be an issue, and the possible effects of CYP3A inducers and inhibitors should also be investigated. Distribution for slowly perfused organs/tissues is considered to be diffusion rate-limited. The Panel recommended that the possibility of low permeability of the capillary endothelium be investigated. Perfusion or flow rate-limited distribution explains the uptake into well perfused organs/tissues. Tissue perfusion rates could be affected at toxic doses. Therefore, PBPK models may need to include fractional distribution of cardiac output to tissue compartments. In addition, the panel felt that the possible role(s) of plasma binding protein(s) should be resolved. Interspecies differences between rat and human in pharmacokinetics should be addressed by studying deltamethrin and permethrin (each enantiomer) in rat/human primary hepatocytes. Subsequent specific human esterase and oxygenase studies could enable precise enzyme identities and possible interactions to be described. The Panel noted that, for deltamethrin, esterase(s) appear to be more efficient in the human than the rat. The parallelogram approach could be improved by consideration of not just liver microsomes/ hepatocytes but also other tissues/organs, such as the small intestine. In sensitivity analysis, the question of how well *in vitro* hepatocyte data reflect *in vivo* metabolism was raised. In constructing a PBPK model, care should be taken in criteria selection so that the iterative process is successful. Further, human exposure biomonitoring for pyrethroid degradation products should be interpreted carefully due to the consumption of plant metabolites that are often identical to mammalian ones.

The Panel indicated that a greater number of time points should be examined for blood concentration following oral pyrethroid dosing. This would help to reduce uncertainty in model

predictions. In addition, the panel encouraged EPA to consider multiple PD endpoints in PBPK modeling. This is especially true given that Type I and Type II pyrethroids may cause different neurotoxic effects, as well as dose/time-related differences for a given pyrethroid. The panel agreed that peak concentration (blood and/or brain) was a useful dose metric for acute signs and that AUC may be more appropriate for chronic effects. Immunotoxicity should be considered a source of important PD endpoints in the development of PBPK model(s). Regional brain concentration could be an important metric, especially if it could be used in conjunction with specific neurotoxic PD endpoints. Pyrethroid concentration in PNS/spinal cord should be considered, given data showing greater concentrations in sciatic nerve than brain for both pyrethroids (lambda-cyhalothrin and permethrin) examined. Metabolites, such as epoxides, may be neurotoxic and/or hepatotoxic. They should not be excluded from PBPK consideration without further investigation. Inhalation and dermal exposure as well as hepatobiliary excretion should be included in the PBPK model(s). Mixture studies, e.g., with deltamethrin and permethrin, could add insight into possible chemical interactions.

Enantiomers of *cis* and *trans* isomers have similar PK properties, although they often have very large PD differences. PK and mammalian toxicity differences for *cis* and *trans* isomers are most marked for Type I pyrethroids, although PD differences are small in insects. *Trans* isomers of Type I pyrethroids are metabolized much more rapidly than *cis* and are often essentially non-toxic in mammals for the endpoints studied (e.g., phenothrin). However, from intracerebral injection studies, it is probable that, in mammals, the lack of toxicity of *trans* Type I isomers is due to reduced PD sensitivity. In contrast, for Type II pyrethroids, such as cypermethrin, there is little difference in PK, PD or toxicity (to mammals or insects) between *cis* and *trans* isomers. Enantiomer conversion (of cypermethrin) can occur in the rat *in vivo*. Plasma protein binding and tissue uptake, through Pgp or other transporters, can also be stereospecific. Due to these differences, the Panel recommended that for model parameterization, deltamethrin and each of the four permethrin enantiomers be studied for metabolism, plasma protein binding and tissue uptake. The Panel encouraged the use of chiral columns with GC-ECD because it would allow quantification of single enantiomers after dosing with a mixture. It appears probable that *cis* and *trans* diastereomers can be modeled separately, based on known PK differences. Based on experimental results (see above), enantiomers (of *cis* and *trans* isomers) should not be lumped together. Any resultant PBPK model should be able to predict the PK behavior of individual components. Based on current knowledge, lumping all stereoisomers together as one chemical should be avoided.

PANEL DELIBERATIONS AND RESPONSE TO CHARGE

The specific issues addressed by the Panel are keyed to the background documents, references, and the charge questions provided by EPA.

Question 1:

The Agency's issue paper describes different aspects of the pharmacokinetic (PK) properties of pyrethroid pesticides. The Agency believes that the important PK properties relevant for PBPK modeling are common among all or most members of this class, such that a 'generic' or family model structure with chemical specific adjustments, as needed, can be used. **Please comment on the evidence which does and does not support the concept of using a generic model structure for the pyrethroid pesticides.**

A generic pyrethroid PBPK model is a good idea, provided compound-specific modifications are applied. The Panel agreed that a generic model should be pursued. The Panel considered the preliminary model provided by EPA, based on deltamethrin data, perhaps too parsimonious because it did not fit especially well for permethrin. Possible reasons for this were discussed in terms of additional metrics that may need to be considered, including the measurement of residues in spinal cord and/or PNS (peripheral nervous system), in addition to brain and blood. However, the Panel observed that blood concentration could be shown to serve as a suitable surrogate for PNS. Differences between permethrin and deltamethrin could be related to pharmacodynamic (PD) differences, i.e., Type I and Type II mechanisms of action. The brain concentration metric is probably an excellent one for Type II pyrethroids but may not fit as well for Type I pyrethroids. In the latter case, nerve targets outside the brain may provide a more significant component of targets for the majority of exposure scenarios. It is therefore possible that separate sub-models will be necessary to describe PBPK data for Type I and Type II pyrethroids. There is also a possibility that competition and/or interaction occurs between different pyrethroids or different isomers of the same pyrethroid. The Panel believed that it was a good idea to have started modeling with relatively "simple" pyrethroids, such as deltamethrin, so that models could then be adapted for more complex ones. There are currently over 20 pyrethroids registered in the US as active ingredients and the Panel believed that current as well as future members of this class should be able to be incorporated into any model(s). Examples include non-ester pyrethroids, such as the rice insecticides etofenprox and silafluofen, which have either an ether or alkyl link in place of the ester, and have very high log P values (> 9). There may be subfamilies of pyrethroids which need to be considered separately for a Family Modeling Approach. Some or all of these could then be combined or lumped together.

While pyrethroids are very lipophilic, the associated extremely low water solubility causes slow dissolution, which tends to result in low oral and dermal bioavailability. Although this may appear counterintuitive since biological cell membrane permeability increases with increasing log P, it is as a result of a combination of factors. The rate of uptake from the gastrointestinal tract is not limited by transcellular movement but by diffusion through water boundary layers and mucus (Abraham et al., 2006). The process is further complicated by the gut contents comprising an aqueous system containing large amounts of protein, and bile acids that emulsify lipids present in the gut lumen. The fraction of non-polar compounds such as the pyrethroids (with low aqueous solubilities and that bind to dissolved organic material) that is

available to drive diffusion across the gut epithelium will be low. The formulation becomes part of the pharmacokinetic system, and may modify it. It is not surprising therefore that the absorption of pyrethroids from the gut into the blood appears to be critically dependent on the method of administration. Large quantitative differences in absorption, clinical signs and toxicity can occur, dependent on the nature and volume of solvent which determines the fraction of the administered dose in solution. In other words, depending on the dosing regimen and vehicle, absorption can appear to be dose-dependent. This makes it critical to standardize dosing regimens and vehicles so as to either maximize absorption/bioavailability or else to best replicate likely human exposure, as required.

It is also important that the administered dose should be as low as possible, without compromising analytical capabilities, in order to avoid complications arising from PD endpoints corresponding to clinical signs impacting the PBPK model(s). The Panel mentioned the degree to which the PBPK model can take into account pharmacodynamics (PD). Minimally, the PK model should be capable of yielding dose metrics considered most likely to be strongly correlated with the processes that cause the adverse effects. This is considered especially important if more than one pyrethroid syndrome is being modeled. It was thought that equitoxic doses of pyrethroids would generally be preferable to equimolar ones. However, it must also be understood that a successful PBPK model must be effective over a range of doses, including ones where neurotoxic signs are observed. The Panel cautioned EPA to remain mindful that high doses can lead to alterations in physiology that could affect the detailed performance of the PBPK models. For example where the toxicological symptoms include gross changes in cardiac output, changes in the pattern of circulation to various organ systems, gut motility, and respiratory function then there would be a marked modification of the pharmacokinetic system and in the values of the parameters for the model. These would be time and dose dependent, and may vary from compound to compound. These time-dependent changes need to be kept in mind when interpreting the outcome of the modeling process. A truly generic model may only be possible for low concentrations, but it would be sensible to investigate, in the first instance, dose ranges for which toxicological doses are available. It should also be borne in mind that the ultimate objective is to assess risk to humans, and so, if possible, dose regimes should reflect expected human exposure.

The metabolism of halogenated pyrethroids appears to be predominantly via ester hydrolysis in humans. This occurs in the liver, although the gastrointestinal tract has not been ruled out as a significant contributor. The metabolism in the gastrointestinal tract should be investigated further. In rodents there is evidence that blood plasma ester hydrolysis also occurs, as a minor pathway compared with hepatic clearance. Oxidative metabolism via hepatic cytochrome 450 (CYP) enzymes is also important, especially in rodents, before and/or after ester hydrolysis. Specific CYP isozymes are being investigated *in vitro* and these data should be included in construction of the model. Isolated rodent and human hepatocyte assays are being established for assessing pyrethroid metabolism *in vitro* and this approach was encouraged by the Panel because both oxidation and ester cleavage could be measured simultaneously. Qualitative differences in metabolic pathways for different pyrethroids must also be incorporated into any PBPK model(s). The inclusion of CYP inhibitors in some pyrethroid formulations was considered to be beyond the scope of the SAP's charge. Because such inhibitors are generally used only with the less toxic, unsubstituted, chrysanthemic acid pyrethroids, the Panel believed that this omission would have minimal impact on the PBPK model output.

It was stressed that toxicity would be caused only in response to bioavailable pyrethroid and, more specifically, to that part of the total bioavailable pyrethroid that is available to the site of action. For example, etofenprox and related non-ester pyrethroids are toxic to insects, but not to fish, because they are so lipophilic that bioavailability via gill uptake is reduced to sub-toxic levels (in fish) (Sieburth et al., 1990). Further, the fraction of bioavailable pyrethroid that is available to the site of action in mammals could be affected by plasma protein binding. The Panel encouraged further investigation of plasma binding protein(s) as a possible modifying process for inclusion in any PBPK model(s). Measurements should be made of binding and tissue uptake for deltamethrin and the four permethrin enantiomers. Archived tissue samples from previously conducted studies could provide adequate biological material for this analysis. Rates for pyrethroid tissue uptake could be quite different in the fetus and neonatal animals compared with the adult. The panel encouraged efforts to study bioavailability differences between adults and neonatal/juvenile animals, using both *in vivo* and *in vitro* approaches. The analysis of breast milk for exposure biomarkers could provide useful information for modeling. This issue could be critical in designing, conducting and interpreting developmental neurotoxicity studies, for example. The Panel liked the idea of modeling rapidly and poorly perfused organs/tissues separately. The Panel also encouraged EPA to incorporate hepatobiliary excretion in any model(s) because there is evidence that this could be significant for pyrethroids. It was pointed out that current PBPK models from the pharmaceutical field also are very adaptable; they have been used to successfully model a diverse range of agents, from macromolecules such as IgG, to metal ions. Specific mechanism-based equations (e.g., the familiar Michaelis-Menten formula) are available to handle the saturation of metabolic enzymes or transporters.

In the search for optimal toxicological endpoints for use in conjunction with PBPK models, the Panel mentioned both neuronal and non-neuronal endpoints. Type I and Type II pharmacodynamic responses are often distinct in the sense that, for example, Type I pyrethroid nerve discharges are not observed with Type II pyrethroids at any dose. Clinical signs can be dependent on route of administration, for example, choreoathetosis is not seen in the mouse after intracerebral dosing, although other routes will cause this sign in rodents. In this context it is necessary to be careful to choose the most appropriate clinical sign as the correlate in PBPK models. For example, reduced motor activity may not be as good an endpoint as tremors or choreoathetosis for PBPK/PD modeling purposes. It is thus possible that different risk assessment-based endpoints will make it necessary to produce different PBPK models. Examples of non-neuronal endpoints included the induction of specific CYP isozymes, PXR receptor activation and possible chronic toxicity. However, for the present, it is most appropriate that a pyrethroid-specific endpoint would include one or more parameters that reflected neuronal dysfunction, since the nervous system appears to be the primary target of pyrethroids. Nonetheless, the Panel encouraged the search for other specific endpoints of pyrethroid toxicity. The panel believed that limited information in the areas described above could complicate using a generic PBPK model for pyrethroids. However, this was not considered by the Panel to be a good reason to forgo the benefits of using a generic model structure for the pyrethroid pesticides.

Question 2:

In the development of PBPK models *in vivo* and *in vitro* data are acquired and used to calibrate and optimize the model. The predictions of the PBPK model are then ideally evaluated against additional *in vivo* data sets. In the case of pyrethroids, there are limited human data

available to calibrate and assess the human PBPK models. The Agency plans to develop a family modeling approach to address this issue. This approach assumes that because pyrethroids share many physical chemical and biological properties, a common model structure can be used for all pyrethroids. The family model approach allows for the assessment of the overall model structure with each iteration. The more iterations through this process, the more confidence is gained in the model's predictive abilities. Thus, the rat deltamethrin model is not only assessed by data from deltamethrin, but is assessed by model fits to data for every other pyrethroid. As our confidence in the rodent family model increases across pyrethroids, our confidence in the use of this modeling approach for rodent to human extrapolation also increases. The Agency is planning to develop equivalent rodent and human *in vitro* databases for metabolic and physiological parameters. The rodent *in vitro* parameters will be assessed by comparing model predictions to *in vivo* data. It is likely that scaling factors will be used in order to incorporate these *in vitro* parameters into the rodent model. When calibrating the human data, the scaling factors used in the rodent models will be used in the human models. **Please comment on this approach and other approaches that could be taken to calibrate and assess these models for use in human risk assessment.**

Comment on Family Modeling Approach

From the evidence in support of a generic PBPK model for pyrethroids (Question 1), it is likely that a useable generic model will emerge. The generic model will have some elements that are fixed for each species (rat and human) and others that are compound specific. A possible scenario is the following for a particular species:

Fixed Elements	Compound-Specific Elements
Tissue compartment volumes	tissue:plasma partition coefficients
Blood flow to compartments	tissue permeability x area product
	GI uptake rate constants
	Elimination clearance constants
	P-glycoprotein transport if any
	intestinal wall metabolic clearance if any, liver or plasma metabolic clearance rates
	reversible binding to blood components

Model parameter sensitivity analysis will identify those elements that strongly affect the magnitude of the dose metric. Modelers may then focus on fixed elements that must be invariant across the family of pyrethroids, and for the compound-specific elements modelers can attempt to evolve relationships between element value and pyrethroid structural features or properties (log P, molecular weight, particular substituents, molecular shape), analogous to a Quantitative Structure-Activity Relationship (QSAR) approach. With model development for successive family members, it may occur that a fixed element shows variability across compounds and in that case the element would become compound-specific or vice versa. In this way, confidence will be gained in the structure of the model. Sub-models will result that will allow prediction of some values for compound-specific elements from chemical properties. For other compound-specific elements, *in vitro* measurements for each compound will be required (e.g., intrinsic metabolic clearance, reversible binding to blood components).

The proposed approach [a family model like that used for the organophosphate (OP) pesticides] is consistent with mode-of action approaches. Unlike the OPs, the "true" molecular target is not known for pyrethroids, although the general target is one or more ion channel(s) in the neuron. Likewise, the effects of modifying nerve conductance pathways during development are also not known in a dose-effect manner. This approach is similar to the approaches used for development of Toxicity Equivalency Factors (TEFs) in dioxins/coplanar PCBs, although in that system, the target is known and the interactions with the target (agonist vs. partial agonist) are acknowledged. In this case, the target is unknown and the basis for a family model is chemical structure and some PK parameters as well as the doses needed to cause specific acute effects.

An evolving family of PBPK models that undergo continuous iterations, as each pyrethroid is studied, seems to be an appropriate and logical approach to tackle the pyrethroid family. Indeed, most PBPK models used to predict the disposition of compounds within specific chemical classes continually adapt and evolve in light of new information and data. For example, the PBPK models for volatile organic chemicals (VOCs) have very similar structures (each VOC exhibits a unique but related set of physicochemical and metabolic properties, which are used to parameterize the model). Therefore, it is not unreasonable to use a similar approach for the pyrethroid family of insecticides. Particularly, since the pyrethroids exhibit similar physicochemical properties, such as lipophilicity, and all are susceptible to metabolic attack by similar enzymes (e.g., oxidative and hydrolytic biotransformation enzymes), the family modeling approach, which undergoes multiple iterations, appears to be a logical strategy.

Prospectively this family approach seems to offer an acceptable probability of success and panel members were generally supportive of the approach. A number of comments, cautions and suggestions were contributed and these are presented in the following paragraphs.

Absorption

In light of results obtained with the deltamethrin model, the absorption processes from the GI tract needs to be better understood for the pyrethroids as a whole. The role of p-glycoprotein transporters in the absorption of pyrethroids within the intestine needs to be addressed. This could be tested directly. For instance, one could potentially use a Caco-2 cell culture system to determine if pyrethroids can be transported/effluxed out of the cultured enterocytes.

Distribution

The delta model incorporates permeability-rate-limited distribution for fat and slowly perfused tissue, which would mainly be muscle. It is possible that this could be due to low permeability of the intact capillary endothelium. From Leon Weiss Cell & Tissue Biology, A Textbook of Histology, 1988, VI Edition, page 377, there are three types of endothelium: continuous, fenestrated, and discontinuous:

> Continuous is found in muscle (all), CNS, exocrine pancreas, gonads, lung, and bone.

Fenestrated is found in GI mucosa, endocrine glands, renal glomerular and peritubular membranes, choroid plexus, and ciliary body. The diaphragms of the fenestrae consist of, it appears, two bilayers of membrane and are not truly open.

Discontinuous is found in liver, hematopoietic organs.

Fenestrae may be sufficiently permeable to pyrethroids to cause tissues with fenestrated capillary endothelia to be perfusion rate limited. But CNS should then be among the tissues that are permeability-rate-limited in distribution as it has continuous endothelium. This seems to be a puzzle. EPA staff indicated during the meeting that the use of permeability-rate-limited distribution was being reconsidered.

Panel members suggested that the appearance of diffusion rate-limited distribution may in fact be due to reversible binding to plasma proteins and other blood constituents, with distribution of unbound pyrethroid actually being perfusion-rate-limited. Two Panel members in particular pointed out the large discrepancy between tissue-to-plasma partition coefficient values of the delta model and the values expected based on the octanol/water partition coefficient of deltamethrin. One explanation for this discrepancy is plasma protein binding. The Panel encouraged EPA staff to characterize plasma protein binding of the pyrethroids and to investigate whether this would explain both the large difference between expected and model-predicted tissue/plasma partition coefficients as well as the apparent permeability-rate-limited distribution in muscle and fat.

Interspecies Differences in Metabolism

Given the tremendous differences in metabolism between rodents and humans, it is clear that utilization of the proposed family approach may be difficult. By using a structure activity approach, it may be possible to group compounds with similar *in vitro* activities. At the present time, enantioselective biotransformation has not been examined in humans. An early study in Dr. John Casida's laboratory indicated limited enantioselectivity of permethrin in mice (Soderlund and Casida, 1977). However, given the structural anomalies between permethrin and other pyrethroids, such as cypermethrin, with three chiral centers, enantiomeric differences may be more pronounced. The Panel recommended that studies with deltamethrin (most toxic) and each permethrin enantiomer be evaluated in rat and human primary hepatocytes. More in-depth studies could also focus on potential enantioselective studies with human carboxylesterases (CE) and CYPs. Variation in CE or CYP expression and polymorphism (ethnic, gender variability) could also be eventually modeled. One Panel member noted that with respect to Type II pyrethroids, recent data (Godin et al., 2006) suggest that the esterase pathway in humans is much more efficient than in rats, a clear species difference that will need to be incorporated into the human models.

The proposed parallelogram approach seems to rely exclusively on measurements of enzyme activity in liver microsomes. EPA should consider the likelihood that appreciable metabolizing activity could be found in (a) liver cytosol, and (b) in organs outside of the liver. For example, Yosigae et al. (1998) made extensive interspecies comparisons of carboxylesterase activities in microsomes from the small intestine for several simple substrates. These and other *in vitro* measurements of enzyme activities and Michaelis-Menten parameters are compiled in a

database on interspecies and interindividual variability in enzyme activity parameters (Hattis and Lynch, 2007).

The Panel recommended that the PK analyses could be conducted in hepatocytes and compared with microsomal results. With respect to intra-species differences comparing animals with humans, the use of pooled microsomes (as often seen in these studies) obscures true variability in enzyme activity, and this variability will need to be accounted for in the model. A database of intra-human variability for metabolizing enzyme activity is available (Hattis and Lynch, 2006).

Sensitivity analysis of the deltamethrin PBPK model indicated that the clearance parameters for oxidative and hydrolytic metabolism had the most influence on model predictions. Since metabolism appears to be a key determinant of pyrethroid disposition in rodents and humans, obtaining accurate *in vitro* metabolism data becomes essential. The 'delta' model used rat microsomes to estimate *in vitro* kinetic parameters for oxidative and hydrolytic biotransformation rates. However, data are now available that suggest hydrolytic metabolism of pyrethroids can also occur in the cytosolic fractions of livers; therefore, using only liver microsomes may underestimate rates of pyrethroid metabolism. Furthermore, the reliability of scaling metabolism data obtained using subcellular fractions as compared with intact cells (e.g., hepatocytes) is questionable. Therefore, rodent and human primary hepatocytes might provide more realistic estimates of pyrethroid metabolic clearance rates than could be obtained from subcellular fractions. Finally, it is necessary to determine whether *in vitro* metabolism rates accurately reflect *in vivo* metabolism rates.

Role for P-Glycoprotein (Pgp)

The oral bioavailability of deltamethrin increases with the size of the oral dose and Mirfazaelian et al. (2006) suggest that an export transporter (such as Pgp) may be operative (p. 440). They went on to note that Pgp and related transporters are active in the hepatocyte canalicular membrane and that biliary elimination of pyrethroids may occur. The molecular weight and log P values of the pyrethroids are consistent with biliary elimination. Pgp is also active in the blood brain barrier; studies in mice with Pgp gene knocked out show much higher brain levels of Pgp substrates than control mice for several drugs. For those pyrethroids (if any) that are Pgp transporter substrates, the generic model may have to incorporate its influence on oral absorption, biliary excretion and distribution into brain. The oral bioavailability influence may also involve intestinal wall first-pass elimination of orally administered pyrethroids. It frequently is the case that Pgp substrates are also CYP3A substrates. Godin et al. (2007) found that two pyrethroids were metabolized by members of the CYP3A family, which further suggests that pyrethroids may be Pgp substrates. When both Pgp and CYP3A are acting on a common substrate in the intestinal absorbing cell, the substrate exposure to CYP3A is enhanced by a recycling mechanism as it is repeatedly passively absorbed from and actively returned to the intestinal lumen. An important consequence of this phenomenon is that for substrates having a large first-pass presystemic elimination, coadministration of a CYP3A blocker (e.g., itraconazole) or inducer (e.g., rifampicin) can have very dramatic effects on the systemic exposure to the substrate, pyrethroids in this case (Backman et al., 1998). Risk analysis may have to take into account the possibility of this "drug interaction" and ontogeny of intestinal Pgp and CYP3A.

Tissue Blood Flow and Toxicity

At toxicologically active dosages the pyrethroids may alter tissue perfusion rates, which would affect the predictive capability of the PBPK model if it were not accounted for. In this regard, not only perfusion rate but also fractional distribution of cardiac output among tissue compartments might be affected by pharmacological and toxicological effects of the pyrethroids.

Iterative PBPK Model Construction

The family modeling approach is, at present, described as a sequence of iterations of PBPK modeling, stepping from one pyrethroid to the next. This description of the iteration in the documents looks somehow oversimplified when suggesting going through the pyrethroid family almost "linearly". One might assume the family approach starts with deltamethrin because of the wealth of data in that case, and then turns to permethrin, for similar reasons. But what then? Does there exist an elaborated strategy for how the iterations will be organized? What will be done if the "iteration" does not lead to improvement at some step? Would one go back some steps and then start anew? More strategic criteria could help to ensure that the iterative approach ends successfully, even if it stops at some stage, because of too many missing data.

Model Calibration and Human Scaling

When considering human dietary exposure to pyrethroids, care should be taken in the interpretation of human biomonitoring data. The presence of phenoxybenzoic acid and dichlorochryanthemic acid, for example, may be partly a result of exposure to these as plant metabolites rather than being an indication of (parent) pyrethroid exposure. Useful human pharmacokinetic data could be obtained from worker exposure studies. Such human exposure could be assessed using dermal patches, urinary monitoring and blood analysis for pyrethroids and/or biomarkers of exposure. A useful human database could thus emerge for testing any rodent-derived PBPK model(s). Efforts should be made to ensure that protocols for such (human) studies include the collection of information that is useful for PBPK modeling.

Other Issues Associated with the EPA's Draft Document

- Noted in Godin (2006) was a transposition of liver weight for humans and rats that should have been 25.7 and 40 g per kg body weight, respectively.
- Appendix B, Figure 1 data are measures of activity of individual isoforms in rats per unit of pure CYP, not % of metabolism attributable to each isoform *in vivo* or *ex vivo* in microsome preparations. This is similar for humans (Figure 2).
- Appendix B, Table 1 shows appreciable and apparently systematic differences in Vmax/Km ratios derived by different methods. The statistical fitting procedures need to be more fully elucidated and perhaps refined. In the simple linear regressions, were low dose data points selected or were all the points used? If the former, which data points exactly were used and on what basis?
- Appendix B, reconstruction calculations: (1) Can the metabolism of the pyrethroids by the mixed set of liver microsomes be reconstructed from the CYP and carboxylesterase concentrations and specific activities? (2) Can the whole body clearance rates of the pyrethroids be reconstructed from the liver microsomal data?

- Appendix C, Figure 2A has a very important result. The brain/blood concentration ratio is much higher at 7 hours than at 4 hours. Investigators also note that brain concentration is the same between the two time points, pointing to differences in blood concentration as the source of the discrepancy (Figure 1A and 1B). Blood concentration should be replotted with a linear y axis; a log regression equation makes no sense. The interpretation must be that the brain concentration must depend on brain uptake at very short times after exposure, when some that would otherwise be bound in blood and elsewhere is available for diffusion. This has significant implications for the modeling. The modelers must distinguish between bound and unbound material in plasma.
- Appendix D (model). Brain concentration vs. time profile does not fit. Brain elimination is appreciably slower in fact than is expected by the model (Figure 4C).
- Humans are exposed to pyrethroids at low concentrations under chronic conditions. More information is becoming available to demonstrate that pyrethroids activate nuclear receptors (e.g., PXR), which can subsequently upregulate cytochrome P450 (CYP) gene expression in cell culture systems. However, it is unclear whether CYP induction following pyrethroid exposure has been demonstrated *in vivo*. How will the PBPK models handle gene expression induction by pyrethroids (or will they?).
- Drug-pyrethroid, pesticide-pyrethroid, disease and lifestyle influences on pyrethroid kinetics and toxicity, and developmental exposures to pyrethroids are potentially important in the development of PBPK models for pyrethroid risk assessment.
- Bayesian model selection approaches along with sensitivity and uncertainty analysis (both structural and parametric) are important components of the family modeling approach.
- One approach for consistent cross-species extrapolation is through the use of the same compartmental structure across multiple species, where corresponding simplified models can be derived using lumping techniques (e.g., see Bjorkman, 2003; Brochot et al., 2005; Nestorov et al., 1998).
- With respect to metabolic differences between animals and humans, do microsomes really reflect all of the tissue's enzyme activity? Can there be some activity in non-microsomal "tissue"?

Other Possible Approaches

Looking at other neurotoxicants that have been regulated under EPA, the organophosphates, carbamates, mercury, and others, there are either a) known mechanisms of action at the molecular level, or b) robust epidemiological data that are based on human behavioral testing. In the latter case, the animal data are used to support the human data, rather than using the animal data scaled to the human system. So, without robust human data, or at least a coordinated system for comparing behavioral effects in rodents with those in humans, more work needs to be done before adopting this approach.

The mode of action, as defined by acute effects, may overlook some of the newer developmental data. The shape of the dose-response curve may look the same for the acute endpoints, but will need to be extended into the low-dose region based, not on modeling, but additional neurobehavioral data.

The Agency might want to view the effects, not as neurotoxic effects, but as 'developmental' effects and review the Agency guidelines for these effects (and testing for PK parameters).

Question 3:

The Agency's issue paper and data provided in Appendix C show that blood and brain concentrations of parent compound in the rat correlate with pyrethroid toxicity as measured by motor activity. At the present time, the Agency plans to evaluate additional metrics (e.g., area under the curve) with additional pyrethroids. Moreover, the Agency plans to test other behavioral measures (e.g., startle response). **Please comment on the available database to assess the dose metric for pyrethroids. Please also comment on what additional experiments, if any, that could further inform the dose metric.**

Comments from the Panel in response to charge question 3 are organized into three sections.

I. Available Database Presented By The Agency

Information in the poster presentation provided in Appendix C of the EPA's background paper is brief. A question was raised about Figure 3, regarding the wide difference in the Hill exponent for blood bifenthrin concentration between 4 and 7 hours after the exposure (i.e., 3.01 versus 0.5). It was also pointed out that the linear versus log scale graphic plot is not ideal for showing Hill model fit. The model fit with brain data appears more reasonable. The Hill coefficient of "1" for the administered dose is important. It indicates that a very simple receptor relationship is involved and that it depends on very fast diffusion of administered material with no threshold. The large uncertainty in estimates suggests the need to repeat the experiment, perhaps using other time points.

II. Dose Metric Selection

The dose metric selection is dependent on the mode of action for the toxicity endpoint(s) of interest. In general, the dose metric should closely reflect the response at the target tissue with demonstrated temporal correlation such that it can be used as a reasonable predictor for the specific endpoint(s) within a range of exposure conditions. For risk assessment purposes, the toxicity endpoint is expected to be fairly sensitive, i.e., occurring at the low dose range before other adverse effects are invoked at higher doses. For neurotoxicity of both Type I and Type II pyrethroids, the dose metric would have a sufficiently good fit with some common endpoints for these structure-activity related chemicals and thus can also be used for assessing their cumulative exposures. An additional desirable feature is that the dose metric can adequately describe a relatively wide range of the dose-response relationship for the common endpoint(s) before reaching a point of altered dose-response relationship (e.g., rate limitation, binding saturation) such that it can no longer serve as a predictor of endpoint(s) of interest.

In terms of neurotoxicity endpoints, the availability of studies on the effects on motor activity for many pyrethroids favors its use in selecting a suitable dose metric for use in PBPK modeling. Studies that correlate motor activity with blood and brain concentrations of parent chemicals undertaken by the Agency showed promising results for further testing with other

pyrethroids. The Panel also supports the Agency's intent to explore the possibility of using startle response as another endpoint for coordinating dose metric selection. Because less information on pyrethroids is available for this endpoint, more effort is expected for collecting data in this area. It was noted that unlike motor activity, the effect on sensory evoked motor reflex in acoustic startle response by a pyrethroid may be bi-directional, enhanced in some cases and inhibited in others. Thus, closer studies on the underlying mechanism may be needed for normalizing this response across the entire group of pyrethroids.

Since the manifestations of pyrethroid neurotoxicity are different for the two groups of pyrethroids, the Agency is encouraged to explore the possibility of selecting dose metrics that correlate to groups of multiple endpoints (e.g., from Functional Observational Battery tests), rather than using only one or two endpoints as proposed by the Agency. Using a group of co-occurring endpoints or mechanistically related syndromes could allow for a more inclusive and robust set of dose-response relationships that is applicable to multiple pyrethroids in cumulative risk assessment.

As a start, the Panel is in general agreement for using blood and brain pyrethroid concentration profiles as dose metrics for pyrethroid neurotoxicity. For pyrethroids that exert toxicity directly by acting primarily on the sodium channel of nerve cells, the expectation that toxic response will be related to the parent compound concentration is reasonable and supported by preliminary data. Both peak concentration and area under the curve (AUC) could provide useful information and most likely be necessary for achieving a satisfactory explanation of toxic effects. AUC represents an opportunity factor that provides a measure of exposure at the site of toxicity. Peak concentration provides a measure of the short-term maximum exposure. It is desirable to investigate the plasma protein binding of pyrethroids. If there is significant plasma protein binding, the unbound plasma concentration may prove superior to the total concentration as it is most directly applicable to the concentration at the site of toxicity. In this case, protein binding should also be included in the model.

The profile of brain concentration appears to be suitable for endpoints that are originated at the central nervous system (CNS), using the dose metric that most directly applies to the concentration at the site of toxicity; i.e., the unbound pyrethroid concentration in equilibrium with pyrethroid bound to sodium channel and perhaps other ion channels in nerve cells. It was noted that brain concentration is only a close surrogate since the pyrethroid concentration in equilibrium with that which is bound to ion channels is likely a small fraction of the total amount of pyrethroid in the brain, with the remainder being bound by other substrates or partitioned into lipids. It is necessary ultimately to determine whether distribution to all portions of the brain is equal or whether there are discrete brain structures that selectively concentrate pyrethroids. This will provide more specific data for use of brain concentration as a metric.

The profile of pyrethroid in the blood is useful as a systemic dose metric and can be used for a wider range of toxicity endpoints. It could serve as a predictor for neurological responses other than from brain, e.g., of spinal origin or at the peripheral tissue level. It can also be used as a general purpose predictor for other relevant toxicities of concern, e.g., developmental neurotoxicity. For acute exposures, an issue is the speed with which pyrethroid equilibrates between the blood and the site of toxicity. The deltamethrin PBPK model currently has pyrethroid distribution into brain as a diffusion-rate-limited process and with a brain/plasma PC = 0.22. The model-predicted brain concentration-time profile closely tracks the plasma

concentration-time profile, with a small lag time separating peak concentrations. If all the pyrethroids behave this way, then plasma-concentration based dose metrics would be suitable and have the advantage of ease of measurement experimentally for model validation. For chronic exposure scenarios, the concentration at the site of toxicity should be at (or near) steady state and directly proportional to the plasma concentration (unbound), so distribution kinetics between CNS and plasma should be less an issue than with acute exposure. As the Agency gains more experience from future studies, the choice of dose metrics may become more specific for the endpoint(s) of concern.

III. Additional Studies Or Data To Further Inform Dose Metric Selection

Some of the areas for future research have already been mentioned previously or in the Panel comments for other charge questions. They are briefly summarized below. Their listing appearance does not imply any priority order.

1. Other behavioral endpoints should be examined to see whether they correlate with brain concentration. It is imperative that enantioselective evaluations be carried out with these tissue concentrations.

2. Anadon et al., 1996, reported marked regional variation in brain concentration of deltamethrin in rat, with regional brain/plasma ratios from ~5 to ~500. This issue should be examined with respect to scaling from rats to human since the proportions of brain regions differ between humans and rats. It was also noted that many behavioral effects are focused in specific brain regions. See work of Heaton et al., 2007.

3. Interspecies endpoint comparison is preferentially done within a framework of broad functional categories, such as sensory, motivational, cognitive, motor, and social variables.

4. Both brain- and plasma-based dose metrics would be affected by transporter in the brain capillary endothelium; e.g. Pgp (P-glycoprotein). Experiments to identify and characterize the effects of transporters on pyrethroid distribution between brain and plasma would be important in this regard.

5. The information presented by the Agency is insufficient to determine what the best dose metric might be for other pyrethroids. It will be necessary to test candidates for relevance to the prediction of pharmacodynamic activity (and pathology).

6. Protein or plasma-based binding of parent/metabolites should be examined. The current distribution scheme, which does not include this possibility, should be examined experimentally.

7. Peripheral nerve concentrations of pyrethroid could be a useful metric, especially for those causing Type I effects. In Summary Table 2.6, both permethrin and cyhalothrin appear to be concentrated more in sciatic nerve than in brain. This indicates that peripheral nerve(s) and/or spinal cord could be sites of interest for any PBPK modeling efforts.

8. There is some evidence that the metabolites may be toxicologically important (Ruzo *et al.,* 1984). The current literature on PXR activation should also be addressed.

9. There have been reports of developmental neurotoxicity that can be more subtle yet important, e.g., neuronal death, actions mediated via pyrethroid metabolites (reviewed by Shafer et al., 2005). It may be necessary to gather data in addition to blood and brain pyrethroid concentrations for developmental effects in animal/human systems. The animal models used to evaluate in utero and neonatal exposures should be extended to include endpoints that could be more sensitive, such as behavioral effects. Additional concerns are:
    - Chronic, low doses (lower than ED50) in developing systems and *in vitro* models that appear to affect migrating neurons (part of the normal ontogeny of brain development in mammals)
    - Peripheral and autonomic nervous systems
    - Effects at specific brain regions

10. Related to the concern for developmental toxicity is the issue of age-sensitivity. According to information summarized by Ray and Fry (2006), neonates can be 4-17 times more sensitive than adults. Pharmacokinetic (PK) data (e.g., ADME) are needed for fetus, neonates and the young for determining whether the adult PK data are sufficient for scaling to these and other potentially sensitive population subgroups. This will also allow the Agency to determine which dose metric is most meaningful and predictive – blood, brain or perhaps cord blood (for the neonate – as with Hg).

11. Inhalation route is not presently in the PBPK model, probably due to the physicochemical properties of the pyrethroids; however fine-particle-based inhalation exposures may need to be examined in the Stochastic Human Exposure and Dose Simulation model (SHEDS) and inhalation of particle-bound substances may become important. It would be better to be aware that these exposure data are missing.

12. Account for dose in skin, not just the dose applied to the skin as the skin serves as a reservoir for continued absorption of compound – this is consistent with the dermal absorption literature for other organics. See Wester et al., 1993. The model will need to account for this source of exposure that is likely diffusion limited (but probably never complete).

13. Hepatobiliary excretion may need to be considered in the development of PBPK models to adequately describe pyrethroid pharmacokinetics. The study cited by the Agency (to discount such effects) was conducted in the 1960s and considered only one pyrethroid (cypermethrin). Other studies, using resmethrin and tetramethrin for example (cited in Casida et al., 1983), have indicated that biliary excretion could be significant. Including hepatobiliary excretion in PBPK model(s) could thus improve predictive properties of such model(s).

14. Immunomodulation is another potentially low dose effect of pyrethroids. Bifenthrin is reported to affect phytohemagglutinin (PHA) activation of homotypic aggregation in

human T-cell lines (Hoffman, 2006). The Agency may want to consider the approach to investigate the sensitivity of immunotoxicity endpoints.

15. In light of the need for assessing cumulative toxicity of chemicals with similar mechanisms of toxicity, it may be desirable for the Agency to conduct a limited set of mixture studies to gain understanding on chemical interactions that impact kinetic processes.

Question 4:

Pyrethroids may have one or more chiral centers resulting in potentially multiple stereoisomers. Some products, such as deltamethrin, are relatively pure single stereoisomers. Others such as cypermethrin may contain as many as eight stereoisomers. There is limited information on the toxicity and pharmacokinetics of the different stereoisomers. The Agency is proposing to evaluate three modeling assumptions. The first approach combines all stereoisomers as one chemical. The second approach includes modeling all the diastereomers and ignores the enantiomers; the third approach includes only the toxic stereoisomers. To evaluate these approaches, the Agency is using permethrin as a model chemical. **Please comment on these three approaches. Are there additional modeling assumptions or approaches that the Agency should consider or that could simplify the modeling?**

This is not an easy question to answer without more data. However, it is important for the success of this project that an adequate answer be found since the selection of the strategy to be used will determine the utility and size of the workload involved.

Pyrethroid insecticides are complex mixtures of up to 8 stereoisomers. Acute toxicity studies have demonstrated the 1-*R-cis* enantiomer is the most toxic isomer in most ester mixtures. However, it should be noted that other enantiomers are also toxic (Liu *et al.* 2005). EPA has focused pilot studies on deltamethrin for which the commercial product comprises mainly (98%) the 1-*R-cis-αS* stereoisomer that, for this compound, is the most toxic form. The use of this compound avoids the problems associated with dealing with mixtures of isomers, and provides a sound basis for initial validation of the general PBPK model. However, since most other pyrethroids are applied as mixtures of isomers there is a need to produce a more generally applicable model that can take into account potential differences in pharmacokinetic behavior of the isomers. This will be important when the PBPK model is used for predicting toxicity, and differential pharmacodynamic activity between isomers is also taken into account.

The differential toxicity of the various isomers can be affected by a number of processes. Pharmacodynamic activity can vary between isomers as can a number of pharmacokinetic processes. This problem is a familiar one in the pharmaceutical industry where many therapeutic agents are chiral and are administered as racemic mixtures. Since individual enantiomers can be biologically active while their antipode lacks activity, there has been heavy investment in developing pharmacokinetic and associated analytical methods to resolve the individual enantiomers. This has provided evidence in the drug PK literature demonstrating that racemic compounds often display PK profiles that are different from those of the pure single enantiomers. There is much information in the literature on the toxicity of various isomers of pyrethroids, but it is not always known whether this is due to differences between pharmacodynamic or pharmacokinetic properties. For instance the *cis* and *trans* isomers of cypermethrin are not

equitoxic when administered at the same dose intracerebrally to rats, but even with this simple system pharmacokinetic processes may be involved. Although there are some generalizations that can be made, there are no hard and fast rules.

Biotransformation can make a major contribution to the differential toxicity of isomers of a single compound. Since marked differences have been observed between the biotransformation pathways by which different isomers are metabolized, this needs to be taken into account. Enantiomers of specific *cis* and *trans* isomers appear to show similar pharmacokinetic properties, but this contrasts with the pharmacodynamic behavior, where the geometric isomers can be similar and the enantiomers can be different (by several orders of magnitude). Pharmacokinetic and toxicity differences between geometric isomers are most marked for Type I pyrethroids, whereas pharmacodynamic differences are small. The *trans* isomers are metabolized much more rapidly than *cis* and are correspondingly much less toxic to mammals. Indeed, some Type I pyrethroids, such as phenothrin, are essentially non-toxic as the *trans* isomer in mammals. For Type II pyrethroids such as cypermethrin, however, there is little difference in pharmacokinetics, pharmacodynamics or toxicity between geometric isomers. Further, recent studies by Wang *et al.* (2006) showed that enantiomeric conversion of cypermethrin enantiomers occurred *in vivo* after injection into rats, though it is not currently known whether this occurs across all pyrethroids. Further there may be interactions between isomers due to competitive inhibition of some important enzymes, and these effects could be investigated realistically using rat and human primary hepatocytes that maintain enzyme integrity.

Detoxification is only one parameter that may vary between enantiomers. Plasma protein and tissue uptake could also be affected, and could make an important contribution to the uncertainties associated with predictions based on the PBPK model. For instance, enantioselective tissue uptake and accumulation of bifenthrin stereoisomers has been observed in vertebrates (Wang et al. 2007). Given that much of the uncertainty of permethrin uptake and distribution using the deltamethrin model could be related to tissue uptake from blood plasma, this seems a logical target to explore. There is some evidence from acute toxicity studies (Liu *et al.*, 2005) of enantioselective toxicity in vertebrates and aquatic insects. It may not be possible to extrapolate enantioselectivity in protein binding or metabolism from rodents to humans. However, once structural motifs that optimize movement to and from plasma binding proteins and in/out of tissues (e.g., central nervous system) can be identified, then groupings of compounds based on enantioselective behavior could be utilized with the model.

The Panel recommended that when parameterization of the model for deltamethrin, where the situation is relatively simple, has been completed, then metabolism, plasma protein binding, and tissue uptake can be investigated using the four permethrin enantiomers to see if there is evidence of marked enantioselective metabolism and disposition. Establishment of an enantiomer ratio for each permethrin PK parameter would be very helpful in making an informed decision on the best practical approach to resolving the chirality dilemma.

Although technical materials contain limited numbers of isomers, and in varying proportions, this situation is complicated by the fact that changes in isomer composition can take place between application in the environment and exposure of animals. The modeling process needs to consider all possible isomers in order to model possible future products as well as existing products. The amount of work involved in obtaining the necessary data could be reduced by using methods based on those developed by Liu *et al.* (2005a, and b) and Wang *et al.*

(2007) who used chiral columns with GC-ECD to measure tissue concentrations of the various isomers of pyrethroids. Given the total concentrations and the isomer ratios this should provide the necessary information for relatively little extra effort. This may obviate the need for pure enantiomers of pyrethroids that are difficult to obtain. If there are interactions between the various isomers, then applying the mixture and separating the isomers from the various tissues (the equivalent of cassette dosing of drugs) would not only provide data that are more toxicologically relevant but also limit the number of experiments to be effected.

There was a consensus among the Panel that the use of permethrin as a model compound could provide a basis for a general methodology, and information on the enantioselective disposition of this compound could inform future work. If it is found that stereoselectivity does not markedly influence disposition, then it may not be necessary to evaluate all stereoisomers and the model can be simplified. However, since, with the exception of deltamethrin, humans are rarely exposed to single isomers, a simultaneous exposure to environmentally relevant mixtures would be preferable providing that the PBPK model can be suitably parameterized using the strategy outlined in the previous paragraph. Indeed, ideally the model would also involve a realistic representation of the method of contact and route of entry since the dosing regime may produce effects on the PK phase of poisoning, and hence the overall toxicity, that are as large as those observed between isomers.

Conclusions Regarding Charge Question 4

This issue of modeling pyrethroid stereochemistry is driven by the analytical capabilities available and the costs and time involved in effecting the necessary program of pharmacokinetic studies, and associated analysis. There is much uncertainty involved in the third approach outlined in Question 4 since, as discussed above, there can be marked differences in the pharmacokinetic behavior and toxicity of the various stereoisomers, there may be interactions between the various isomers, and the different technical products vary in composition. For these reasons the Panel could not support the approaches of either treating the mixtures of isomers as one compound or of considering only the most toxic isomers. Any model developed must be able to handle not only existing products, but also new products that may contain different mixtures of isomers.

The second approach of modeling the *cis* and *trans* diastereomers has some merit because these two isomers are known to exhibit different pharmacokinetic behavior. Moreover, the *cis* and *trans* isomers are not equitoxic when administered intracerebrally at the same dose. Therefore, knowledge of the PK behavior of each isomer would be beneficial. If resources are limited, then as many enantiomers as possible could be lumped on the basis of knowledge (gained from the work on permethrin) of what is driving enantioselective activity. However, the downside to this approach will be the loss of resolution in terms of modeling stereo-specific processes, if they occur.

Since the three dimensional configuration of pyrethroids is a critical determinant of toxicity, then increased resolution in the models would be beneficial. If the levels of permethrin in blood and tissues could be resolved into their component stereoisomers, a much greater level of mechanistic information would be gained, and the model would be much more widely applicable (even for effects at target sites outside the nervous system; e.g., induction effects on xenobiotic metabolizing enzymes in the liver). If the ultimate goal is to assess the risk of these

pesticides and eliminate 10-fold safety factors, then the risk of each product (consisting of a number of isomers) needs to be evaluated initially as a single entity. The PBPK model should predict the pharmacokinetic behavior of the individual components, thus reducing uncertainties associated with predictions of toxicity based on the pharmacokinetic behavior.

Since currently available analytical methods can adequately separate the enantiomers as well as the *cis* and *trans* diastereoisomers of pyrethroids, then the first approach (i.e., combining all stereoisomers as one chemical) should be excluded. The cassette dosing strategy applied to permethrin should inform future decisions on the feasibility and necessity of modeling all isomers of each pyrethroid.

References:

Abraham, M.H. Zhao, Y.H. Le, J. Hersey, A. Luscombe, C.N. Reynolds, D.P. Beck, G. and Cooper, I. 2006. On the mechanism of human intestinal absorption. Europ. J. Med. Chem. 37:595-605.

Anadón, A. Martinez-Larrañaga, M.R. Fernandez-Cruz, M.L. Diaz, M.J. Fernandez, M.C. Martinez, M.A. 1996. Toxicokinetics of deltamethrin and its 4'-HO-metabolite in the rat. Toxicol Appl Pharmacol 141:8-16.

Backman, J.T. Kivisto, K.T. Olkkola, K.T. Neuvoven, P.J. 1998. The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. Eur J Clin Pharmacol 54(1):53-58.

Bjorkman, S. 2003. Reduction and lumping of physiologically based pharmacokinetic models: prediction of the disposition of fentanyl and pethidine in humans by successively simplified models. J Pharmacokinet Pharmacodyn 30(4):285-307.

Brochot, C. Toth, J. Bois, F.Y. 2005. Lumping in pharmacokinetics. J Pharmacokinet Pharmacodyn 32(5-6):719-736.

Casida, J.E. Gammon, D.W. Glickman, A.H. and Lawrence, L.J. 1983. Mechanisms of selective action of pyrethroid insecticides. Ann Rev Pharmacol Toxicol 23:413-438.

Godin, S.J., Scollon, E.J., Hughes, M.F., Potter, P.M., DeVito, M.J., and Ross, M.K. 2006. Species differences in the *in vitro* metabolism of deltamethrin and esenvalerate: differential oxidative and hydrolytic metabolism by humans and rats. Drug Metabolism and Disposition 34:1764-1771.

Godin, S.J. Crow, J.A. Scollon, E.J. Hughes, M.F. DeVito, M.J. and Ross, M.K. 2007. Identification of rat and human cytochrome P450s and a rat serum esterase which metabolize deltamethrin and esfenvalerate. Drug Metabolism and Disposition (In press).

Hattis, D. and Lynch, M. 2006. "Interspecies Differences and Human Interindividual Variability in Tissue-Level Pharmacokinetic Parameters. Task 3—Final Report—Description and Analyses of the Data Bases." Report by Clark University to the U.S. Environmental Protection Agency Under Purchase Order No. EP05W002147, October 2006. Available at http://www2.clarku.edu/faculty/dhattis

Hattis, D. and Lynch, M. K. 2007. Empirically Observed Distributions of Pharmacokinetic and Pharmacodynamic Variability in Humans—Implications for the Derivation of Single Point Component Uncertainty Factors Providing Equivalent Protection as Existing RfDs. in Toxicokinetics in Risk Assessment, J. C. Lipscomb and E. V. Ohanian, eds., Informa Healthcare USA, Inc., 2007, 69-93.

Heaton, K.J. Palumbo, C.L. Proctor, S.P. Killiany, R.J. Yurgelun-Todd, D.A. White, R.F. 2007. Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. Neurotoxicology 28:761-769.

Hoffman, N. Tran, V. Daniyan, A. Ojugbele, O. Pryor, S. C. Bonventre, J. A. Flynn, K. and Weeks, B. S. 2006. Bifenthrin activates homotypic aggregation in human T-cell lines. Med Sci Monit 12(3):BR87-94

Liu, W. P. Gan, J. Y. Schlenk, D. Jury, W. A. 2005a. Enantioselectivity in environmental safety of current chiral insecticides. Proc. Natl. Acad. Sci. U.S.A. 102, 701-706.

Liu, W. P. Gan, J. J. Qin, S. J. 2005b. Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. Chirality 17, S127-S133.

Mirfazaelian, A. Kim, K-B. Anand, S.S. Kim, H.J. Tornero-Velez, R. Bruckner, J.V. and Fisher, J.W. 2006. Development of a physiologically based pharmacokinetic model for deltamethrin in the adult male Sprague-Dawley rat. Toxicol. Sci., 93, 432-442.

Nestorov, I.A. Aarons, L.J. Arundel, P.A. Rowland, M. 1998. Lumping of whole-body physiologically based pharmacokinetic models. J Pharmacokinet Biopharm 26(1):21-46.

Ray, D. E. and Fry, J. R. 2006. A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacology & Therapeutics 111(1):174-193.

Ruzo, L.O. Gammon, D.W. and Casida, J.E. 1984. Neurophysiological activity and toxicity of pyrethroids derived by addition of methylene, sulfur or oxygen to the chrysanthemate 2-methyl-1-propenyl substituent. Pestic.Biochem.Physiol. 21(1):84-91.

Shafer, T. Meyer, D. Crofton, K. 2005. Developmental Neurotoxicity of Pyrethroid Insecticides: Critical Review and Future Research Needs. Environ. Hlth. Perspect. 113:123-136.

Sieburth, S.M. Lin, S.Y. Engel, J.F. Greenblatt, J.A. Burkart, S.E. and Gammon D.W. 1990. Silane analogs of MTI-800: biology and chemistry. pp. 142-150 in Recent Advances in the Chemistry of Insect Control II. Ed. L. Crombie, Royal Society of Chemistry, London.

Soderlund, D.M. and Casida, J.E. 1977. Effects of pyrethroid structure on rates of hydrolysis and oxidation by mouse liver microsomal enzymes. Pestic. Biochem. Physiol., 7, 391-401.

Wang, Q. Qui, J. Zhu, W. Jia, G. Li, J. Bi, C. and Zhou, Z. 2006. Stereoselective degradation kinetics of theta cypermethrin in rats. Environ. Sci. Technol..40: 721-726.

Wang, L. Liu, W. Yang, C. Pan, Z. Gan, J. Xu, C. Zhao, M. Ma, Y. Schlenk D. 2007. Estrogenic potential and bioaccumulation of bifenthrin enantiomers. Environ. Sci. Technol. 41, 6124-6128.

Weisskopf, M. Hu, H. Mulkern, R.U. White, R. Aro, A. Oliveiva, S. Wright, R.O. 2004. Cognitive deficits and magnetic resonance spectroscopy in adult monozygotic twins with lead poisoning. Environ. Hlth. Perspect. 112:620-625.

Wester, R.C. Maibach, H.I. Sedik, L. Melendres, J. Wade, M. 1993. Percutaneous absorption of PCBs from soil: *in vivo* rhesus monkey, *in vitro* human skin, and binding to powdered human stratum corneum. J Toxicol Environ Health. 39:375-82.

Yoshigae, Y. Imai, T. Horita, A. Matsukane, H. Otagiri, M. 1998. Species differences in stereoselective hydrolase activity in intestinal mucosa. Pharma. Res. 15 (4) 626-631.